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HLA and KIR Frequencies in Sicilian Centenarians

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Abstract

Several studies suggest that human longevity appears to be linked inextricably with optimal functioning of the immune system, suggesting that specific genetic determinants may reside in loci that regulate the immune response, as human leukocyte antigen (HLA) and killer cell immunoglobulin-like receptor (KIR) genes. It has been suggested that longevity is associated with positive selection of alleles (i.e., HLA-DR11) or haplotypes (i.e., HLA-B8,DR3) that confer resistance to infectious disease(s). On the other hand, the cytolytic activity of natural killer (NK) cells is controlled by activating and inhibitory cell-surface receptors, including KIR. The genetic diversity of the KIR loci with respect to successful aging has been analyzed only in one study performed in the Irish population. Although two KIR genes (*2DS3*, *2DL5*) displayed an initial increased frequency in the aged group, the significance of this association was lost when repeated in a second cohort. We have evaluated by polymerase chain reaction–sequence-specific primers (PCR-SSP) HLA-DRB1 and KIR receptors/HLA ligands frequencies in centenarians and controls from Sicily. Our results demonstrate an increase of the HLA *DRB1*18* allele in male centenarians ($p = 0.0266$, after Bonferroni correction). Concerning KIR, no significant difference was observed after Bonferroni correction. However, our findings suggest that HLA/KIR/longevity associations are population specific, being heavily affected by the population-specific genetic and environmental history. This kind of study is important to better understand aging and longevity, hence enhancing the planning of antiaging strategies.

Introduction

Human longevity appears to be inextricably linked with optimal functioning of the immune system, suggesting that specific genetic determinants may reside in loci that regulate the immune response, as human leukocyte antigen (HLA), cytokine networks, and killer cell immunoglobulin-like receptor (KIR).[1] The studies performed on the association between longevity and HLA (the human major histocompatibility complex [MHC]) are generally difficult to interpret, owing to major methodological problems. However, some of these studies, which are well designed and performed and support suggestions derived from the mouse studies on MHC effects on longevity.[2,3] These studies suggest that longevity is associated with positive selection of alleles (i.e., *HLA-DR11*) or haplotypes (i.e., *HLA-B8,DR3*) that confer resistance to infectious diseases, respectively, via peptide presentation or via antigen nonspecific control of immune response. It is noteworthy that these associations are mostly gender related.[3,4]

In our previous case–control studies, we have demonstrated that both HLA-DR and -DQ alleles are not associated with longevity in the Sardinian population. On the other hand, association studies are subjected to a number of possible confounding factors (as part of the homogeneity of the population in terms of geographic origin).[5,6]

Another crucial role is played by the KIRs in the regulation of innate immune response. They are glycoproteins expressed on the cell surface of natural killer (NK) cells and subsets of T cells. These polymorphic receptors interact with specific motifs on HLA class I molecules, modulate NK cytolytic activity, and are encoded by genes located on chromosome 19q13.4.[7–9]

The KIR gene family represents a highly polymorphic class of receptor-encoding genes, which have displayed considerable allelic diversity, and it is postulated that KIR polymorphism arises from both the occurrence of random point mutations and recombination between homologous KIR genes.[10,11] At present, approximately 16 expressed KIR genes have been identified and these are classified into four major groups (*KIR2DS*, *3DS*, *2DL*, *3DL*) on the basis of the structural organization and function of the receptors they encode.[12] In addition, two KIR pseudogenes have been identified.[9,13] These are named *KIR2DP1* and *KIR3DP1*,

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reflecting the high sequence homology they share with two-domain and three-domain KIR genes, respectively.

On the basis of gene content, two groups of KIR haplotype have been defined as A and B. Common to both groups of haplotypes are the "framework genes": *KIR3DL2*, *KIR3DL3*, and *KIR2DL4*.[14] Group A haplotypes contain not more than nine loci, which include *KIR2DL1*, *2DL3*, *2DL4*, *2DS4*, *3DL1*, *3DL2*, *3DL3*, *2DP1*, and *3DP1*. In contrast, the group B haplotypes are more diverse and have several genes coding activating receptors.

Several studies have associated KIR genes with disease susceptibility, immune responsiveness, and events following allogenic transplantation. Recent reports have implicated KIRs in affecting the outcome of hematopoietic stem cell transplantation.[15] The genetic diversity of the KIR loci with respect to successful aging has been analyzed only in one study performed in the Irish population. Although, two KIR genes (*2DS3*, *2DL5*) displayed an initial increased frequency in the aged group, the significance of this association was lost when repeated in a second cohort.[16]

To validate (in our homogeneous population) the gender-related associations between HLA-DRB1 genes and longevity observed in other Caucasoid populations, and to ascertain whether a particular repertoire of KIR receptors is associated with successful aging, we have evaluated HLA-DRB1 and KIR genotypes in centenarians and controls from Sicily.

Materials and Methods

Subjects

We have genotyped 77 centenarians and 299 healthy controls for HLA-DRB1 and 44 centenarians and 57 healthy controls for KIR receptors/HLA ligands, all from Palermo and surrounding municipalities in western Sicily, Italy. The age of Sicilian centenarians was verified by archival records at the municipal offices and/or church registries. We paid particular attention to the concordance between reported age and personal chronologies (age of marriage and of military service for men, age of first and last pregnancy for women, age of children, among others). This group did not have any cardiac risk factors or major age-related diseases (e.g., coronary heart disease, severe cognitive impairment, severe physical impairment, clinically evident cancer, or renal insufficiency), although some had decreased auditory and visual acuity, as would be expected.[17] Most biochemical parameters, including cholesterol and triglycerides, were in the normal range. The healthy control group of 299 subjects was recruited amongst students or staff personnel who were checked and judged to be in good health on the basis of their clinical history and blood tests (complete blood cell count, erythrocyte sedimentation rate, glucose, urea nitrogen, creatinine, electrolytes, C-reactive protein, liver function tests, iron, proteins, cholesterol, triglycerides). Because immigration and intermarriage have historically been rare, the Sicilian ethnicity of all participants was established if all four grandparents were born in western Sicily. The study was approved by the University Hospital Ethics Committee, and written informed consent was obtained from all participants.

HLA and KIR typing

The salting-out method was used to extract the DNA, following the standard protocol.[18] The DNA samples were typed for class II HLA alleles and KIR receptors /HLA ligands by polymerase chain reaction–sequence-specific primer (PCR-SSP; One Lambda, Inc. Canoga Park, CA) and KIR-TYPE/Epitop-TYPE (BAG Health Care GmbH, Lich, Germany). The KIR-TYPE kit was designed to identify 14 KIR genes (*2DL1, 2DL2, 3DL1, 2DL4, 2DL5, 2DS1, 2DS2, 2DS3, 2DS4, 2DS5, 3DL1, 3DL2, 3DL3, 3DS1*), 2 pseudogenes (2DP1 and 3DP1) and the common variants of *KIR2DL5* (*KIR2DL5A*, *KIR2DL5B*), the *KIR2DS4* allele (*001/002 and *003), and *KIR3DP1* allele (*001/002 and *003), whereas the Epitop-TYPE kit was used to detect the alleles of the HLA specificities HLA-Cw Asn80, HLA-Cw Lys80, HLA-B Bw4Threo, HLA-B Bw4 Iso, and HLA-A Bw4. The PCR reaction was amplified using a PCR thermal sequencer (Mycicler, Biorad, Milan, Italy). After the PCR process, the amplified DNA fragments were separated by agarose gel electrophoresis (2–2.5%), 0.5–1% Tris/borate/EDTA buffer, and ethidium bromide (0.5 mg/mL). All the subjects positive for the HLA-DR3 subtype, HLA-DRB1*17, were typed for HLA-B8 by micro-SSP (One Lambda, Inc.) to identify carriers of HLA-B8,DR3(DR17) haplotype, which is part of the 8.1 ancestral haplotype (AH 8.1) HLA-A1, Cw7, B8, TNFAB*a2b3, TNFN*S, C2*C, Bf*s, C4A*Q0, C4B*1, DRB1*0301(DR17), DRB3*0101, DQA1*0501, DQB1*0201.[4]

Statistical analysis

Allele frequencies were evaluated by gene count, and 2×2 tables were constructed to determine the statistical significance (chi-squared test with Yates correction) of differences in allele frequency for the HLA-DR polymorphisms and KIR genes between centenarians and controls. The p values obtained were multiplied for the number of alleles under study (Bonferroni correction), i.e., 14 for DR typing and 19 KIR typing. The data were tested for the goodness of fit between the observed and expected genotype values and their fit to Hardy–Weinberg equilibrium (HWE).

Results

The frequencies of the of HLA-DRB1* alleles and KIR genes under investigation were consistent with those predicted by the HWE. Table 1 shows the HLA-DRB1* allele frequencies in 299 controls and 77 centenarians. We found an increase of HLA DRB1*18 allele in male centenarians ($p = 0.0019$), the increase remained significant after Bonferroni correction ($p = 0.0266$). Concerning the other alleles, no significant differences were observed between centenarians and controls (both in women and in men). A sample of subjects (38 healthy controls and 44 centenarians) was typed for HLA-B8, but there were no significant differences between centenarians and controls concerning the HLA-B8,DR3 phenotype (data not shown).

Concerning KIR genes, Table 2 shows the KIR gene frequencies in 57 healthy controls and 44 centenarians. Only a significant decrease of the 2DP1 KIR gene in centenarians was observed ($p = 0.03$), and it was no longer significant after Bonferroni correction. No significant difference was obtained stratifying the subjects by gender (data not shown). Table 3 shows HLA ligands frequencies observed in 57 healthy controls and 44 centenarians. No significant difference was observed in centenarians and controls for HLA ligands.

Table 1. HLA-DRB1* Allele Frequencies in 299 Controls (171 Women and 128 Men) and in 77 Centenarians (51 Women and 26 Men) from Sicily

	Controls			*Centenarians*		
HLA	*All*	*Women*	*Men*	*All*	*Women*	*Men*
DRB1*01	43 (14.4%)	25 (14.5%)	18 (14%)	9 (12%)	7 (13.7%)	2 (8.7%)
DRB1*15	74 (24.7%)	39 (22.6%)	35 (27.3%)	12 (16.2%)	7 (13.7%)	5 (21.7%)
DRB1*16	20 (6.6%)	8 (4.6%)	12 (9.3%)	3 (4%)	1 (2%)	2 (8.7%)
DRB1*17	70 (23.4%)	40 (23.2%)	30 (23.4%)	15 (20%)	12 (23.5%)	3 (13%)
DRB1*18	2 (0.6%)	2 (1.1%)	0	6 (8.1%)	3 (5.8%)	3 (13%)[a]
DRB1*4	62 (20.7%)	32 (18.6%)	30 (23.4%)	15 (20%)	11 (21.5%)	4 (17.4%)
DRB1*10	12 (4%)	6 (3.4%)	6 (4.6%)	3 (2.7%)	3 (5.9%)	0
DRB1*11	117 (39.1%)	73 (42.4%)	44 (34.3%)	27 (36.5%)	19 (37.2%)	8 (34.8%)
DRB1*12	10 (3.3%)	10 (5.8%)	0	3 (4%)	2 (4%)	1 (4.3%)
DRB1*13	43 (14.4%)	26 (15.1%)	17 (13.3%)	17 (23%)	13 (25.5%)	4 (17.4%)
DRB1*14	21 (7%)	13 (7.5%)	8 (6.2%)	7 (9%)	4 (7.9%)	3 (13%)
DRB1*7	37 (16.1%)	24 (11.6%)	13 (10.1%)	9 (12%)	5 (9.8%)	4 (17.4%)
DRB1*08	4 (1.3%)	2 (1.1%)	2 (1.5%)	1 (1.3%)	1 (1.9%)	0
DRB1*09	1 (0.3%)	1 (0.5%)	0	2 (2.7%)	1 (1.9)	1 (4.3)

[a]$p = 0.0019$ by chi-squared Yates correction, $p = 0.0266$ after Bonferroni's correction.
HLA, Human leukocyte antigen.

Analyzing the distributions of "A" haplotype, "B" haplotype, and "A/B" haplotype, there are not significant differences (data not shown). However, the A haplotype predominates in the Sicilian population (26% vs. 0% in healthy controls).

Discussion

Longevity is the result of several interacting factors, including genetic, environmental, and behavioral components.[19] Several studies suggest that human longevity is inextricably linked to the optimal function of the immune system. Hence, genetic determinants of longevity might reside in the polymorphisms for genes that regulate immune responses as HLA and KIR.[1] HLA polymorphisms have been the focus of a vast number of aging association studies, in contrast to only two studies of KIR genes.[16] Conflicting results have been obtained regarding HLA, suggesting that the observed age-related differences in the frequency of HLA antigens are due to bias. The studies performed on the association between longevity and HLA are generally difficult to interpret, owing to major methodological problems. However, as reported Caruso et al.,[2,3] some that were well designed and performed suggest an HLA effect on longevity. In studies performed in Caucasoids, an increase in HLA-DRB1*11 in Dutch women over 85 years was observed.[20] The same laboratory performed a further study and, by using a "birthplace-restricted comparison" in which the origin of all the subjects was ascertained, the authors were able to confirm that aging in women was positively associated with HLA-DR5.[21] Two French studies confirmed the relevance of HLA-DR11 to longevity in aged populations.[22,23] This increase is consistent with the protective effects of this allele in viral diseases, as HLA-DR5, or its subtype HLA-DR11, frequencies have been shown to be decreased in some viral diseases.[2,3]

An association between longevity and the haplotype AH 8.1 seems to emerge. An excess of this AH in the oldest old men has been reported in French and in North Ireland populations.[24,25] This association appears to be gender specific. In fact, a Greek study showed a significant decrease of

Table 2. KIR Gene Frequencies Observed in 57 Healthy Controls and 44 Centenarians

KIR genes	*Controls* (n = *57*)	*Centenarians* (n = *44*)	p *value*
2DL1	56 (98.2%)	42 (95.5%)	
2DL2	40 (70%)	28 (63.6%)	
2DL3	39 (68.4%)	30 (68%)	
2DL4	56 (98.2%)	43 (97.7%)	
2DL5A	10 (17.5%)	10 (22.7%)	
2DL5B	18 (31.5%)	10 (22.7%)	
2DS1	28 (49.1%)	19 (43.2%)	
2DS2	36 (63.1%)	29 (66%)	
2DS3	30 (52.6%)	24 (54.5%)	
2DS4001/002	10 (17.5%)	8 (18%)	
2DS4003/007	43 (75.4%)	32 (72.7%)	
2DS5	24 (42.1%)	16 (36.3%)	
3DL1	51 (89.5%)	39 (88.6%)	
3DL2	54 (94.7%)	42 (95.5%)	
3DL3	56 (98.2%)	44 (100%)	
3DS1	25 (43.8%)	21 (47.7%)	
2DP1	55 (96.5%)	37 (84%)	0.03[a]
3DP1001/004	2 (3.5%)	4 (9%)	
3DP1003	53 (93%)	37 (84%)	

[a]Uncorrected p value by chi-squares Yates correction.
KIR, Killer cell immunoglobulin-like receptor.

Table 3. The HLA Ligands Frequencies Observed in 57 Healthy Controls and 44 Centenarians

Group	*Controls* (n = *57*)	*Centenarians* (n = *44*)	p *value*
C1	16 (28%)	19 (43%)	N.S.
C2	17 (30%)	11 (25%)	N.S.
C1,C2	24 (42%)	14 (32%)	N.S.

HLA, Human leukocyte antigen; N.S., not significant.

AH 8.1 in aged women.[26] Thus, immune dysfunctions of AH 8.1 should contribute to early morbidity and mortality in elderly women, to more susceptibility to autoimmune diseases than men, and to longevity in elderly men.[2–4]

These associations are gender-related, but it is not unexpected on the basis of available data on the genetics of longevity, showing that the association of longevity with particular alleles may be found only in one gender.[27] However, in a longitudinal study, in which a total of 919 subjects aged 85 and older were HLA-typed and followed up for at least 5 years, no HLA association with mortality was found. [21] Positive studies need confirmation in different Caucasoid populations with different ethnic backgrounds and/or replication in a new cohort of oldest old from the same population. In Sardinian centenarians, we did not observed the associations demonstrated in the other well-planned and designed studies discussed above.[5] One study set out to specifically confirm the previously reported increase in the frequency of AH 8.1 in aged men of the northern Irish population,[25] but did not reveal any statistically significant haplotype frequency differences between the aged cohort of individuals compared to the younger controls. However, a striking decrease was observed when the aged women (13.1%) were compared to the control women (17.8%).[28]

A pivotal role in the innate immune response is also played by NK cells in successful aging. In particular, some studies have showed that decreased NK cell function is associated with an increased incidence of infectious diseases both in aged humans [29] and in mice,[30] highlighting the importance of a well-preserved NK cell function in old age. The elderly (>85 years) with low numbers of NK cells were reported to have three times the mortality risk in the first 2 years of follow up than those with high NK cell numbers.[31] In addition, high NK cytotoxicity in aged people is related both to a low incidence of respiratory tract infections and to good development of protective antibody titers in response to influenza vaccination.[32] Hence, preserved NK cytotoxicity should be considered a biomarker of healthy aging and longevity, whereas low NK cytotoxicity is a predictor of morbidity and mortality due to infections.

However, concerning KIR genes, the potential role of KIR diversity in immunosenescence and longevity has been investigated only in the Irish population. Although, two KIR genes (*2DS3*, *2DL5*) displayed an initial increased frequency in the aged group, the significance of this association was absent when repeated in a second cohort.[16] Both activating and inhibitory KIR genes encode receptors with immune functions that clearly have the potential to optimize NK cell activity in the elderly, thereby contributing to prolonged life span. Hence, the increased prevalence of such a KIR gene in the healthy aged may reflect a role for the activating receptor it encodes in providing an improved level of innate immune protection against virus infection and cancer.

In this study we evaluated the frequencies of genes HLA-DRB1 and KIR in centenarians and controls from Sicily. Our results demonstrate an increase of HLA DRB1*18 allele in male centenarians compared to controls even after Bonferroni correction. The frequency of the HLA-B8,DR3 haplotype is very low and not significant both in centenarians and in controls as well as in literature data.[33] In regard to KIR genes, only a significant decrease of the *2DP1* KIR gene in centenarians was observed, which was no longer significant after Bonferroni correction. As in other Caucasian populations, the A haplotype was found to be prevalent in Sicilian population. Thus, results obtained show that only an HLA allele (DRB1*18) is associated with longevity. To confirm this association, a further study in a new cohort of centenarians and controls is needed. On the other hand, no significant association between successful aging and KIR gene content was observed in this Sicilian population.

Although, the present study does not support a role for KIR diversity in aging, considerably more research is required to elucidate the precise role that NK cells play in longevity. It is conceivable that epistatic interactions between particular HLA and KIR haplotypes may contribute to the generation of a dynamic immune response that supports successful aging, whereas certain other haplotype combinations may produce suboptimal levels of immunity and reduced life span.

Present and previous findings suggest that HLA/KIR/longevity associations are population specific, being heavily affected by the population-specific genetic and environmental history. On the other hand, our group has performed several studies showing that inflammatory gene variants are associated with longevity.[34,35] In our opinion, inflammatory genes are more relevant than immune response genes in the control of successful aging. However, this kind of study is important to better understand aging and longevity, hence enhancing the planning of antiaging strategies.[36–38]

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