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Short Communication

# −174 G/C interleukin-6 gene polymorphism and increased risk of multi-infarct dementia: a case-control study

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**Abstract**

The aim of this study was to evaluate the association between the −174 G/C polymorphism of interleukin-6 (IL-6) gene promoter and multi-infarct dementia (MID). We studied a group of 122 patients affected by MID and 134 age- and sex-matched controls and evaluated classical risk factors for MID, as well as the distribution of IL-6 alleles and genotypes by polymerase chain reaction and restriction enzyme analysis. The distribution of IL-6 genotypes was 63 GG, 47 GC, 12 CC in patients with MID and 29 GG, 58 GC, 47 CC in control subjects. The GG genotype was significantly more common in the MID group ($P < 0.0001$), while the CC genotype was more common in control patients ($P < 0.0001$). Logistic regression analysis indicated that the presence of GG genotype significantly increases the risk of MID (odds ratio 9.1 [3.1–26.1], $P < 0.0001$). This study indicates a strong association between the −174 G/C polymorphism of the IL-6 gene and MID. Our data support the hypothesis that IL-6 and inflammatory mechanisms are important in the pathophysiology of the vascular changes responsible for cognitive deterioration. 

*Keywords:* IL-6; Gene polymorphism; Inflammation; Multi-infarct dementia

## 1. Introduction

Multi-infarct dementia (MID) is the second most common cause of dementia after Alzheimer's disease (AD) (Clarfield, 1988). MID is caused by multiple small infarcts from small or medium-sized vessel disease. It occurs more often in persons who have hypertension and/or diabetes mellitus or who abuse tobacco. However, the mechanisms for clinical cognitive deterioration in patients with cerebral ischemia are not completely understood. Several recent observations hint at the role of inflammatory processes in the development of brain ischemia and multi-infarct cognitive impairment, as demonstrated by the accumulation of inflammatory cells and mediators in the ischemic brain (del Zoppo et al., 2000). The inflammatory process following focal

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cerebral ischemia is thought to be initiated by pro-inflammatory mediators such as tumor necrosis factor (TNF)-α, interleukin (IL)-1β, interleukin (IL)-6, and interleukin (IL)-8 (Li et al., 2001). In particular, IL-6 participates in the acute-phase response that follows cerebral ischemia and there is an association between high plasma levels of IL-6 and early neurological worsening (Vila et al., 2000). These data are consistent with the observation that elevated levels of IL-6 in plasma increase the risk of future myocardial infarction in an apparently healthy population (Ridker et al., 2000).

Recently, a G/C polymorphism in position −174 of the promoter region of the IL-6 gene has been reported (Fishman et al., 1998). This polymorphism is functionally important, since plasma concentration of IL-6 is influenced by the G/C genotype: indeed, the GG genotype correlates with higher circulating IL-6 concentrations, as shown in healthy Caucasians and in Italians undergoing surgical coronary revascularization (Fishman et al., 1998; Burzotta et al., 2001). However, how the −174 G/C polymorphism modulates IL-6 plasma levels remains controversial, since one study has reported that the CC genotype is associated with higher levels of IL-6 in the blood of patients affected by abdominal aortic aneurysm (Jones et al., 2001).

Recent studies have evaluated the relationship between the G/C IL-6 gene polymorphism and both carotid artery atherosclerosis and AD (Rauramaa et al., 2000; Bhojak et al., 2000; Bagli et al., 2000). This study was designed in order to assess whether the −174 G/C polymorphism of the IL-6 gene promoter is a risk factor for MID.

## 2. Patients and methods

### 2.1. Patients

Patients and controls were recruited among subjects consecutively admitted to the Department of Medicine of the A. Gemelli University Hospital of Rome, Italy, from November 1, 2000 to September 31, 2001. Diagnosis of probable MID was in accordance with the criteria established by the National Institute of Neurological Disorders and Stroke-Association pour la Recherche et l'Enseignement en Neurosciences (NINDS-AIREN): impairment of memory and other two or more cognitive domains; clinical and neuroradiologic evidence of cerebrovascular disease; and a relationship between the two disorders, inferred by onset of dementia within 3 months following a recognized stroke or abrupt and stepwise deterioration of cognitive functions (Roman et al., 1993). Cognitive function in MID patients was evaluated by a team of neurologists with formal neuro-psychological testing and mini-mental state examination (MMSE) (Folstein et al., 1975). All patients underwent brain imaging evaluation by computer tomography (CT) scan (G.E. Sytec SRi, G.E. Yokogawa Medical Systems, Nakano, Japan). Neuroradiologic diagnosis of MID was done when multiple bilateral ischemic cortical–subcortical lesions were present. The hachinski ischemic score (HIS) was also used to aid in distinguishing between MID and dementia from neurological disorders, such as AD (Hachinski et al., 1975). In addition, in order to exclude metabolic causes of dementia, peripheral venous blood samples were taken for determination of vitamin $B_{12}$, folic acid, and thyroid-stimulating hormone (TSH) serum levels, complete blood count, and electrolyte measurement.

Subjects matched for age and sex, without dementia and with no clinical and instrumental evidence of cerebral ischemia, were used as controls. Controls had no relationship with cases and no family history of dementia. Their cognitive function was evaluated by the same team of neurologists with formal neuro-psychological testing and MMSE. All control subjects underwent brain imaging evaluation by CT scan, which did not show the presence of ischemic lesions nor cerebral atrophy. All subjects with AD or dementia of metabolic origin were excluded. Subjects with suspected mixed dementia (MID and AD), defined in accordance with the NINDS-AIREN criteria (patients with possible AD but neuroradiologic evidence of cerebral infarct or history of stroke) (Roman et al., 1993) were excluded as well. Subjects with family history of AD were also excluded by this study. In both patient and control groups, subjects affected by tumors, chronic inflammatory diseases, and autoimmune diseases were excluded.

At the end of the recruitment period, a total of 122 patients with MID and 134 controls were enrolled. All

Table 1
Demographic and clinical data in patients with multi-infarct dementia (MID) and controls

| | MID ($n = 122$) | Controls ($n = 134$) | $P$ |
|---|---|---|---|
| Age (years) | 76.5 ± 8.2 | 76.1 ± 7.2 | 0.6 |
| Male:female ratio | 57:65 | 62:72 | 0.94 |
| Hypertension | 94 (77.0%) | 69 (51.5%) | <0.0001 |
| Hypercholesterolemia | 41 (33.6%) | 42 (31.3%) | 0.69 |
| Diabetes | 38 (31.1%) | 24 (17.9%) | 0.014 |
| CAD[a] | 52 (42.6%) | 35 (26.1%) | 0.005 |
| PAOD[b] | 28 (22.9%) | 14 (10.4%) | 0.007 |
| Smoking (current) | 9 (7.3%) | 15 (11.2%) | 0.29 |
| Smoking (former) | 25 (20.5%) | 35 (26.1%) | 0.28 |

[a] Coronary artery disease.
[b] Peripheral artery occlusive disease.

subjects were born and resident in Italy and belonged to independent pedigrees. For all individuals, a complete medical history was collected including smoking habit, hypertension, hypercholesterolemia, alcohol consumption, presence of diabetes, and drug treatment. The study protocol was accepted by the Ethics Committee of our University Hospital.

### 2.2. Genetic testing

DNA was extracted from peripheral blood and assayed by polymerase chain reaction (PCR) for the detection of IL-6 gene, using the published primer set: 5′-TGACTTCAGCTTTACTCTTTGT-3′ (sense primers) and 5′-CTGATTGGAAACCTTATTAGG-3′ (antisense primers). The conditions of the PCR reaction were as previously described (Fernandez-Real et al., 2000). The amplified sequence was digested by SfaNI restriction enzyme (New England BioLabs, Beverly, Massachusetts, USA) at 37 °C overnight. The digested products were electrophoresed in 2% agarose gel and visualized by ethidium bromide staining.

### 2.3. Statistical analyses

Demographic and clinical data between groups were compared by $\chi^2$ test and by t-test. Genotype and allele frequencies were compared by $\chi^2$ test. Odds ratios were calculated with 95% confidence interval (CI). To estimate the association between genotype and MID, a logistic regression model was used. All analyses were done by using Intercooled STATA 6.0 for Windows (Statistics/Data Analysis, Stata Corporation, College Station, Texas, USA). Statistical significance was established at $P < 0.05$.

## 3. Results

The demographic and clinical data of MID and control subjects are shown in Table 1. There were no significant differences between groups in terms of age ($P = 0.6$), sex ($P = 0.94$), smoking habit ($P = 0.2$), and hypercholesterolemia ($P = 0.69$). In contrast, other classical risk factors for MID, such as hypertension ($P < 0.0001$), diabetes ($P = 0.014$), positive history of coronary artery disease (CAD) ($P = 0.005$), and presence of peripheral artery occlusive disease (PAOD) ($P = 0.007$), were significantly more frequent in patients than controls.

All MID patients enrolled in the study had normal serum values of folic acid (16.8 ± 1.3 nmol/l), vitamin $B_{12}$ (342.2 ± 38.6 pmol/l), TSH (1.4 ± 0.3 mUI/l), sodium (141.1 ± 2.1 mmol/l), and potassium (4.3 ± 0.5 mmol/l). Likewise, red cell ($4.8 \pm 0.5 \times 10^{12}\,l^{-1}$), leukocyte ($5.5 \pm 0.9 \times 10^{9}\,l^{-1}$), and platelet count ($258 \pm 12 \times 10^{9}\,l^{-1}$) were normal in all patients.

Table 2 shows mean ± standard deviation (SD) of MMSE and HIS scores in MID patients and controls. The overall mean MMSE score was 17.6 ± 4.7 in the patient group and 27.6 ± 1.6 in the control group. Among the 122 patients with dementia, 49 (40.2%) had early cognitive deterioration (mean MMSE score = 21.9 ± 0.7), 61 (50%) had mild cognitive

Table 2
Mini-mental state examination (MMSE) and hachinski ischemic score (HIS) in patients with multi-infarct dementia (MID) and controls

| | MMSE score[a] (mean ± SD) | HIS score[b] (mean ± SD) |
|---|---|---|
| MID patients ($n = 122$) | 17.6 ± 4.7 | 12 ± 2.0 |
| Early dementia ($n = 49$) | 21.9 ± 0.7 | |
| Mild dementia ($n = 61$) | 16.1 ± 2.1 | |
| Severe dementia ($n = 12$) | 6.1 ± 1.4 | |
| Controls ($n = 134$) | 27.6 ± 1.6 | |

[a] Normality = 24–30, Early dementia = 21–23, Mild dementia = 11–20, Severe dementia = 0–10.
[b] Multi-infarct dementia ≥ 7, Neurodegenerative dementia ≤ 4.

deterioration (mean MMSE score = 16.1 ± 2.1), and 12 (9.8%) had severe cognitive deterioration (mean MMSE score = 6.1 ± 1.4). The mean HIS score in patients with dementia was 12 ± 2.1.

The distribution of IL-6 genotypes and alleles in cases and controls is shown in Table 3. Genotypes were in Hardy–Weinberg equilibrium. In the 122 patients with MID, the genotypes distribution was 63 GG, 47 GC, 12 CC and differed significantly from that observed in the 134 control subjects: 29 GG, 58 GC, 47 CC. The frequency of the GG genotype in patients with dementia was more than 2 times higher than in controls (51.6% vs. 21.6%, $P < 0.0001$). In contrast, the frequency of the CC genotype was almost 4 times higher in control subjects with no dementia (35.1% vs. 9.8%, $P < 0.0001$). Likewise, allele distribution was significantly different between the two groups: the G allele frequency was 70.9% in the patient group and 43.3% in controls; the C allele frequency was 29.1% in patients and 56.7% in controls; the ratio between the G and C allele frequency was 2.4 in the patients with MID and 0.7 in controls ($P < 0.00001$).

Table 3
IL-6 genotype and allele distribution in patients with Multi-infarct Dementia (MID) and controls

| | MID ($n = 122$) | Controls ($n = 134$) | $P$ |
|---|---|---|---|
| *Genotypes* | | | |
| G/G | 63 (51.6%) | 29 (21.6%) | <0.0001 |
| G/C | 47 (38.5%) | 58 (43.3%) | 0.439 |
| C/C | 12 (9.9%) | 47 (35.1%) | <0.0001 |
| *Alleles* | | | |
| G | 173 (70.9%) | 116 (43.3%) | |
| C | 71 (29.1%) | 152 (56.7%) | |
| G/C ratio | 2.4 | 0.7 | <0.00001 |

A logistic analysis (Table 4) showed that GG genotypes is an independent risk factor for MID in our population. Patients carrying the GG genotype have a risk more than 9 times higher than CC homozygous patients to develop MID [odds ratio 9.1 (3.1–26.1), $P < 0.0001$].

## 4. Discussion

Our study shows for the first time an association between the −174 G/C polymorphism of the IL-6 gene promoter and MID. As expected, patients with MID had a higher incidence of cardiovascular risk factors compared to controls. In particular, hypertension, diabetes, CAD, and PAOD were significantly more frequent in MID patients. Interestingly, we also found a significant difference in the distribution of IL-6 genotypes between the two groups. In our population, subjects carrying the G allele show increased risk of MID, while the C allele seems to play a protective role. In particular, subjects homozygous for the G allele have a risk 9.1 times higher to develop MID compared to CC homozygous patients.

Our data strengthen the hypothesis that inflammatory mechanisms play a crucial role in the pathogenesis and/or evolution of cerebrovascular diseases. Although the role of inflammatory cells and cytokines is still uncertain, a large body of evidence suggests that lymphocytes, macrophages, and several forms of soluble inflammatory mediators are important in arteriosclerosis, myocardial infarction, stroke, and cerebrovascular diseases. MID is characterized by activation of the microglia (Akiguchi et al., 1997), macrophages are abundant in atherosclerotic plaque, and infiltration of inflammatory cells to the surface of

Table 4
Risk factors for multi-infarct dementia (MID) based on logistic regression analysis

| | Odds ratio | 95% Confidence interval | *P* |
|---|---|---|---|
| G/G genotype | 9.1 | 3.1–26.1 | <0.0001 |
| G/C genotype | 2.4 | 0.8–7.0 | 0.08 |
| Diabetes | 3.2 | 1.3–7.7 | 0.007 |
| CAD[a] | 1.4 | 0.6–3.2 | 0.352 |
| Male sex | 0.9 | 0.4–2.1 | 0.865 |
| Age | 1.0 | 0.9–1.0 | 0.487 |
| Hypertension | 1.6 | 0.7–3.6 | 0.211 |
| Hypercholesterolemia | 1.3 | 0.5–3.0 | 0.479 |
| PAOD[b] | 4.2 | 1.6–11.2 | 0.003 |

[a] Coronary artery disease.
[b] Peripheral artery occlusive disease.

carotid plaques may be a critical step in promoting plaque rupture (Golledge et al., 2000). In addition, atherosclerosis might be considered a chronic inflammatory disorder (Ross, 1999) and elevated levels of IL-6 and other acute-phase proteins are found in patients with coronary syndromes (Miyao et al., 1993; Biasucci et al., 1996). Finally, increased intrathecal production of granulocyte macrophage-colony stimulating factor (GM-CSF), a cytokine stimulating microglial cell growth and exerting inflammatory properties, has been found in patients with MID (Tarkowski et al., 2001).

Interestingly, the same mechanisms seem to be involved in the pathophysiology of neurodegenerative diseases (Brod, 2000). A recent report has described the association between TNF-α polymorphism and both MID and AD (McCusker et al., 2001). However, as described in the Section 2, we paid particular attention to distinguish between MID and AD, and all subjects with AD, suspected mixed dementia, or family history of AD were excluded.

Several studies on IL-6 have focused on the detection of the protein in serum or tissue. However, IL-6 protein levels are influenced by many pathologic and physiologic conditions, such as infections, autoimmune disorders, malignancies, trauma, ischemia, and drug treatment (Van Snick, 1990; Papanicolaou et al., 1998; Marian, 2001; Ikonomidis et al., 1999). Therefore, a single measurement might be not predictive of disease in a given individual. For this reason, we focused on a polymorphism regulating gene expression and plasmatic levels of IL-6 (Terry et al., 2000; Fishman et al., 1998; Burzotta et al., 2001; Jones et al., 2001).

This study has some potential limitations. It has been designed as a case-control study and a possible survival bias cannot be excluded for the group of patients with MID. In addition, our patients have a higher incidence of CAD and PAOD than controls, and the IL-6 gene polymorphism might be associated with ischemic vascular disorders in general. Likewise, it is possible that the observed association is with cerebral ischemia only or cerebral ischemia and dementia. The distribution of G/C genotype that we found in our control group is different from that reported in a previous study performed on a randomly selected Italian population (Bonafè et al., 2001). However, our control group was specifically composed by hospitalized subjects with no clinical and instrumental evidence of dementia, without family history of dementia, and not affected by tumors, autoimmune, or chronic inflammatory diseases. Therefore, the control subjects investigated in our study might be not representative of the general Italian population. Finally, we cannot exclude a role played by a gene or by several genes in linkage disequilibrium with the IL-6 gene, nor that a variant of another gene, closely linked to the IL-6 gene, causes the observed association. In particular, there is strong linkage disequilibrium between the IL-6 gene promoter polymorphism and the variable number of tandem repeat polymorphism of the 3′ flanking region of the IL-6 gene (Bagli et al., 2000).

In conclusion, we provide evidence that the −174 G/C polymorphism of the IL-6 gene promoter is a risk

factor for MID. Although further studies are needed, our data support the hypothesis that IL-6 is important in the pathophysiology of MID and suggest a possible role for anti-inflammatory therapy in the prevention and treatment of this disease.

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