Role of polymorphisms of CC-chemokine receptor-5 gene in acute myocardial infarction and biological implications for longevity

Inflammation is involved in the pathophysiology of atherosclerosis and its clinical complications, such as AMI. Several pro-inflammatory molecules have been identified in atherosclerotic lesions. Chemokines and their receptors seem to play a pivotal role in the genesis and progression of atherosclerosis.[1] CCR5 is a β-chemokines receptor involved in the migration of monocytes, NK cells and some T cells to the inflammation site. Recent evidence has suggested its involvement in human diseases, such as infectious and age-related inflammatory diseases including atherosclerosis.[2,3] A possible association between *CCR5* gene (NM-U83326) variants and AMI has, therefore, been suggested. Accordingly, CCR5Δ32 variant, a non functional allele resulting from a 32-bp deletion in exon 4 (rs333), seems to have a protective role against AMI and coronary heart diseases (CHD), determining a slower progression of atherosclerotic lesion as a consequence of an attenuated inflammatory response. However, conflicting results have been obtained regarding the CCR5 role in atherosclerosis.[3-5] In this study, we evaluated whether CCR5Δ32 variant and two other functional single nucleotide polymorphisms (SNPs) in *CCR5* gene promoter[6,7] might be associated with AMI. We also analyzed their distribution in centenarians, since our previous studies have demonstrated that alleles associated with AMI susceptibility are not included in the genetic background favoring longevity.[8] We enrolled 133 young subjects (<45 years) selected among AMI patients admitted to the Intensive Coronary Therapy Unit of Palermo University Hospital. Diagnosis was based on identification of electrocardiograph and serum enzyme activity changes confirmed by echocardiography and coronary angiography. All 133 patients suffered from AMI with or without ST-segment elevation. However, accurate information about the number of occluded arteries was not available. They were consecutively enrolled during the last three years immediately before they were discharged, i.e. 4-10 days after AMI occurrence. In this sample of patients, no mortality was observed during the hospital admission. Smokers accounted for 74% of patients, there was family CVD history in 52.6%, type 2 diabetes in 18%, obesity in 34%, hypertension in 29%, hypercholesterolemia in 61%, and hypertriglyceridemia in 43%. A matched control group consisted of 136 subjects in good health according to 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). Among young controls, 21.3% were moderate smokers; no other cardiac risk factor was present. A second control group consisted of 123 Sicilian centenarians (> 99 years), whose age was confirmed from records at the City Hall and/or Church registries. No cardiac risk factors or age-related diseases were observed in centenarians, although some had reduced auditory and visual acuity (as expected[9] most biochemical parameters including cholesterol and triglycerides, were in the normal range). Because immigration and intermarriage have historically been rare, Sicilian ethnicity of all participants was established by all four grandparents having been born in Sicily.

The study was approved by the University Hospital Ethics Committee, and written informed consent was obtained from all participants. DNA was genotyped for CCR5Δ32 variant and two SNPs of promoter *CCR5* gene region, 59029A/G and 59353C/T, as published.[6,7] Differences in allele and genotypic frequencies of three CCR5 variants among three groups were evaluated by gene count and χ^2 test. ODD ratio (OR) with confidence interval (CI) was also calculated. Due to the small number of AMI women patients, a logistic regression to test the association of pro-inflammatory wild type CCR5 genotype with AMI taking into account the cardiac risk factors was only carried out in male patients and controls. We found significant differences in frequency of CCR5Δ32 genotypes between controls and patients (p=0.005 by χ^2 test; OR=0.16, 95%CI=0.046-0.56, p=0.003 by Fisher's exact test). Therefore, there was a significantly higher prevalence of Δ32 allele in controls than AMI patients (p=0.0007, by χ^2 test with Yates' correction; OR=0.14 95%CI=0.04-0.48, p=0.0008 by Fisher's exact test). By contrast, no statistical differences were observed in the genotype distributions and the allele frequencies of 59029A/G and 59353C/T promoter SNPs between AMI patients and matched controls (*Table 1, online supplement*).

The genetic distribution and allele frequency of *CCR5Δ*32 deletion in 123 Sicilian centenarians is shown in Table 1. To focus the attention on significant genotypic and allele differences in three cohorts, this table also reports the CCR5Δ32 data of two groups previously described. Significant differences were found in genotypic frequency among the three groups (p=0.003 by χ^2 test). Accordingly, the same results were obtained for *CCR5*Δ32 allele frequency among the three groups (p=0.00006 by χ^2 test). The CCR5Δ32 allele was underrepresented in AMI patients and over-represented in centenarians, with intermediate values in age-matched controls. After adjustment for risk factors, significant differences in pro-inflammatory wild type CCR5 allele were observed between male AMI patients and matched con-

Table 1. Genotype distributions and allelic frequencies of CCR5Δ32 deletion in 133 AMI patients, 136 age-matched controls and 123 centenarians from Sicily. 2x2 comparisons between the different groups with odd ratio (OR) and 95% confidence interval).

Genotypes	wt/wt	wt/Δ32	Δ32/Δ32
AMI patients (n=133)	130	3	0
Age-matched controls (136)	119	14	3
Centenarians (n=123)	104	13	6
Alleles (%)	wt	Δ32	
AMI patients (n=133)	263 (98.8%)	3 (1.2%)	
Age-matched controls (136)	252 (92.6%)	20 (7.4%)	
Centenarians (n=123)	221(89.8%)	25 (10.2%)	

Comparing the genotype distribution of CCR5Δ32 genotypes of centenarians with those of AMI patients and age-matched controls, significant differences by χ^2 test (3×3 table) were found in the frequency among the various groups (p=0.003). Accordingly, analyzing by χ^2 test (2×3 table) the CCR5Δ32 allele frequency of three groups, a significant difference was obtained (p=0.00006). In particular, the CCR5Δ32 allele was underrepresented in patients with AMI and overrepresented in centenarians, with intermediate values in age-matched controls. After adjustment for smoking habits, family history of CHD, and the presence of type 2 diabetes, obesity, hypertension, hypercholesterolemia, and hypertriglyceridemia, significant differences in genotype frequencies persisted between male AMI patients and age-matched controls (p=0.0003, OR 3.3 (2.5-8.6) and between male AMI patients an centenarians (p=0.00001, OR 7.6 (3.01-9.8)).

trols (p=0.0003, OR 3.3 (2.5-8.6) and between male AMI patients and centenarians (p=0.00001, OR 7.6 (3.01-9.8)) (Table 1). These results therefore indicate that pro-inflammatory wild-type CCR5 genotype is an independent risk factor for developing AMI in the Sicilian population. Results from our study of a homogeneous population suggest that CCR5Δ32 variant is associated with AMI. Association studies are influenced by a number of possible confounding factors, such as, among others, the total number of patients and controls and the homogeneity of the population in terms of geographical origin. False associations might occur if the controls are not ethnically matched with the patients. We compared people belonging to the same homogeneous population from Sicily. Therefore, we think that, although based on a relatively reduced number of patients and controls, our results might more reliable than studies performed on larger cohorts of patients from northern Europe and the United States which are ethnically matched but only refer to Caucasians in general.

Genetic traits significantly contribute to the CVD risk and literature data indicate that innate immunity alleles may increase the risk of disease. Accordingly, common gene polymorphisms regulating high inflammatory molecule production have been associated with atherosclerosis. Conversely, those associated with a positive control of inflammation might play a protective role against atherosclerosis. However, contrasting results have been obtained in different populations, including the molecule under study.[1,4,5,10,11]

Since centenarian offspring seem to have a significant reduction in CVD prevalence,[8] we performed centenarian genetic studies to clarify the role of key genetic components influencing AMI. We have demonstrated in previous studies that alleles associated to CHD susceptibility are not included in the genetic background favoring longevity. So, genetic background promoting pro-inflammatory responses may play an opposite role in CVD and longevity.[8] In agreement with our hypothesis the results show that the role of CCR5Δ32 variant confers AMI resistance and promotes longevity in Sicilians.

In the clean modern Western environment, with reduced pathogen load and improved control of severe infections by vaccinations and antibiotics, the CCR5Δ32 anti-inflammatory genotype might result in an increase in longevity, since it reduces the risk of atherosclerosis.[10,12]

Carmela Rita Balistreri,[1] Giuseppina Candore,[1] Marco Caruso,[2] Egle Incalcaterra,[1,2] Claudio Franceschi,[3] Calogero Caruso[1]

[1]Gruppo di Studio sull'Immunosenescenza, Dipartimento di Biopatologia e Metodologie Biomediche, Università di Palermo; [2]Dipartimento di Medicina Interna, Malattie Cardiovascolari e Nefrologiche, Università di Palermo; [3]Istituto Nazionale di Riposo e Cura per Anziani, Ancona, and Dipartimento di Patologia Sperimentale, Università di Bologna, Bologna, Italy

Key words: atherosclerosis, AMI, CCR5, CCR5Δ32, inflammation, longevity

Funding: this work was supported by grants from the Italian Ministry of Education, University and Research to GC and CC. Funds from the Italian ministry of Health (Markers genetici di sindrome coronarica acuta e valutazione della L-arginina nella prevenzione di eventi ischemici; effetti della dieta mediterranea e di carboidrati a basso indice glicemico sulla sindrome metabolica) to CC are also acknowledged. EI is a PhD student of Pathobiology at Palermo University (directed by CC) and this paper is in partial fulfilment of her PhD requirements.

Correspondence: Giuseppina Candore, PhD, Dipartimento di Biopatologia e Metodologie Biomediche, Università di Palermo, corso Tukory 211, 90134 Palermo, Italy. Phone: international +39.091.6555911. Fax: international +39.091.6555933. E-mail: gcandore@unipa.it or crbalistreri@unipa.it

References

1. Candore G, Vasto S, Colonna-Romano G, Lio D, Caruso M, Rea IM, et al. Cytokine gene polymorphisms and atherosclerosis. In Koen Vandenbroeck: editors. Cytokine Gene Polymorphisms in Multifactorial Conditions. ISBN: 0849336198. Florida CRC press 2006. p. 363-78.
2. Locati M, Bonecchi R, Corsi MM. Chemokines and their receptors: roles in specific clinical conditions and measurement in the clinical laboratory. Am J Clin Pathol 2005;123 Suppl:S82-95.
3. Balistreri CR, Candore G, Grimaldi MP, Listì F, Vasto S, Orlando V, et al. CCR5 receptor: biologic and genetic implications in age-related diseases. Ann N Y Acad Sci 2007;1100:162-72.
4. Petrkova J, Cermakova Z, Lukl J, Petrek M. CC chemokine receptor 5 (CCR5) deletion polymorphism does not protect Czech males against early myocardial infarction. J Intern Med 2005;257:564-6.
5. Pai JK, Kraft P, Cannuscio CC, Manson JE, Rexrode KM, Albert CM, et al. Polymorphisms in the CC-chemokine receptor-2 (CCR2) and -5 (CCR5) genes and risk of coronary heart disease among US women. Atherosclerosis 2006;186:132-9.
6. Mummidi S, Ahuja SS, McDaniel BL, Ahuja SK. The human CC chemokine receptor 5 (CCR5) gene. Multiple transcripts with 5'-end heterogeneity, dual promoter usage, and evidence for polymorphisms within the regulatory regions and noncoding exons. J Biol Chem 1997;272: 30662-71.
7. Balistreri CR, Grimaldi MP, Vasto S, Listi F, Chiappelli M, Licastro F, et al. Association between the polymorphism of CCR5 and Alzheimer's disease: results of a study performed on male and female patients from Northern Italy. Ann N Y Acad Sci. 2006;1089:454-61.
8. Candore G, Balistreri CR, Grimaldi MP, Listi F, Vasto S, Caruso M, et al. Opposite role of pro-inflammatory alleles in acute myocardial infarction and longevity: results of studies performed in a Sicilian population. Ann N Y Acad Sci 2006;1067:270-5.
9. Lio D, Malaguarnera M, Maugeri D, Ferlito L, Bennati E, Scola L, et al. Laboratory parameters in centenarians of Italian ancestry. Exp Gerontol 2008;43:119-22.
10. Licastro F, Candore G, Lio D, Porcellini E, Colonna-Romano G, Franceschi C, et al. Innate immunity and inflammation in ageing: a key for understanding age-related diseases. Immun Ageing 2005;2:8.
11. Vasto S, Candore G, Balistreri CR, Caruso M, Colonna-Romano G, Grimaldi MP, et al. Inflammatory networks in ageing, age-related diseases and longevity. Mech Ageing Dev 2007;128:83-91.
12. Caruso C, Candore G, Colonna-Romano G, Lio D, Franceschi C. Inflammation and life-span. Science 2005; 307: 208-9.

Citation: Balistreri CR, Candore G, Caruso M, Incalcaterra E, Franceschi C, Caruso C. Role of polymorphisms of CC-chemokine receptor-5 gene in acute myocardial infarction and biological implications for longevity. Haematologica 2008 Apr; 93(4):637-638. doi: 10.3324/haematol.12239