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Role of VEGF gene variability in longevity: A lesson from the Italian population

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Abstract

Vascular endothelial growth factor (VEGF) gene polymorphisms have been associated with an increased risk of developing a wide variety of disorders from diabetes to neurodegenerative diseases suggesting functions not confined to its vascular effects originally described. Based on the VEGF protective roles undisclosed in pathological conditions, we evaluate whether VEGF variability might be a determinant also for longevity. Four polymorphisms (−2578C/A, −1190G/A, −1154G/A and −634G/C) within the VEGF gene promoter region in 490 unrelated Italian healthy subjects have been analysed. Significant changes of allele, genotype (−2578/AA versus −2578/CC: OR = 2.08, $p = 0.007$; −1190/AA versus −1190/GG: OR = 2.01, $p = 0.011$) and haplotype (AAGG: 10.4% versus 14.9%, $p = 0.03$) frequency distributions were observed between young/elderly (25–84 years old) and long-lived (85–99 years old) subjects. These results suggest that VEGF gene variability can be inserted among the genetic factors influencing the lifespan.

Keywords: Aging; Vascular endothelial growth factor; Genetic risk factor; Longevity

1. Introduction

Longevity represents a very complex phenomenon due to the interaction among genetic, environmental and lifestyle factors. Identification of genetic and non-genetic factors involved in aging has progressed extensively in the recent years because of increased interest in defining the determinants of human life expectancy. It has been suggested that genes and biochemical factors likely to be implicated in aging-related disorders may have an important role also in human longevity. Among genetic markers, several variants in pro- or anti-inflammatory cytokines and in vascular factors have been shown to affect successful aging and longevity (Panza et al., 2004; Salvioli et al., 2006).

Vascular endothelial growth factor (VEGF) is an important angiogenesis cytokine that undergoes transcriptional and post-transcriptional induction by hypoxia. It has been implicated in several pathological conditions, from tumour proliferation to inflammatory and ischemic processes (Carmeliet, 2003; Ferrara et al., 2003). VEGF has been originally identified on the basis of its vascular effects, but recently has been recognised as an important signalling molecule in the nervous system, as well as in neurodegenerative disorders and in peripheral neuropathies (Rosenstein and Krum, 2004; Storkebaum and Carmeliet, 2004). An age-dependent decline in VEGF expression has been reported in different tissues, however the molecular alterations responsible for this reduction have not been

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elucidated. A number of polymorphisms localised within the VEGF gene promoter region have been linked to increased susceptibility to multiple angiogenesis-dependent diseases and age-related neurological disorders in humans (Del Bo et al., 2005; Lambrechts et al., 2003). Individuals homozygous for the haplotypes involving the −2578AA, −1154A/G and −634GG variants had lower serum VEGF levels and increased risk of developing amyotrophic lateral sclerosis (ALS). C(−2578)A, C(−1198)T, G(−1190)A and G(−1154)A polymorphisms have been correlated with the risk of developing Alzheimer's disease (AD). In addition, the same polymorphisms are able to alter basal and post-stimulation promoter activity in human transfected MCF7 cells (Stevens et al., 2003). Finally, an association has been reported between −1154 and −2578 genotypes and VEGF production in the peripheral blood mononucleated cells of healthy subjects (Shahbazi et al., 2002). All these effects are dependent on several sequence polymorphisms, suggesting that VEGF gene variability may contribute to the inherited predisposition to VEGF-mediated pathological conditions.

Aim of the present study is to explore the VEGF gene promoter variability as a genetic determinant for longevity in an Italian population.

2. Materials and methods

2.1. Subjects

A total of 490 unrelated Italian individuals (40.2% males) born and resident in Northern Italy, ranging from 25 to 99 years of age, were enrolled for this study. Clinical records were obtained for each participant. The presence of moderate/severe dementia in subjects aged more than 80 years was excluded by the mini-mental scale examination (MMSE) test at time of blood collection. Subjects having a MMSE score ≤ 22 points were not included. Two groups of subjects were included in the study. Three hundred and four individuals (ranging from 25 to 99 years of age) were volunteers recruited either at nursing homes or at the Alzheimer Unit of "IRCCS Foundation Ospedale Maggiore Policlinico, Mangiagalli and Regina Elena", Milan, (non-consanguineous patients' kindreds); 105 out of 304 subjects were aged more than 65 years; all of them had a MMSE > 26, CDR = 0 and Hachinski Ischemic score < 4 at time of sampling. In addition, 186 subjects (ranging from 80 to 99 years of age) were participants in the ongoing Monzino 80-plus study, a prospective door to door population-based survey among all 80 years or older residents in the lower Olona Valley, some 20 km to the north of Milan (Tettamanti et al., 2006). An informed consent, approved by the Institutional Review Board of the "IRCCS Foundation Ospedale Maggiore Policlinico, Mangiagalli and Regina Elena", Milan, was obtained by all participants.

2.2. Genetic analysis

Total DNA was isolated from peripheral blood according to standard protocols. Three genomic DNA regions containing portions of the VEGF promoter were amplified by PCR (for primer sequences, see Supplementary data). Amplification protocol was the following: 5 min at 94 °C for the first cycle, denaturation at 94 °C for 30 s, annealing at 55–60 °C for 30 s, extension at 72 °C for 40 s for the subsequent 35 cycles, and a final extension at 72 °C for 5 min. To detect the polymorphism C(−2578)A, fragments were directly electrophoresed on a 3% agarose gel because of an 18-nucleotide insertion always associated with the −2578A allele, whereas CC homozygotes do not contain this insertion. The polymorphism G(−634)C was detected through PCR followed by *Mwo*I digestion. −1190G/A and −1154G/A variants were detected through direct sequencing analysis of PCR fragments using BigDyeTerminator TM protocol on an automated 3100ABI Prism Genetic Analyzer (Applied Biosystem, Foster City, CA).

All subjects were genotyped for the APOE status, as described (Del Bo et al., 1997).

2.3. Statistical analysis

Two-tailed Pearson's Chi-square and Fisher's exact tests were used to compare genotype and allele frequency distributions and corresponding odds ratios (ORs) and 95% confidence intervals (95% CIs) were calculated. For haplotype definition, we used the algorithm developed by the haploview program (http://www.broad.mit.edu/mpg/haploview), which uses a two-marker expectation-maximization (EM) to define the eventual blocks, to estimate the maximum-likelihood values and to calculate the D' values (Barrett et al., 2005). Haplotype phase and population frequency were inferred using a standard EM algorithm with a partition-ligation approach for blocks with greater than 10 markers. We also adjusted for multiple testing through a permutation approach on single marker. For each permutation, status was shuffled among both young/elderly and long-lived individuals, while the genetic data for each subject was not altered. The permutations were repeated 10,000 times.

3. Results

We screened 490 unrelated subjects from Italy for four well-known polymorphisms localised in the promoter and 5′ UTR region of VEGF gene. To carry out a comparative analysis of the genotypes, the whole cohort was divided into three age groups: (i) 166 young subjects (ranging from 25 to 65 years of age; mean ± standard deviation (S.D.): 47.4 ± 11.2 years; 45.5% males); (ii) 158 elderly subjects (ranging from 66 to 84 years of age, mean ± S.D.: 75.7 ± 5.7 years; MMSE: available only in 35 subjects aged more than 80 years: 25.6 ± 1.8 points; 50.4% males); (iii) 166 long-lived