Opposite effects of interleukin 10 common gene polymorphisms in cardiovascular diseases and in successful ageing: genetic background of male centenarians is protective against coronary heart disease

D Lio, G Candore, A Crivello, L Scola, G Colonna-Romano, L Cavallone, E Hoffmann, M Caruso, F Licastro, C M Caldarera, A Branzi, C Franceschi, C Caruso

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Key points

- Centenarians escape, or at least delay, age associated diseases that normally cause mortality at earlier ages. Considerable evidence supports involvement of genetic components to longevity. Accordingly, siblings of centenarians have a marked increased probability of living to 100 years of age. The major trait of the offspring of centenarians is a significantly reduced prevalence of cardiovascular diseases.
- Patients with atherosclerosis have a proinflammatory genotype, and tight control of inflammatory reactions might decrease the incidence of atherosclerosis. Gene polymorphisms for proinflammatory cytokines seem to contribute considerably to the risk of coronary heart disease, including acute myocardial infarction, so alleles associated with susceptibility to acute myocardial infarction are expected to be less represented in genetic backgrounds that favour longevity. On the other hand, in Italian centenarian men, the frequency of the genotype associated with interleukin 10 (–1082GG) is associated with significantly increased production of the antiinflammatory cytokine interleukin 10.
- In two different populations from north and south Italy, analysis of genotype distributions showed a significantly higher frequency of the –1082GG genotype among oldest old participants than in controls and patients with acute myocardial infarction. Conversely, the frequency of the –1082AA genotype, associated with low production of interleukin 10, was significantly higher in patients with acute myocardial infarction than in controls and oldest old participants. High production of interleukin 10 thus is protective for acute myocardial infarction and a determinative parameter for longevity.
- People with exceptional longevity possess genetic factors that modulate ageing processes and, in particular, factors protective for cardiovascular disease. This supports the opinion that a genetic background protective against cardiovascular diseases is a component of longevity.
- Our immune system has evolved to control pathogens, so proinflammatory responses are likely to be programmed by evolution to resist fatal infections, and low production of interleukin 10 is associated with an increased resistance to pathogens. Increased concentrations of interleukin 10, however, might better control inflammatory responses induced by chronic vessel damage and reduce the risk of atherogenetic complications. These conditions might result in an increased chance of long life in an environment with a reduced load of antigens (that is, pathogens).

Centenarians escape, or at least have a delay in, age associated diseases that normally cause mortality at earlier ages. Considerable evidence supports the involvement of genetic components to longevity. Accordingly, the siblings of centenarians have a markedly increased probability of living to 100 years of age. A recent study showed that the offspring of centenarians had a markedly reduced prevalence of age associated diseases, particularly cardiovascular disease and cardiovascular risk factors. On the other hand, this is not unexpected in view of the fact that cardiovascular diseases account for about 50% of all deaths worldwide.^{1–3}

For a long time, hypercholesterolaemia has been claimed to be the most important risk factor for atherogenesis. Nonetheless, inflammatory processes, coupled with dyslipidaemia and formation of atheroma, have been shown to play an important role in the progression of atherosclerosis. In fact, patients with atherosclerosis have a proinflammatory

phenotype, so control of inflammation might have a role in protecting against the complications of atherosclerosis, including acute myocardial infarction.^{4–6} Acute myocardial infarction may occur as a result of erosion or uneven thinning and rupture of fibrous caps, often at the shoulder of lesions, where macrophages enter, accumulate, and are activated and where apoptosis may occur.^{4–9}

Genetic traits contribute significantly to the risk of coronary heart disease,¹⁰ so a number of studies have looked at the hypothesis that variations in genes of the immune system may increase the risk of disease.^{11–12} Differences in the genetic regulation of inflammatory processes might partially explain why some people but not others develop the disease and why some develop a greater inflammatory response than others. Accordingly, common gene polymorphisms that control high production of inflammatory molecules have been associated with atherosclerosis, and good control of inflammation might play a protective role against atherosclerosis.^{11–13}

In addition, a variety of studies has shown that interleukin 10 (IL-10) plays a crucial role in the regulation of inflammation. The main function of IL-10 seems to be to limit and ultimately terminate the inflammatory signal in inflammatory cells such as monocytes and macrophages.^{14, 15} The gene for IL-10 is located on chromosome 1 at 1q31–32 and is highly polymorphic.¹⁶ Stimulation of blood samples from humans with bacterial lipopolysaccharide showed large interindividual variations of production of IL-10, which suggests a genetic component of approximately 75%. Interindividual differences in the regulation of production of IL-10 may be critical with respect to the final outcome of an inflammatory response: that is, within physiological or pathological limits.^{13, 17} Furthermore, production of IL-10, independent of the interaction of other cytokine gene products,¹⁸ is controlled genetically by polymorphisms in its own promoter sequence.¹⁶

We previously reported that the frequency of the homozygous genotype of –1082G for IL-10, included in the –1082G/–819C/–592C haplotype,¹⁹ which is associated with increased production of interleukin and better control of inflammation, is significantly increased in Italian centenarian men.^{20–22} To evaluate whether the IL-10 genotype might be a component of a genetic background protective against cardiovascular diseases, we genotyped for interleukin 10 –1082/–819/–592 single nucleotide polymorphisms in two cohorts of men affected by acute myocardial infarction from north and south Italy, two groups of age matched controls, and two groups of oldest old men from the same geographical areas.

METHODS

In this study, we analysed two cohorts of men affected by acute myocardial infarction and unrelated controls matched for age. The cohort comprised 142 patients (mean age 67 years, range 55–80 years) affected by acute myocardial infarction, who were diagnosed at the cardiac unit of Bologna University Hospital, and 153 controls (mean age 67 years, range 65–73 years) from Emilia-Romagna, who had no clinical history of age related disease. The second cohort comprised 90 younger patients (mean age 41 years, range 23–46 years) affected by acute myocardial infarction, who were diagnosed at the cardiac unit of Palermo University Hospital, and 110 healthy controls (mean age 38 years, range 20–55 years) from Sicily. The diagnosis of acute myocardial infarction was based on typical electrocardiographic changes and standard laboratory findings confirmed by echocardiography and coronary angiography.

As a further control, we genotyped samples obtained from two groups of oldest old men (>95 years old and centenarians) without clinical history of cardiovascular diseases: 57 from the same geographical area of Bologna and 52 from Sicily. Age had been verified by researching archival records in the city hall or church registries, or both, with attention paid to the concordance between reported age and personal chronologies (such as age of marriage, age at military service, and age of children). The project was approved by the ethics committees of the two university hospitals, and informed consent was obtained from each participant.

We collected blood specimens in sterile tubes containing tripotassium ethylenediaminetetraacetic acid, and we extracted and processed DNA for IL-10 gene analysis. We identified three different biallelic polymorphisms at –1082 (G→A), –819 (C→T), and –592 (C→A) nucleotides with the –1082, –819, and –592 haplotype specific typing method described by Koss et al.¹⁹ Briefly, we mixed 12 couples of 3' and 5' allele specific sequence primer pairs separately in a 13 µl total volume that contained DNA template, 2.00 mM

magnesium chloride, 9.8 mM ammonium sulphate, 39.6 mM Tris, 200 µM dNTPs, and 0.2U Taq-polymerase. Cycling was performed at 96°C for 1 minute, followed by five cycles at 96°C for 25 seconds, 70°C for 45 seconds, and 72°C for 45 seconds, 20 cycles at 96°C for 25 seconds, 65°C for 50 seconds, and 72°C for 45 seconds, and five cycles at 96°C for 25 seconds, 55°C for 60 seconds, and 72°C for 120 seconds. Products of polymerase chain reaction that potentially contained the –592/–819, –592/–1082, or –819/–1082 possible allele combinations were detected by electrophoresis on 2% agarose.

Interleukin 10 haplotype and genotype frequencies were evaluated by gene count. The data were tested for goodness of fit between the observed and expected genotype values (χ^2 test) and their fit to Hardy-Weinberg equilibrium. Chi squared tests (3×2 tables, or 2×2 table with Yates' correction) were performed to calculate significant different haplotype or genotype distribution between patients with acute myocardial infarction, age matched controls and oldest old controls. As multiple comparisons were made, Bonferroni's correction was applied and p values were multiplied by the number of haplotypes detected (that is, 3). The strength of the statistical association was expressed by odds ratio of risk and 95% of confidence intervals.

RESULTS

Direct typing of IL-10 haplotypes confirmed the presence of the only three possible allele combinations characteristic of IL-10 polymorphisms in Caucasian people,^{23–25} which allowed us to present the results as –1082/–819/–592 haplotype frequencies.

Table 2 shows the genotype frequencies for –1082/–819/–592 IL-10 haplotypes in men with acute myocardial infarction, age matched controls, and oldest old controls from north and south Italy. Significantly different distributions of –1082/–819/–592 haplotypes were observed among controls and old (mean age 67 years) men with acute myocardial infarction (p = 0.0027), among oldest old and old patients with acute myocardial infarction (p = 0.0003) and among controls and oldest old (p = 0.039) from north Italy. Marginally significant different distributions of –1082/–819/–592 haplotypes were observed among controls and young (mean age 41 years) men with acute myocardial infarction (p = 0.069) and controls and oldest old (p = 0.078), whereas a highly significant difference was observed among oldest old and patients with acute myocardial infarction (p = 0.0006) from South Italy.

Table 3 shows IL-10 –1082G→A genotype frequencies in men with acute myocardial infarction, age matched controls, and oldest old controls from north and south Italy. Significant different distributions of –1082G→A genotypes

Table 1 Characteristics of patients from Bologna and Sicily

Characteristic	Patients (%)	
	Bologna	Sicily
Positive familial history of coronary heart disease	30	60
Smoking		
Current smoker	28	70
Current and former smoker	69	89
History of type 2 diabetes	14	17
History of obesity	10	35
Hypertension	57	31
Blood cholesterol levels >220 mg/dl	20	60
Triglycerides >160 mg/dl	24	42

Table 2 Genotype frequencies for -1082/-819/-592 IL-10 haplotypes in men with acute myocardial infarction, age matched controls, and oldest old controls from north and south Italy. Values are numbers (percentages)

Participants	No. of participants	Genotype					
		GCC/GCC	GCC/ACC	GCC/ATA	ACC/ACC	ACC/ATA	ATA/ATA
North Italy							
"Old" patients with acute myocardial infarction	142*†	30 (21.1)	35 (24.6)	17 (12.0)	20 (14.1)	26 (18.3)	14 (9.9)
Male controls	153‡	48 (31.4)	41 (26.8)	34 (22.2)	12 (7.8)	10 (6.5)	8 (5.2)
Oldest old men	57	34 (59.6)	10 (17.5)	8 (14.0)	2 (3.5)	2 (3.5)	1 (1.8)
South Italy							
"Young" men with acute myocardial infarction	90**††	17 (18.9)	17 (18.9)	12 (13.3)	16 (17.8)	19 (21.1)	9 (10.0)
Male controls	110‡‡	26 (23.6)	30 (27.3)	26 (23.6)	10 (9.1)	10 (9.1)	8 (7.3)
Oldest old men	52	25 (48.1)	12 (23.1)	11 (21.2)	2 (3.8)	3 (5.8)	1 (1.9)

Haplotypes were determined with sense and anti-sense primers specific for the three single nucleotide polymorphisms considered, which allowed direct typing of the existing haplotypes. For comparison of genotype distribution, χ^2 test with Yates' correction was performed (Graphpad Instat, Graphpad Software, San Diego, CA, USA). Bonferroni's correction was applied multiplying the obtained p values by three possible haplotypes.

*p=0.0027 (vs. male controls); †p=0.0003 (vs. oldest old men); ‡p=0.039 (vs. oldest old men); **p=0.069 (vs. male controls); ††p=0.0006 (vs. oldest old men); ‡‡p=0.078 (vs. oldest old men)

were observed among controls and men with acute myocardial infarction ($p = 0.0003$), among controls and oldest old ($p = 0.0024$) and among oldest old and men with acute myocardial infarction ($p = 0.0003$) from north Italy. In the same population, the analysis of the single genotype distribution showed a higher frequency of the -1082GG homozygous genotype in oldest old men than controls ($p = 0.0002$; odds ratio 3.20 (95% CI 1.72 to 6.07)) and men with acute myocardial infarction ($p = 0.0002$; 3.10 (2.90 to 20.20)). The frequency of the -1082AA homozygous genotype was significantly higher in men with acute myocardial infarction than in controls ($p = 0.0001$; 3.00 (1.78 to 5.04)) and oldest old men ($p = 0.0001$; 7.60 (2.90 to 20.20)). A significant different distribution of -1082G→A haplotypes was seen in controls and men with acute myocardial infarction ($p = 0.0069$), among controls and oldest old men ($p = 0.019$) and among oldest old and men with acute myocardial infarction ($p = 0.0003$) from south Italy. In the same population, the analysis of the single genotypes distribution showed a significant increase of -1082GG homozygous genotype frequency in oldest old men with

respect to the controls ($p = 0.0034$; 3.20 (1.72 to 6.07)) and men with acute myocardial infarction ($p = 0.0005$; 3.97 (1.86 to 8.48)). Finally, the frequency of -1082AA was significantly higher in men with acute myocardial infarction than in controls ($p = 0.0007$; 2.80 (1.54 to 5.08)) and oldest old ($p = 0.0001$; 8.99 (3.27–24.70)).

DISCUSSION

The highly polymorphic IL-10 gene is located on chromosome 1 at 1q31–32.¹⁶ Several polymorphisms located close to or within the IL-10 gene can influence the transcription levels of mRNA coding for the protein. Three polymorphisms in the proximal region of IL-10 gene have been described,²⁶ but conflicting results have been reported on the influence of self-standing or reciprocal linkage on IL-10 transcription.²⁷ Three well documented haplotypes, involving three single nucleotide polymorphisms at -1082 (G→A), -819 (C→T), and -592 (C→A) nucleotides of promoter, have been identified in Caucasians,^{16, 20, 23–25, 28–33} and our results confirm the above mentioned results in Italian population samples. In the original paper of Turner *et al.*,²⁰ the -1082 SNP was

Table 3 Interleukin 10 -1082G→A genotype frequencies in men with acute myocardial infarction, age matched controls, and oldest old controls from north and south Italy. Values are numbers (percentages)

Participants	No of participants	Genotype		
		GG	GA	AA
North Italy				
"Old" patients with acute myocardial infarction	142*†	30 (21.1)	52 (36.6)	60 (42.2)
Male controls	153‡	48 (31.4)	75 (49.0)	30 (19.6)
Oldest old men	57	34 (59.6)	18 (31.6)	5 (8.8)
South Italy				
"Young" patients with acute myocardial infarction	90**††	17 (18.9)	29 (32.2)	44 (48.9)
Male controls	110‡‡	26 (23.6)	56 (50.9)	28 (25.5)
Oldest old men	52	25 (48.1)	23 (44.2)	6 (11.5)

The genotype percentages were obtained considering the following haplotype combinations: (A) -1082G/-819C/-592C homozygous participants for -1082GG genotype; (B) -1082A/-819C/-592C -1082G/-819C/-592C and -1082A/-819T/-592A -1082G/-819C/-592C heterozygous participants for -1082GA genotypes; (C) -1082A/-819C/-592C and -1082A/-819T/-592A homozygous and -1082A/-819C/-592C -1082A/-819T/-592A heterozygous participants for -1082AA genotypes. For the comparison of genotype distribution, χ^2 test with Yates' correction was performed (Graphpad Instat, Graphpad Software, San Diego, CA, USA). Bonferroni's correction was applied by multiplying the obtained p values by three possible haplotypes.

*p=0.0003 (vs. male controls); †p=0.0003 (vs. oldest old men); ‡p=0.0024 (vs. oldest old men); §p=0.0069 (vs. male controls); ¶p=0.0003 (vs. oldest old men); **p=0.019 (vs. oldest old men).

claimed to influence IL-10 production independently from polymorphisms at the other positions. In another study, however, GCC haplotype was associated with higher production of IL-10 by peripheral blood mononuclear cells and the ATA haplotype with lower production.²⁸ Recently, levels of IL-10 mRNA were tested by real time reverse transcription polymerase chain reaction in 123 healthy donors. The authors found statistical differences in mRNA concentrations between the polymorphic variant GCC/GCC and the low producer genotypes. The G allele at position -1082 was the most important genetic factor in the regulation of constitutive IL-10 mRNA levels. Similarly, the authors found an association of this single nucleotide polymorphism with serum concentrations >2 pg/ml.³⁴ In any case, genotypes that are low producers of IL-10 seem to control inflammatory responses and affect susceptibility to inflammatory and infectious disease.^{13 21 22 28 35–37} The single nucleotide polymorphisms under study thus are relevant functionally.

Several pieces of evidence indicate an involvement of IL-10 in the development of atherosclerosis.³⁸ Interleukin 10 has several antiatherogenic effects, including inhibition of adhesion of low density lipoproteins (LDL) activated monocytes to endothelium and downregulation of fibrinogen biosynthesis. Interleukin 10 can limit the progression of experimental atherosclerosis. Knockout mice deficient for IL-10 have a 30-fold higher susceptibility to atherosclerosis, induced either by high cholesterol diet or by cardiac allograft vasculopathy setting. The transfection of adenovirus mediated overexpression of IL-10 into LDL receptor knockout mouse strongly inhibits otherwise rapidly growing atherosclerotic lesions.^{38–42} Interleukin 10 is detectable in human atherosclerotic plaques,⁴ and it might play a regulatory role in the progression of atherosclerosis lesions. Furthermore, serum levels of IL-10 may be an important prognostic determinant in patients with acute coronary syndromes.⁴³ Plasma levels of IL-10 were lower in patients with unstable form of angina pectoris than in patients with the stable form. The predictive value of IL-10 also showed a significant interaction with the well known risk factor of C reactive protein (CRP) levels. Patients positive for CRP with high serum levels of IL-10 had a lower cardiac risk than patients positive for CRP with low levels of IL-10.⁴⁴ The candidate gene under study thus is likely involved in the phenotype of patients with acute myocardial infarction.

Published case-control studies did not find any association with IL-10 in patients with atherosclerotic related disease or myocardial infarction.^{32 45 46} The cause of the discrepancies between previous reports and our results is not clear; however, association studies are influenced by a number of possible confounding factors, such as the total number of patients and controls and the homogeneity of the population in terms of geographical origin. Errors might occur if controls are not matched ethnically with patients. We compared people belonging to the same homogeneous populations from north Italy and Sicily. Thus, although our study is based on a relatively smaller number of patients and controls, it is more matched genetically than studies from larger cohorts in other investigations. On the other hand, acute myocardial infarction is a multifactorial disease, so different single genes are expected to strongly contribute to general risk, which also depends on environmental interactions. Accordingly, a recent study showed that the low IL-10 producer -1082AA genotype was associated with elevated levels of serum CRP and was predictive for a higher cardiovascular morbidity in patients undergoing dialysis compared with those with the -1082GG genotype.⁴⁷ Inflammatory mediators are induced in all patients with chronic renal failure because of uraemia and renal replacement therapy. While inflammation in patients with the IL-10 high-producer genotype effectively may be

limited, this is not the case in patients with the low-producer genotype. The different results obtained in our Italian patients thus might also depend on the differential importance of classic risk factors for atherosclerosis and acute myocardial infarction in the Italian population, as the traditional Mediterranean diet may affect the incidence and prevalence of acute myocardial infarction.^{48 49}

It is intriguing that in another population with different style of life—Ashkenazi Jews—people with exceptional longevity and their offspring had a genetic background that conditioned significantly larger particle sizes of high density lipoprotein and LDL. Again, this phenotype was associated with a lower prevalence of hypertension, coronary heart disease, metabolic syndrome, and increased homozygosity for the I405V variant in the cholesteryl ester transfer protein gene, which is involved in regulation of lipoprotein and its particle sizes.^{50 51} Previous and present results thus suggest that people with exceptional longevity possess genetic factors that modulate ageing processes and, in particular, protective cardiovascular disease factors, which supports the opinion that a genetic background protective against cardiovascular diseases is a component of the trait of longevity.²

In addition, centenarians, who have overcome the age related risk, may be a better control group for case-control studies focussed on age associated diseases with multifactorial aetiology. It is, in fact, noteworthy that the -1082GG genotype, which is associated with an increased production of IL-10 and has been associated with the possibility of reaching the extreme limits of human lifespan in Italian men,^{21 22} was underrepresented in young and old patients with coronary heart disease, with the -1082AA genotype being overrepresented.

Finally, our data prompt consideration of the role that antagonistic pleiotropy plays in diseases and longevity.⁵² In fact, our immune system has evolved to control pathogens, so proinflammatory responses are likely to be evolutionarily programmed to resist fatal infections, and low production of IL-10 is associated with an increased resistance to pathogens. However, increased levels of IL-10 might better control inflammatory responses induced by chronic vessel damage and reduce the risk of atherogenic complications. These conditions might result in an increased chance of long life survival in an environment with reduced antigen (that is, pathogen) load.⁵³

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Authors' affiliations

D Lio, G Candore, A Crivello, I Scola, G Colonna-Romano, I Cavallone, C Caruso, Gruppo di Studio dell’Immunosenescenza, Dipartimento di Biopatologia e Metodologie Biomediche, University of Palermo, Palermo, Italy
F Licastro, C Franceschi, Dipartimento di Patologia Sperimentale, University of Bologna, Bologna, Italy
I Cavallone, C Franceschi, Istituto Nazionale di Riposo e Cura per Anziani di Ancona, Ancona, Italy
M Caruso, E Hoffmann, Dipartimento di Medicina Interna, Malattie Cardiovascolari e Nefrourologiche, University of Palermo
A Branzi, Istituto di Cardiologia, University of Bologna
C M Caldarera, Dipartimento di Biochimica, University of Bologna

Correspondence to: C Caruso, Gruppo di Studio dell’Immunosenescenza, Dipartimento di Biopatologia e Metodologie Biomediche, Corso Tukory 211, 90134 Palermo, Italy; marcoc@unipa.it

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