Neurol Sci (2002) 22:455–458

ORIGINAL

D. Intiso • P. Crociani • D. Fogli • E. Grandone • G. Cappucci • F. Di Rienzo
P. Di Viesti • P. Simone • P. Tonali

Occurrence of factor V Leiden mutation (Arg506Gln) and anticardiolipin antibodies in migraine patients

Received: 5 October 2002 / Accepted in revised form: 22 January 2002

Abstract The occurrences of factor V Leiden mutation (Arg506Gln) and antiphospholipid antibodies (APA) in migraine patients have been reported, but the findings are controversial. We investigated the presence of factor V Leiden and the serum level of anticardiolipin antibodies (aCL) in a consecutive series of 70 migraine patients (47 women; mean age, 34.1 years). Of these, 40 patients had migraine with aura. A matched sample of 70 healthy people was considered as the control group. Heterozygous genotype for factor V Leiden mutation was detected in 4 (5.7%) migraine patients (of which 2 had migraine with aura) and in 2 (2.8%) subjects of the control group. Although proportionally more migraine patients harbored the factor V Leiden mutation, this difference was not statistically significant, perhaps due to the small number of patients involved. We found normal serum levels of aCL in all migraine patients. Further studies and a long-term follow-up are warranted to determine the significance of this genetic abnormality in migraine.

Key words Migraine • Factor V Leiden • Antiphospholipid antibodies • Anticardiolipin antibodies • Coagulation

D. Intiso (✉) • P. Crociani • D. Fogli • F. Di Rienzo
P. Di Viesti • P. Simone
Department of Neurology
IRCCS "Casa Sollievo della Sofferenza"
Viale dei Cappuccini 1, I-71013
San Giovanni Rotondo (FG), Italy

E. Grandone • G. Cappucci
Atherosclerosis and Thrombosis Unit
IRCCS "Casa Sollievo della Sofferenza"
San Giovanni Rotondo (FG), Italy

P. Tonali
Department of Neurology
Catholic University, Rome, Italy

Introduction

Epidemiological surveys have shown that migraine is a risk factor for ischemic stroke in young people [1, 2], particularly in young women [3, 4], but the physiopathological mechanism is unknown. An intriguing hypothesis suggests that a prothrombotic condition characterizes migraine attacks predisposing to cerebral ischemia, particularly migraine with aura. Indeed, plasma hypercoagulability follows the derangement of the coagulation process due to the presence of factors affecting the coagulation cascade, such as factor V Leiden mutation and anticardiolipin antibodies (aCL), a quantifiable subgroup of antiphospholipid antibodies (APA) which is an independent risk factor for stroke [5]. Previous studies investigated the relationship between APA and migraine, but the results were controversial [6–9].

An alteration of system C and a high frequency of resistance to activated protein C have been recently detected in patients suffering from migraine with aura [10–13]. Resistance to activated protein C has emerged as an important hereditary cause of venous thromboembolism and is suspected to predispose young people to ischemic stroke and myocardial infarction [14–17]. A genetic alteration consisting in a single adenine-for-guanine point mutation in the gene coding for coagulation factor V results in the replacement of arginine by glutamine at position 506 in protein structure (Arg506Gln). This mutation, known as factor V Leiden, makes the activated form of factor V relatively resistant to degradation by activated protein C. To further clarify the possible role between derangements of the coagulation cascade and migraine, we investigated the presence of factor V Leiden

mutation and the serum level of anticardiolipin antibodies (aCL) in a series of migraine patients and controls.

Patients and methods

Migraine patients attending the Headache Centre of our department were consecutively enrolled after giving informed consent. Migraine was diagnosed according to criteria of the International Headache Society. Subjects with ascertained autoimmune disease, vasculitis, abnormalities of the coagulation process or antiphospholipid syndrome were excluded. These exclusion criteria were also used for the control group.

Clinical and neurological evaluations were performed as was a complete migraine history including site, intensity and frequency of migraine attacks. In order to analyze the coagulation process, prothrombin time (PT), partial-thromboplastin time (PTT), and serum levels of antithrombin III, fibrinogen, and aCL were measured. All patients had a cerebral magnetic resonance imaging (MRI) evaluation on a 0.5 tesla Toshiba instrument. An age- and sex- matched sample of healthy controls without migraine was recruited from people working in our hospital.

The presence of factor V Leiden mutation (Arg506Gln) was ascertained by DNA polymerase chain reaction (PCR). Blood samples taken by antecubital venepuncture were diluted 1:10 in 0.1 M sodium citrate and the samples were stored at -80°C until DNA extraction. Leukocyte DNA was obtained from frozen blood standard techniques [18]. A 220-base-pair DNA fragment of the factor V Leiden gene that includes nucleotide 1691 was amplified by PCR. After endonuclease digestion with Mnl I, the fragments were analyzed by electrophoresis through 4% agarose gels in Tris-acetate EDTA buffer containing 0.5 mg/ml ethidium bromide and were visualized using ultraviolet light [19, 20].

Anticardiolipin antibodies (aCL) were titrated according to the method described elsewhere [21] and assayed for IgG idiotype by enzyme-linked immunosorbent assay (ELISA). Values are reported as number of IgG phospholipid (GPL) units; one GPL unit is defined as the cardiolipin binding of 1 μ/ml of an affinity-purified aCL IgG preparation from standard serum.

Statistical analysis was performed using chi-square and Fisher's exact tests.

Results

Seventy migraine patients and 70 age- and sex-matched controls were enrolled in the study (Table 1). Of the 70 migraine patients, 40 had migraine with aura.

Heterozygous genotype for factor V Leiden mutation was detected in 4 (5.7%) migraine patients (2 with and 2 without aura) and in 2 (2.8%) controls (Table 2). The difference between migraine patients and controls was not significant. There were no differences in term of site, frequency and intensity of migraine attacks between migraine patients with and without the factor V Leiden mutation (data not shown). None of the migraine patients showed a high serum titre of aCL (Table 3). Furthermore PT, PTT and serum levels of antithrombin III and fibrinogen were normal in all patients (data not shown). Cerebral MRI scans disclosed no abnormalities in migraine patients (not shown).

Table 1 Clinical characteristics of migraine patients and control group

	n	Age, years[a]	Males, n (%)
Migraine patients	70	34.1 (9.8)	23 (33)
Migraine with aura	40	36.6 (10.1)	12 (30)
Migraine without aura	30	31.5 (12.3)	11 (37)
Controls	70	33.5 (8.6)	24 (34)

[a] Values are means (SD)

Table 2 Factor V Leiden mutation in migraine and control groups. The difference between migraine patients and controls is not significant (p=0.40; odds ratio=2.00; 95% CI, 0.28–22.68)

	Heterozygous subjects, n (%)
Migraine patients	4 (5.7)
Migraine with aura	5 (5.0)
Migraine without aura	2 (6.6)
Controls	2 (2.8)

Table 3 Serum levels of anticardiolipin antibodies (aCL) in migraine and control groups. Values are means (SD)

	aCL (GPL units)[a]
Migraine patients	2.28 (1.9)
Migraine with aura	2.47 (2.0)
Migraine without aura	2.19 (1.9)
Controls	2.15 (1.7)

[a] 1 GPL unit = 1

Discussion

A high frequency of factor V Leiden mutation (Arg506Gln) has been recently detected in young patients suffering from ischemic stroke [10]. Most of these patients also had a history of migraine attacks; consequently, a relationship between migraine and this genetic alteration was suggested. A recent study found increased frequency of activated protein C resistance due to factor V Leiden mutation and protein S deficiency in patients suffering from migraine with aura and in young adults with ischemic stroke, suggesting that this genetic abnormality may be a shared risk factor in both disorders [11]. Indeed, migraine is a risk factor for ischemic stroke in the young [1–4], but the physiopathological pathways which precipitate ischemia are unclear. Platelet hyperaggregability and potential derangement of the coagulation process may characterize the early phase of migraine attacks producing a prothrombotic condition, which may favor cerebral ischemia. In this way, factors interfering with the coagulation cascade such as APA, in particular aCL, and factor V Leiden mutation could have an important role. Several hypotheses have been proposed about the mechanism of APA producing a prothrombotic condition including interference with antithrombin III activity, inhibition of free kallikrein, interference with endothelial cell function and inhibition of prostacyclin from blood vessels [22–24].

We did not discover any alteration of serum aCL levels, as in previous research that did not find a relationship between migraine and APA, including lupus anticoagulant and aCL [7–9]. On the other hand, we detected a higher occurrence of factor V Leiden mutation in migraine patients than in the control group, although this difference was not significant. Recent research reported a high frequency of factor V Leiden mutation in patients suffering from migraine with aura [11, 12]. However, we found the factor V Leiden mutation in migraine patients, independent of the type of migraine attack. The difference between these findings could be due to the small number of patients involved. A previous study by Leone et al. [25] of a small migraine sample gave results similar to ours. On the other hand this picture could be coincidental and simply reflect the similar incidence of factor V Leiden mutation in migraineurs and in the general population. The carrier frequency of factor V Leiden mutation in a healthy control population ranges from 3% to 7% in Europe and the USA and may be as high as 15% in some selected groups [26–29]. In our control group, the occurrence of factor V Leiden mutation was 2.8%, a finding similar to that of previous studies [10, 26–29], but less than in that in migraine patient (5.7%), even if the difference was not significant. However this finding, as well as the lack of any difference for high serum levels of aCL between migraineurs and controls may be due to low power of our study.

In conclusion, our data overlap with the results of previous studies which did not find increased serum levels of aCL in migraine patients. On the other hand, a high occurrence of factor V Leiden mutation was detected in patients suffering from migraine. Studies with larger numbers of patients and a long-term follow-up are warranted to determine if this genetic abnormality has a specific role in migraine patients.

Sommario *È stato riportato in letteratura che i pazienti affetti da emicrania possono presentare la mutazione genetica (Arg506Gln) del fattore V della coagulazione, nota come fattore V Leiden, nonché alterazione dei livelli sierici degli anticorpi antifosfolipidi, anche se questo dato è controverso. Noi abbiamo studiato una serie consecutiva di pazienti emicranici per valutare sia la presenza di fattore V Leiden che di anticorpi anticardiolipina (aCL). Settanta pazienti emicranici (47 donne; età media, 34.1 anni, SD 9.8) e un gruppo di controllo sovrapponibile per sesso ed età sono stati inseriti nello studio. Dei pazienti emicranici, 40 presentavano emicrania con aura e 30 emicrania senza aura. La mutazione (Arg506Gln) nella forma genotipica eterozigotica fu riscontrata in 4 (5.8%) pazienti emicranici (rispettivamente in 2 pazienti affetti da emicrania con aura e 2 pazienti con emicrania senza aura) e in 2 (2.8%) soggetti del gruppo di controllo. Nessun paziente emicranico presentò alterazione dei livelli sierici di anticorpi anticardiolipina. Nel nostro studio non abbiamo trovato incrementi dei livelli di aCL sia nei pazienti emicranici che nel gruppo di controllo. Al contrario una maggiore frequenza di mutazione del fattore V Leiden può essere riscontrata nei pazienti emicranici, ma ulteriori studi con un campione più vasto e controlli nel tempo sono necessari per chiarire il ruolo e il significato di questo dato.*

References

1. Buring JE, Hebert P, Romero J et al (1995) Migraine and subsequent risk of stroke in the Physicians' Health Study. Arch Neurol 52:129–134
2. Merikangas KR, Fenton BT, Cheng SH, Stolar MJ, Risch N (1997) Association between migraine and stroke in a large scale epidemiological study of the United States. Arch Neurol 54:362–368
3. Tzourio C, Tehindrazanarilevo A, Iglèsias S et al (1995) Case-control study of migraine and risk of ischemic stroke in young women. BMJ 310:830–833
4. Carolei A, Marini C, DeMatteis G and the Italian National Research Council Study Group on Stroke in the Young (1996) History of migraine and risk of cerebral ischemia in young adults. Lancet 347:1503–1506
5. – (1993) Anticardiolipin antibodies are independent risk factor for first ischemic stroke. Antiphospholipid Antibodies in Stroke Study (APASS) Group. Neurology 43:2069–2073
6. Shuaib A, Barklay L, Lee MA, Suchowersky O (1989) Migraine and antiphospholipid antibodies. Headache 29:42–45
7. Montalbàn J, Cervera R, Font J et al (1992) Lack of association between anticardiolipin antibodies and migraine in sys-

temic lupus erythematosus. Neurology 42:681–682
8. Hering R, Couturier EGM, Steiner TJ, Asherson RA, Rose FC (1991) Anticardiolipin antibodies in migraine. Cephalalgia 11:19–21
9. Tietjen GE, Day M, Norris L, Aurora S, Halvorsen A, Schultz LR, Levine SR (1998) Role of anticardiolipin antibodies in young persons with migraine and transient focal neurologic events: a prospective study. Neurology 50:1433–1440
10. Kontula K, Ylikorkala A, Miettinen H, Vuorio A, Kauppinen-Makelin R, Hamalainen L, Palomaki H, Kaste M (1995) Arg506Gln factor V mutation (factor V Leiden) in patients with ischemic cerebrovascular disease and survivors of myocardial infarction. Thromb Haemost 73(4):558–560
11. D'Amico D, Moschiano F, Leone M, Ariano C, Ciusani E, Erba N, Grazzi L, Ferraris A, Schieroni F, Bussone G (1998) Genetic abnormalities of the protein C system: shared risk factors in young adults with migraine with aura and with ischemic stroke? Cephalalgia 18(9):618–621
12. Corral J, Iniesta JA, Gonzalez-Conejero R, Lozano ML, Rivera J, Vicente V (1997) Migraine and prothrombotic genetic risk factors. Cephalalgia 17(59):605–607
13. Soriani S, Trabetti E, Casartelli A, Pignatti PF, Arnaldi C, Borgna-Pignatti C (1997) Frequency of factor V Leiden in juvenile migraine with aura. Cephalalgia 17:329 (abstract)
14. Rosendaal FR, Koster T, Vandenbroucke JP, Reitsma PH (1995) High risk of thrombosis in patients homozygous for factor V Leiden (activated protein C resistance). Blood 85:1504–1508
15. Ridker PM, Hennekens CH, Lindpaintner K, Stampfer MJ, Eisenberg PR, Miletich JP (1995) Mutation in the gene coding for coagulation factor V and the risk of myocardial infarction, stroke, and venous thrombosis in apparently healthy men. N Engl J Med 332:912–917
16. Svensson PJ, Dahlback B (1994) Resistance to activated protein C as a basis for venous thrombosis. N Engl J Med 330:517–522
17. Price DT, Ridker PM (1997) Factor V Leiden mutation and the risks for thromboembolic disease: a clinical perspective. Ann Intern Med 127:895–903
18. Miller SA, Dykes DD, Polesky NF (1988) A simple salting out procedure for extracting DNA from human nucleated cells. Nucleic Acid Res 16:1212
19. Margaglione M, D'Andrea G, Giuliani N, Brancaccio V, De Lucia D, Grandoni E, De Stefano V, Tonali P, Di Minno G (1999) Inherited prothrombotic conditions and premature ischemic stroke, sex difference in the association with factor V Leiden. Arterioscler Thromb Vasc Biol 19:1751–1756
20. Margaglione M, Brancaccio V, Giuliani N, D'Andrea G, Cappucci G, Iannacone L, Vecchione G, Grandone E, Di Minno G (1998) Increased risk of venous thrombosis in carriers of the prothrombin A20210 gene variant. Ann Intern Med 129:89–93
21. Loizou S, McCrea JD, Rudge AC et al (1986) Measurement of anticardiolipin antibodies by an enzyme-linked immunosorbent assay: standardization and quantitation of results. Clin Exp Immunol 62:739–744
22. Cosgriff TM, Martin BA (1981) Low functional and high antigenic antithrombin III level in a patient with lupus anticoagulant and recurrent thrombosis. Arthritis Rheum 24:94–96
23. Angles-Cano E, Sultan Y, Clavel JP (1979) Predisponing factors to thrombosis and systemic lupus erythematosus. J Lab Clin Med 94:312–323
24. Carreras LO, Vermulen JG (1982) Lupus anticoagulant and thrombosis. Possible role of inhibition of prostacyclin formation. Thromb Haemost 48:38-40
25. Leone M, Ciusani E, Ariano C, Moschiano F, Grazzi L, D'Amico D et al (1996) Point mutation (Arg506Gln) of factor V in migraine with and without aura. Cephalalgia 16:395 (abstract)
26. Rees DC, Cox M, Clegg JB (1995) World distribution of factor V Leiden. Lancet 346:1133–1134
27. Ridker PM, Miletich JP, Hennekens CH, Buring JE (1997) Ethnic distribution of factor V Leiden in 4047 men and women. Implication for venous thromboembolism screening. JAMA 277:1305–1307
28. Svensson PJ, Dahlbach B (1994) Resistance to activated protein C as a basis for venous thrombosis. N Engl J Med 330:517
29. Bertina RM, Koeleman BPC, Koster T et al (1994) Mutation in blood coagulation factor V associated with resistance to activated protein C. Nature 369:64–67