LETTERS

The androgen receptor CAG repeat and serum testosterone in the risk of Alzheimer's disease in men

We recently reported[1] that the glutamine (CAG) repeat polymorphism of exon 1 of the androgen receptor was associated with Alzheimer's disease in men from the Oxford region. If this androgen receptor polymorphism indeed affects Alzheimer's disease risk rather than being in linkage disequilibrium with the true risk factor, then we should see the effect through androgen receptor actions. The association should, for instance, be influenced by ligands of the androgen receptor. Androgen receptor isoforms are the main, perhaps sole, receptors for the principal mammalian androgens, testosterone and 5α-dihydrotestosterone. On binding ligand, the receptor moves from the cytoplasm to specific compartments of the nucleus ("nuclear foci"), where it interacts with coactivators and corepressors in the cell specific regulation of numerous genes. We therefore looked for an interaction in Alzheimer's disease risk of the androgen receptor CAG polymorphism with serum concentrations of total testosterone.

We studied 207 elderly men, 79 with sporadic Alzheimer's disease (mean (SD) onset age, 70 (9) years) and 128 controls (mean age, 75 (10) years), all from the cohort of the Oxford Project to Investigate Memory and Ageing (OPTIMA). Of the 79 Alzheimer cases, 45 were necropsy confirmed as having Alzheimer's disease by CERAD criteria (40 "definite" and five "probable") and 34 were diagnosed as "probable Alzheimer's disease" by NINCDS-ADRDA criteria. All 128 controls were without detectable cognitive dysfunction and with CAMCOG scores of more than 80. Serum concentrations of total testosterone and androgen receptor allele lengths were determined as previously described.[1 2] Testosterone concentrations were divided into tertiles (69 subjects in each tertile): the upper tertile was >19 nmol/l, the middle between 13 and 19 nmol/l, and the lower ⩽13 nmol/l. Short androgen receptor alleles were ⩽20 CAG repeats. Logistic regression analysis was by *R*.

Results

As expected, short androgen receptor alleles[1] and low testosterone[2] were associated with Alzheimer's disease. Controlling for age, odds ratios of Alzheimer's disease were 2.1 (95% confidence interval, 1.1 to 4.1) (p = 0.025) for short androgen receptor alleles, and 2.2 (1.01 to 4.6) (p = 0.04) for the lower *v* the upper tertile of testosterone. There was a significant trend in the association with Alzheimer's disease by testosterone tertile (χ^2_{trend}, $p<0.05$), indicating a possible dose related effect of testosterone.

Table 1 shows the unadjusted odds ratios of Alzheimer's disease for each combination of long or short androgen receptor alleles with each testosterone tertile, taking long alleles with upper tertile testosterone as reference. The odds ratio for short androgen receptor alleles with lower tertile testosterone was 4.2 (1.4 to 13) (p = 0.01). Combining the two at risk subgroups—that is, carriers of short androgen receptor alleles with middle or lower tertile testosterone—versus all the others gave an odds ratio of Alzheimer's disease of 2.3 (1.1 to 4.8) (p = 0.03), when adjusted for age and for carrier status of apolipoprotein E ε4.

Table 1 Odds ratios of Alzheimer's disease in men, according to serum level of total testosterone and androgen receptor CAG allele length

AR CAG allele*	Testosterone tertile†	Subjects (n) AD	Subjects (n) Controls	Unadjusted odds ratio (95% CI)
Long	Upper	13	40	1 (reference)
Long	Middle	12	26	1.4 (0.6 to 3.6) (NS‡)
Long	Lower	21	29	2.2 (1.0 to 5.2) (NS)
Short	Upper	7	9	2.4 (0.7 to 7.7) (NS)
Short	Middle	15	16	2.9 (1.1 to 7.4)§
Short	Lower	11	8	4.2 (1.4 to 13) §

*Long alleles are >20 CAG repeats.
†Testosterone tertiles are: upper >19 nmol/l, middle >13 and ⩽19 nmol/l, lower ⩽13 nmol/l.
‡NS, not significant at p = 0.05.
§Odds ratio for carriers of short AR alleles with middle or lower tertile testosterone *v* all others = 2.3 (1.1 to 4.8), adjusted for age and carrier status of apolipoprotein E ε4.
AD, Alzheimer's disease; AR, androgen receptor; CI, confidence interval.

Comment

A difficulty with the study of complex diseases such as sporadic Alzheimer's disease lies in the numerous interactions of each risk gene with other genes, with age and sex, and with the environment. This results in a weak overall effect of each gene and in conflicting results of genetic studies. To resolve the difficulty, we need to examine the interactions that define the "relevant subset" of people at risk for each susceptibility gene.[3]

The association of the glutamine (CAG) repeat polymorphism of the androgen receptor with Alzheimer's disease appears limited to men.[1] This is unsurprising, given the role of the androgen receptor as the main receptor for testosterone and 5α-dihydrotestosterone. We should therefore expect an interaction with testosterone in Alzheimer's disease risk. Low serum testosterone has been associated with poor cognitive performance in elderly men[4] and with Alzheimer's disease.[2] Testosterone exerts various neuroprotective actions[5 6] through the androgen receptor. The CAG polymorphism of the androgen receptor has been associated with the risk of Alzheimer's disease in men[1] and with other brain disorders.[7]

Polyglutamine tracts play an important role in the activity of many transcription factors. The androgen receptor tract is in exon 1, which carries the transactivation domain. Transcriptional activity of the androgen receptor decreases with increasing length of the tract, even within the normal range.[8] This effect is cell specific, which suggests that it may reflect interactions with other proteins. The androgen receptor coactivator, ARA24, has been found to be less effective with expansion of the androgen receptor polyglutamine tract. Binding of testosterone to the androgen receptor changes the latter's conformation, releasing it from its cytoplasmic compartment and allowing nuclear translocation. This also permits interactions with coactivators, as well as between the N- and C-terminal domains of the androgen receptor. The polyglutamine tract is involved in several of these interactions.

Our results suggest that the combination of short androgen receptor alleles with lower levels of serum testosterone may increase the risk of Alzheimer's disease for men. Further study is needed to clarify whether these two potential risk factors interact or act independently. There is a growing appreciation of, first, the influence of sex steroids on Alzheimer's disease risk[2] and, second, the role of sex in Alzheimer's disease genetics.[1 3 9] This study therefore merits replication in other, carefully characterised, all male cohorts. To demonstrate an interaction, large numbers would be needed, either through a collaborative study, using meta-analytical techniques for pooling, or eventually through a meta-analysis.

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Subthalamic nucleus stimulation in a parkinsonian patient with previous bilateral thalamotomy

Stereotactic surgical ablation of the thalamic nucleus (thalamotomy) has long been applied to parkinsonian tremor, rigidity, and levodopa induced dyskinesias.[1–3] Chronic high frequency deep brain stimulation (DBS) of the thalamus, the globus pallidus internus (GPi), and the subthalamic nucleus (STN) has been widely used as an alternative to ablative surgery in the treatment of Parkinson's disease (PD). STN stimulation has become increasingly popular because it can result in a striking improvement of motor symptoms and the ability for PD patients to pursue the activities of daily living.[4 5] We report that STN stimulation markedly alleviated axial motor symptoms in a PD patient who had undergone bilateral thalamotomy more than 20 years earlier.

This 70 year old right handed woman presented for the first time to our university hospital in early 2002. Around 1968, she developed right sided tremor and bradykinesia and was diagnosed with PD. Although she initially responded well to medication, her symptoms progressed, with worsening motor fluctuations including dyskinesia. Left and right thalamotomy, performed in 1976 and 1982 respectively, resulted in marked improvement of her tremor and dyskinesia. She received optimised medication and for the next 10 years was able to continue her job as a secretary. However, she developed slowly worsening postural instability with hesitant and shuffling gait, and suffered occasional falls.

Admission examination in March 2002 disclosed no neurological signs apart from parkinsonism. Under the administration of carbidopa/levodopa (4×5.0/50 mg/day), cabergoline (3×1.0 mg/day), amantadine hydrochloride (3×50 mg/day), and L-threo-DOPS (3×100 mg/day), neither rest tremor nor rigidity were apparent. Her bradykinesia was slight and more pronounced on the left side. Among her parkinsonian disabilities, axial symptoms including postural instability and gait disorder were the most pronounced. In the sitting position, her upper body was

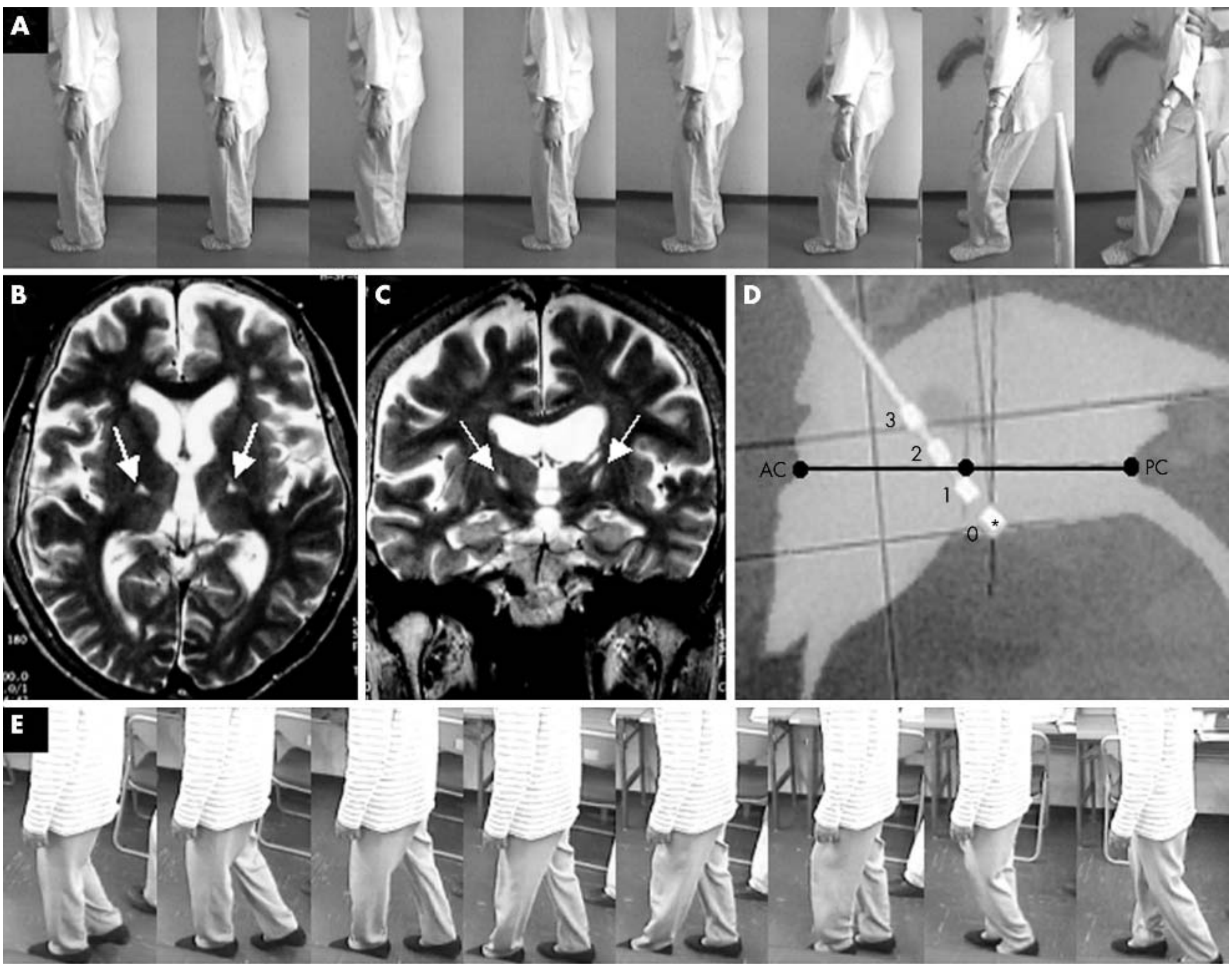

Figure 1 (A) Serial photographs (from left to right) taken before surgery for STN DBS. Although the patient is trying to walk backwards, she cannot step backwards due to hesitation, and finally falls. (B, C) Axial (B) and coronal (C) T2 weighted MR images show surgical lesions (arrows) in the bilateral thalamic nuclei that were made more than 20 years earlier. Under local infiltrating anaesthesia, a quadripolar deep brain stimulation (DBS) electrode (model 3387; Medtronic Inc., Minneapolis, MN, USA) was implanted in the right STN with the aid of MRI, imaging of the third ventricle, and microelectrode guidance. A Leksell MRI compatible stereotactic apparatus was used. The optimal target was determined to be 2 mm posterior and 12 mm lateral to the midpoint of the anterior to posterior commissure (AC−PC) line, and 4 mm below the AC−PC line. The location of the electrode was checked radiologically and the most ventral contact was placed exactly on the target point (D). As stimulation tests, performed for 3 days, confirmed the beneficial effects of DBS, a programmable pulse generator (Soletra, Model 7426; Medtronic Inc.) was implanted and connected to the DBS electrode. Her postoperative course was uneventful. (D) Location of the electrodes superimposed on the lateral view of the selective third ventriculography. The target point is indicated by asterisk. AC, anterior commissure; PC, posterior commissure. (E) Serial photographs (from left to right) taken under STN stimulation. The patient can walk backwards easily without shuffling or hesitant steps.

bent to the left. Without assistance she could not easily stand up, and her feet quickly froze when she attempted to walk forward. Shuffling hesitant steps were more marked on the left side and were particularly evident when turning, initiating gait, and walking backwards (fig 1A). They were not alleviated by visual aids such as stripes on the floor or by the use of an inverted walking cane. Her facial expression and speech were slightly affected and she manifested micrographia. On medication, her total and Part III motor scores on the Unified Parkinson's Disease Rating Scale were 38 and 19, respectively. Magnetic resonance imaging (MRI) revealed no obvious abnormalities except for the surgical lesions produced 20 years earlier in the bilateral thalamic nuclei (fig 1B, C). Because she had experienced occasional transient drug induced psychoses that disappeared when the dosages were reduced, we concluded that it was not possible to improve her symptoms by pharmacotherapy alone and decided to perform stereotaxy. Prior informed consent was obtained from the patient and her family.

STN stimulation using two ventral contacts (contacts 0 and 1) gave rise to a striking improvement of her axial symptoms. Contacts 0 and 1 were used as cathode (fig 1D) and the pulse generator as anode. After extensive trials, the optimal stimulation parameters were determined to be 130 Hz frequency, 60 µsecond pulse width, and 1.8 V and 2.6 V amplitude at the first and final session, respectively. Under stimulation, she was able to stand up with ease and without assistance, and could initiate gait fluently. Her shuffling hesitant steps almost disappeared even when turning and walking backwards (fig 1E). Compared to preoperative baselines, her total and Part III motor scores were reduced from 38 and 19 to 11 and 5, respectively. She continued to take the same medication at the same doses as before and the beneficial effects of STN stimulation were unchanged at 9 months post-treatment.

Medically intractable PD has been addressed with different types of surgery. Our patient initially underwent staged bilateral thalamotomy. Although this procedure produced long lasting benefits and her tremor, rigidity, and dyskinesias were improved, bilateral, even staged, thalamotomy carries significant risks and is no longer considered a viable treatment option.[2] Nonetheless, we recognised the long lasting effectiveness of bilateral thalamotomy; our patient was operated on 20 years earlier by Dr. Narabayashi.[1] However, because of the recent aggravation of her axial parkinsonian symptoms, for which thalamotomy is generally thought to be ineffective,[2] she was referred to our hospital for further treatment.

While STN DBS can markedly improve all levodopa responsive motor features of parkinsonism including axial symptoms,[6] STN DBS appears to exhibit greater anti-parkinsonian effects than GPi DBS. Therefore, we chose STN stimulation for our patient. We initially thought that bilateral surgery was necessary. However, we found that unilateral (right) STN DBS contralateral to the more severely affected side markedly alleviated her axial symptoms, and led to the almost complete disappearance of her shuffling, hesitant steps and falling. At present, we do not know whether she will eventually require additional contralateral (left) STN DBS.

In patients with PD, various combinations of different stereotactic interventions have produced varying results and there is currently no consensus regarding their efficacy [for examples, see references 7 and 8]. Based on the experience reported here, we suggest that PD patients with a prior successful thalamotomy may be good candidates for STN stimulation.

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Testosterone deficiency in a Parkinson's disease clinic: results of a survey

It has been shown recently that male patients with Parkinson's disease who have testosterone deficiency may have symptoms resembling non-motor parkinsonian symptoms.[1 2] Because of the similarity between the non-motor symptoms of Parkinson's disease and the symptoms of testosterone deficiency, clinicians may fail to recognise and treat testosterone deficiency in patients with Parkinson's disease.[1] The identification of testosterone deficiency may have a significant impact on the long term course of the disease, as symptoms mistakenly labelled as non-motor parkinsonian manifestations could be relieved more effectively by testosterone replacement than by other treatments.

In this study we examined the prevalence of testosterone deficiency and testosterone deficiency symptoms among a group of patients with Parkinson's disease presenting to our movement disorders clinic, to assess how common undiagnosed symptomatic testosterone deficiency was in this population. A mail-back survey was administered to all the patients seen in the clinic after a 12 month period where patients were seen, examined, and entered into a database. The surveys were returned by 91 of 137 male patients with Parkinson's disease (66%). The diagnosis of idiopathic Parkinson's disease was confirmed by a movement disorders specialist who applied the UK Brain Bank criteria[3] and currently recommended guidelines for the diagnosis of Parkinson's disease.[4] We included in the survey two validated scales—the St Louis testosterone deficiency questionnaire[5] and the Beck depression inventory.[6 7] Additionally, historical information was obtained including the number of antidepressants the patients were exposed to in a lifetime, number of current antidepressants, history of testosterone deficiency, history of prostate cancer, and history of hormone replacement therapy. Forty two male patients with Parkinson's disease who returned questionnaires had previously been identified as testosterone deficient by measurements of plasma testosterone concentrations.

The results of the survey are summarised in table 1. The average age of the male patients was 62 years. Nine per cent of the study population were on testosterone gel replacement therapy. Fifty of the 91 patients with Parkinson's disease were screened with free testosterone levels during the 12 month period of the study. Half of the Parkinson's disease patients (n = 25) who were screened for testosterone deficiency had a level of <70 pg/ml and were defined as having "low" testosterone. Ninety per cent of all male patients with Parkinson's disease had a positive St Louis testosterone deficiency questionnaire (positive answers to more than three questions).

Comment

The results of our survey indicate that testosterone deficiency is common in the elderly male population seen in a movement disorders clinic setting, and the prevalence is similar to that previously reported in the Baltimore longitudinal study of aging in the normal elderly population[8] and in Parkinson's disease.[1] As the non-motor symptoms of Parkinson's disease—including depression, anxiety, fatigue, decreased libido, sexual dysfunction, and a decreased enjoyment in life—directly overlap with those seen in male testosterone deficiency, separating testosterone deficiency from the non-motor symptoms of Parkinson's disease can be difficult. This separation is important because specific treatment for testosterone deficiency is available and because these symptoms may not respond satisfactorily to antidepressant therapy.

Previous observations of refractory non-motor symptoms of Parkinson's disease that responded to testosterone replacement suggested that patients were on or had been exposed to an increased number of antidepressants.[1] Seidman and Rabkin found that testosterone deficiency—as also seen in thyroid hormone deficiency—may blunt responsiveness to antidepressants,[9] prompting the investigators to examine whether patients with testosterone deficiency took more antidepressants than those without testosterone deficiency, as well as to examine whether deficiency scores correlated with the number of antidepressants. Our data did not suggest a difference in antidepressant use in the testosterone deficient Parkinson's disease

Table 1 Characteristics of 91 male patients with Parkinson's disease who returned the questionnaire

Characteristic		All (n = 91)	Free T <70 pg/ml (n = 19)	Free T >70 pg/ml (n = 23)
Age (years)				
Mean		64	71	61
SD		10	9	10
Range		40 to 89	55 to 89	44 to 83
HRT (% of patients)		10%	21%	4%
Free testosterone level (pg/ml)				
<70		19 (45%)	19 (100%)	0 (0%)
>70		23 (55%)	0 (0%)	23 (100%)
Mean		82.1	49.0	109.4
SD		41.6	13.6	36.7
Range		4.1 to 196.1	4.1 to 65.7	71.2 to 196.1
St Louis score				
⩾3		85 (93%)	18 (95%)	22 (96%)
<3		6 (7%)	1 (5%)	1 (4%)
Mean		5.9	6.6	5.8
SD		2.3	2.2	2.2
Range		0 to 10	2 to 10	2 to 10
BDI score				
Mean		9.2	10.5	9.3
SD		6.0	6.7	5.4
Range		0 to 31	2 to 22	0 to 19
Number of antidepressants				
Current:	0	51 (56%)	7 (37%)	13 (57%)
	1	37 (41%)	11 (58%)	10 (43%)
	>1	3 (3%)	1 (5%)	0 (0%)
Lifetime:	0	35 (38%)	4 (21%)	9 (39%)
	1	27 (30%)	10 (53%)	4 (17%)
	2	15 (16%)	1 (5%)	6 (26%)
	3	10 (11%)	3 (16%)	2 (9%)
	>3	4 (4%)	1 (5%)	2 (9%)

BDI, Beck depression inventory; HRT, hormone replacement therapy; T, testosterone.

population; however, a prospective study will need to be done to confirm this observation. Overall, however, depression scores were not high in this study, which may either reflect aggressive treatment of depression in our Parkinson group, or suggest that testosterone deficiency does not present as major depression. Additionally, the study did not specifically screen for patients who were refractory to antidepressant treatment, and for those who had previously received aggressive treatment for depression. We suspect that testosterone deficiency, like thyroid deficiency, does affect the efficacy of antidepressants, but better prospective studies will be needed to examine this question.

Testosterone deficiency is a common, treatable, and largely unrecognised form of co-morbidity in Parkinson's disease, and as demonstrated by this study is common in a movement disorders clinic setting. It may go undiagnosed when the symptoms are attributed to the non-motor manifestations of Parkinson's disease. Additionally, a lack of a history of refractoriness to antidepressants, or lack of a positive depression screening questionnaire, should not dissuade practitioners from checking testosterone levels, as antidepressant responsive "depressive symptoms" seem to be common in testosterone deficiency.

Prospective epidemiological studies on this topic need to be undertaken, as this analysis of clinic patients suffered from both the bias of the researchers interested in testosterone deficiency, and the failure to get 100% return of the surveys. Additionally, a control group will need to be included in future analyses, and better screening devices with increased specificity for patients with Parkinson's disease and testosterone deficiency will need to be developed. The issue of which type of testosterone assay is best, and how much the testosterone level matters if a patient is symptomatic, will also need to be examined.

Every practitioner who sees patients with Parkinson's disease should be aware of this common treatable co-morbidity. The diagnosis of testosterone deficiency should be confirmed and prostate cancer excluded before initiating treatment.

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LBP-1c/CP2/LSF gene polymorphism and risk of sporadic Alzheimer's disease

The ε4 allele of apolipoprotein E (ApoE) accounts for an estimated 45–60% of the genetic risk for late onset sporadic Alzheimer's disease, suggesting that it may be possible to identify other genetic loci that could account for the remaining risk associated with this disease. Recently, a biallelic polymorphism (G/A) in the 3′ untranslated region (UTR) of the transcription factor LBP-1c/CP2/LSF (for brevity, CP2) has been implicated in Alzheimer's disease susceptibility, with the 3′-UTR A allele being associated with a reduction in the risk of sporadic Alzheimer's disease.[1–3] The CP2 gene is a plausible candidate for influencing Alzheimer's disease risk: it is located near the LDL receptor related protein gene within the Alzheimer's disease linkage region on chromosome 12; it controls the expression of several genes (α2 macroglobulin, glycogen synthase kinase-3β); and it interacts with different proteins (serum amyloid A3, interleukin 1α, tumour necrosis factor α, and Fe65 protein) and viruses (herpes simplex virus type I or human immunodeficiency virus) that are probably linked to Alzheimer's disease pathogenesis.[1 4] In the present study, we investigated the potential association of the CP2 polymorphism in a sample of sporadic early onset and late onset cases along with age and sex matched control subjects from southern Italy.

The Alzheimer's disease group consisted of 166 patients (62 men and 104 women) from the Apulia region with a mean (SD) actual age of 69.4 (10.3) years, including 95 patients with sporadic late onset disease (age at onset ⩾70 years; mean age 78.1 (4.9) years; 64 women and 31 men), and 71 patients with sporadic early onset disease (age at onset <70 years; mean age 63.7 (4.3) years; 50 women and 21 men). A clinical diagnosis of probable Alzheimer's disease was made according to the NINCDS/ADRDA criteria.[5] The age at

Table 1 Genotype and allele frequencies of LBP-1c/CP2/LSF gene 3′ UTR polymorphism in patients with Alzheimer's disease and non-demented age and sex matched controls

	Age at onset and at collection (SD) (years)	Genotype, n (frequency (95% CI))			Allele, n (frequency (95% CI))	
		GG	GA	AA	G	A
All cases of Alzheimer's disease (n = 166)	67.6 (10.8)	147 (0.89 (0.93 to 0.84))	19 (0.11 (0.16 to 0.07))	0 (0.0 (0.0–))	313 (0.94 (0.97 to 0.92))	19 (0.06 (0.08 to 0.03))
All controls (n = 225)	71.3 (11.4)	216 (0.96 (0.99 to 0.93))	9 (0.04 (0.07 to 0.01))	0 (0.0 (0.0–))	441 (0.98 (0.99 to 0.97))	9 (0.02 (0.03 to 0.01))
LOAD (⩾70 years) (n = 95)	76.1 (4.4)	86 (0.91 (0.95 to 0.83))	9 (0.9 (0.17 to 0.05))	0 (0.0 (0.39 to 0.0))	181 (0.95 (0.98 to 0.91))	9 (0.05 (0.09 to 0.03))
Controls ⩾70 years (n = 193)	77.8 (5.2)	184 (0.95 (0.98 to 0.91))	9 (0.05 (0.09 to 0.03))	0 (0.0 (0.02 to 0.0))	377 (0.98 (0.99 to 0.96))	9 (0.02 (0.04 to 0.01))
EOAD (<70 years) (n = 71)	61.5 (4.5)	61 (0.86 (0.92 to 0.76))	10 (0.14 (0.24 to 0.08))	0 (0.0 (0.05 to 0.0))	132 (0.93 (0.96 to 0.88))	10 (0.07 (0.13 to 0.04))
Controls <70 years (n = 32)	64.1 (4.3)	32 (1.0 (1.00 to 0.89))	0 (0.0 (0.11 to 0.0))	0 (0.0 (0.11 to 0.0))	64 (1.0 (1.00 to 0.94))	0 (0.0 (0.06 to 0.0))

CI, confidence interval; EAOD, early onset Alzheimer's disease; LOAD, late onset Alzheimer's disease; n, number of individuals genotyped.

onset of Alzheimer's disease symptoms was estimated by semistructured interviews with the patients' caregivers.

The non-demented age, sex, and ethnically matched control group comprised 225 unrelated caregivers (72 men and 153 women), spouses, friends, neighbours, or volunteers, consecutively examined between June 1998 and October 2002 in our centre. Their mean age at the time of the study was 71.3 (10.4) years. The healthy subjects included 193 individuals of ⩾70 years of age (130 women and 63 men) and 32 of <70 years (23 women and nine men).

The ascertainment, diagnosis, and collection of cases and controls has been described in detail elsewhere.[6] The study protocol was approved by the ethics committee of the University of Bari. Informed written consent was obtained from all subjects or their relatives before blood samples were collected. Genomic DNA was extracted from peripheral blood samples using Cod 1796828 (Roche Diagnostics kit). APOE genotypes were determined as previously described.[7] CP2 polymorphism was analysed on a Lightcycler system using specifically designed hybridisation probes (sensor probe: 5′-GCGTTTCATGCCCAGTGGC-fluorescein; anchor probe: red 640–GCTCC TTCCTTCACCTCTGAAAACGG-phosphate;TIB Molbiol). Polymerase chain reaction (PCR) amplification was undertaken using 200 ng of genomic DNA, 50 pmol each primer (5′-GACAGAATTTCGCTCTTGTTGCC-3′, reverse primer; 5′-TCAGGTTCTTGCAGACCTTCAA-3′ forward primer), 3 pmol each probe, 2.5 mM $MgCl_2$, 1×DNA master hybridisation probes (Roche Diagnostics). The amplification conditions were 95℃ for two minutes, and 38 cycles of 94℃ for five seconds, 58℃ for 15 seconds, and 72℃ for 10 seconds. After amplification, the temperature was raised to 94℃ for 30 seconds, lowered to 40℃ at 20℃/s of temperature transition rate, and held at 40℃ for one minute. A melting curve analysis profile was obtained by raising the temperature to 80℃ at 0.05℃/s while collecting fluorescence data continuously. The melting temperatures were 60℃ for the 3′-UTR G allele and 66℃ for the 3′-UTR A allele.

Statistical analysis was done using Pearson χ^2 tests to make genotype and allele comparisons, and a test for data agreement using Hardy-Weinberg principles. Allele frequencies were determined by allele counting. To express variances of allele and genotype frequencies, we used 95% confidence intervals (CI), calculated by Wilson's formulae. Differences among age at onset of Alzheimer's disease symptoms in relation to different CP2 genotypes were calculated using the Mann–Whitney test. To evaluate whether the association between Alzheimer's disease and CP2 genotypes was homogeneous in all ApoE strata we used a permutation based exact logistic model by LogXact procedure implemented in the SAS system. (Proc-LogXact 5 by CYTEL Software Corporation, Cambridge, Massachusetts, USA). The odds ratios and the 95% CI between Alzheimer subjects with and without at least one A or G allele were calculated. In most cases χ^2 (by SAS FREQ procedure, version 8.2) or z tests were calculated by asymptotic p values, while exact p values (by Proc-StatXact version 5.0) were used when the data in comparisons were smallest. Any statistics were calculated for the AA genotype, because they cannot be computed when the number of non-empty rows or columns in 2×2 contingency tables is 1. The threshold of significance was set at $p<0.05$.

Results

The CP2 genotype and allele frequencies in the whole Alzheimer's disease sample and age and sex matched non-demented controls are shown in table 1. The genotype distributions were in Hardy-Weinberg equilibrium in both Alzheimer's disease and control subjects (cases: Pearson $\chi^2 = 0.61$, $p = 0.43$; controls: $\chi^2 = 0.09$, $p = 0.761$). Statistically significant differences were found in CP2 genotype frequencies between cases and controls (GG *v* GA and AA, and GA *v* GG and AA: Pearson $\chi^2 = 7.97$, Bonferroni $p<0.05$, $df = 1$). A statistically significant increase in A allele frequency was found in the Alzheimer's disease sample compared with the controls (Pearson $\chi^2 = 7.670$, $p = 0.006$). In particular, the presence of the A allele was associated with Alzheimer's disease with an odds ratio of 2.97 (95%CI, 6.66 to 1.33). When we subdivided the whole Alzheimer's disease sample into early onset and late onset groups, no statistically significant differences were found in CP2 genotype frequencies between the Alzheimer patients and the controls, while the A allele showed a statistically significant increase only in the Alzheimer patients who were less than 70 years old (Pearson $\chi^2 = 4.740$, exact $p = 0.03$). Furthermore, the Alzheimer patients bearing the A allele had a mean age of onset lower than those carrying the G allele (mean age at onset: A allele, 64.8 (12.2) years; G allele, 68.0 (9.4) years), although this difference was not statistically significant ($z = 0.9$, $p>0.05$). We did not find any significant differences in rates between CP2 alleles and Alzheimer's disease among ApoE allele strata.

Comment

The major finding of the present study is that the A allele of the 3′-UTR CP2 gene polymorphism increases the risk of sporadic Alzheimer's disease (OR = 2.97), without interaction with ApoE alleles. After stratification for age at onset, this effect was statistically significant only in patients with early onset disease (<70 years), whereas in late onset disease (⩾70 years) there was a difference in the A allele frequency between affected subjects and controls (though this did not reach statistical significance). Lambert *et al* reported an association between the CP2 polymorphism and sporadic Alzheimer's disease in French and British populations, and a similar trend in a north American population.[1] The combined analysis of the three independent populations suggested a protective effect of the A allele (OR = 0.58), that decreased with age (OR = 0.43 before 70 years; OR = 0.52 between 70 and 80 years; OR = 0.83 after 80 years). More recently, Taylor and colleagues found similar results, detecting a significant protective effect of the A allele (OR = 0.59) in 216 neuropathologically confirmed patients with late onset disease and 301 controls from the United Kingdom.[2] Finally, Luedecking-Zimmer *et al* found that the frequency of the A allele was higher in controls than in cases (0.07 *v* 0.05), suggesting a moderate protective effect of the CP2 polymorphism against the risk of Alzheimer's disease (OR = 0.65).[3]

To the best of our knowledge, this is the first report suggesting a risk of Alzheimer's disease linked to the CP2 A allele, and the contrasting results of our study are, at present, difficult to explain. However, Lambert *et al* did not observe a significant protective effect of the A allele in the US population,[1] and we recently provided a novel finding that the ApoE ε4 allele frequency decreases according to a geographic trend from northern to southern Europe.[7] We hypothesise that the variability in the association between the A allele and Alzheimer's disease can be related to ethnic and geographical variations: from 0.09 to 0.07 of A allele frequency in healthy controls from the UK, France, and north America, to only 0.02 in southern Italy.[1 2] It is also possible that a moderate effect associated with the CP2 polymorphism is caused by its non-random association with a functional mutation present somewhere in the gene. Finally, it is possible that there is linkage disequilibrium with another biologically relevant locus on chromosome 12. The possible role of the A allele as a risk factor for sporadic Alzheimer's disease is supported by the lower mean age at onset of Alzheimer's disease in patients with the A allele than those carrying the G allele, though this difference was not significant. We found no interaction between CP2 polymorphism and ApoE alleles in relation to Alzheimer's disease risk, and this finding is consistent with previous results.[1 2]

In conclusion, our data support CP2 as a candidate gene for sporadic Alzheimer's disease, suggesting further studies on larger, ethnically and geographically different populations to clarify the role of this gene in Alzheimer's disease pathogenesis.

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Vestibular stimulation in mania: a case report

Caloric vestibular stimulation is a common clinical procedure, routinely employed during testing of vestibulocochlear nerve function. The procedure involves stimulation of vestibular afferents by the application of cooled water to the tympanic membrane. Vestibular afferents are distributed widely to areas of the diencephalon and cortex, including areas believed to be involved in the regulation of mood. In accordance with these observations, imaging studies have shown widespread though largely contralateral hemispheric activation following the procedure.[1]

Caloric vestibular stimulation has been associated with a rapid but short lived improvement in stroke induced functional deficits,[2] but the effect of the procedure on psychiatric symptomatology has not been reported. In the case described here, an improvement in manic symptoms was observed after caloric vestibular stimulation in a 29 year old woman with a 10 year history of bipolar affective disorder. The patient was admitted to an acute psychiatric ward with several weeks of increasingly elevated and irritable mood. Her symptoms fulfilled DSM-IV criteria for a manic episode. Resistance to therapeutic drug use and intolerance of side effects had limited effective management of her condition. Previous episodes of mania had often responded to ECT. At the time of admission her treatment regimen included olanzapine and carbimazole. Carbimazole had been started following the identification of abnormal thyroid function tests on routine testing.

The patient did not respond to increases in antipsychotic drugs or to a course of right unilateral ECT given three times a week. She withdrew consent for ECT when no improvement was noted after five treatments. At this point, a review of published reports suggested that left caloric vestibular stimulation might reduce the severity of the manic symptoms through modulation of mood related neural circuits. A trial was proposed and informed consent obtained. The severity of the patient's manic symptoms was measured using the Young mania rating scale (YMRS).[3] The severity of her symptoms before caloric vestibular stimulation was felt by staff to represent her general level of symptoms during the past two months.

Otological examination before the caloric stimulation revealed an intact tympanic membrane and a clear external auditory canal. A flexible tube (14 gauge) was attached to a 50 ml syringe and introduced into the left auditory canal to a depth of 2 cm; 50 ml of cold water (4℃) were then introduced into the canal over a period of two to three minutes. Run off was collected in a kidney dish. The procedure was repeated after 72 hours.

The YMRS was applied by nursing staff involved in the patient's care at the following times: before vestibular stimulation, and at 10 minutes, 20 minutes, 60 minutes, 6 hours, 24 hours, and 48 hours after the procedure.

The procedure was well tolerated; the patient described minimal local discomfort and a sense of vertigo. Horizontal nystagmus occurred towards the right. Within two minutes of termination of the procedure the patient described a slowing of thoughts and speech and a lowered mood. She remained on the examination couch until all sensation of vertigo had passed (approximately 10 minutes). During this period she was calm, cooperative, and appropriate in behaviour. There was an obvious reduction in speed and volume of speech and a reduction in spontaneous laughter and movement. These observations corresponded to a reduction in YMRS score of 32 (pre-stimulation) to 10 (post-stimulation).

Upon returning to the ward, she remained appropriate in her behaviour and interactions with staff and other patients. The patient described a lasting lowering of mood and slowing of thoughts and quickly became embarrassed when reminded of some of the behaviours she had shown before stimulation. Staff noted a gradual increase in her manic symptoms from approximately 24 hours post-stimulation, and after 72 hours her YMRS score was similar to that observed before the procedure (fig 1). The vestibular stimulation was readministered, and a dramatic and sustained partial reduction in symptoms again occurred, followed by a slow return towards baseline.

Comment

This case describes an impressive and relatively sustained improvement in manic symptoms following left caloric vestibular stimulation. It is possible that the power of suggestion, or a "placebo" effect, contributed to the observed effect. Care was taken not to relay to the patient a sense of expectation of an improvement in mood, and extra contact with staff following the procedure was minimised. It is unlikely that the immediate improvement in symptoms reflected a change in behaviour secondary to adverse effects of the procedure. Vertigo was the only side effect experienced by the patient, and all sense of vertigo had resolved within 10 minutes of the procedure. The use of the YMRS provided a standard for comparison of the severity of her symptoms before and after stimulation and served to illustrate a marked reduction in manic symptoms.

Caloric vestibular stimulation represents a novel approach to the treatment of mania. It is possible that it exerts its effect on mood through stimulation of mood related neural circuits. Following caloric vestibular stimulation, functional magnetic resonance imaging shows widespread, mainly contralateral activation of diencephalic and cortical regions which include the basal ganglia, insula,

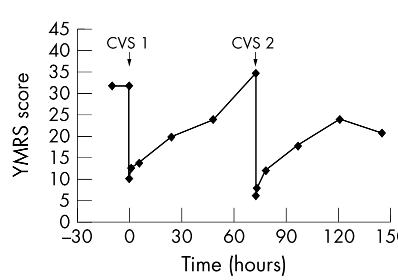

Figure 1 Severity of manic symptoms, represented by score on the Young mania rating scale (YMRS) as a function of time. Left caloric vestibular stimulation was given at 0 hours and again at 72 hours.

cingulate gyrus, prefrontal, and parieto-temporal areas.[1] These areas have also been implicated in disorders of mood, and some laterality of mood is suggested by neuroimaging and lesion studies that link depression to left cerebral impairment and mania to right cerebral impairment.[4 5] Thus impulses transmitted by vestibular afferents in response to caloric vestibular stimulation may reach previously underactive neural pathways, so restoring a balance to previously imbalanced mood circuits.

Transient resolution of stroke induced deficits has been documented following caloric vestibular stimulation.[2] These effects have lasted only minutes, and patients who have responded to the procedure have shown a reduced response to subsequent stimulations. The sustained response observed in this case may have been because neuronal hypofunctioning was present in the absence of overt neuronal damage as occurs following stroke.

This case report, which requires replication, describes a sustained reduction in manic symptoms following left caloric vestibular stimulation; this may have occurred through the activation of previously hypofunctioning neural circuits. Whether the observed improvement in symptoms corresponds to a normalisation of cerebral perfusion, as illustrated by positron emission tomography and functional magnetic resonance imaging, remains to be seen. Further research in the area may yield an alternative treatment for mood disorders, and provide an avenue for clarification of the neural pathways involved in the regulation of mood.

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Hand weakness onset Guillain–Barré syndrome

Landry's 19th century report gives the impression that Guillain–Barré syndrome (GBS) is characterised by ascending weakness. This clinical picture now is called "Landry's ascending paralysis."[1] Indeed, muscular weakness in GBS does usually begin in the legs, progressing to the trunk, arms, and cranial regions.[2] However, several clinical variants are now recognised in which weakness initially begins in other areas.[2] Four patients with acute polyneuropathy were reported initially to have had muscle weakness in the hands.[3] In two of these, *Campylobacter jejuni* infection had preceded the neurological symptoms, and serum anti-GM1 antibody was detected in the others.

Table 1 Comparison of clinical and serological features

	Initial symptoms: Hand weakness (n = 33)	Initial symptoms: Others (n = 431)	p Value	OR	95% CI
Age (median, (range))	43 (14 to 78)	44 (0 to 88)	NS		
Sex (M/F)	22/11	262/169	NS		
Preceding symptoms:					
Diarrhoea	19 (58%)	151 (35%)	$<$0.001	2.5	1.0 to 6.3
Upper respiratory tract infection	11 (33%)	174 (40%)	NS		
Cranial nerve involvement	9 (27%)	156 (36%)	NS		
Neck weakness	14 (42%)	221 (51%)	NS		
Sensory deficit	10 (30%)	211 (49%)	0.03	0.27	0.1 to 0.6
IgG antibodies against:					
All gangliosides tested	24 (73%)	182 (42%)	0.03	2.3	1.1 to 5.1
GM1	20 (61%)	106 (25%)	$<$0.001	3.4	1.4 to 3.8
GM1b	15 (45%)	111 (26%)	0.01	2.4	1.4 to 4.8
GM2	1 (3%)	2 (0.4%)	NS		
GD1a	10 (30%)	64 (15%)	0.01	2.5	1.1 to 4.7
GalNAc-GD1a	6 (18%)	37 (9%)	NS		
GD1b	9 (27%)	75 (17%)	NS		
GT1a	3 (9%)	41 (10%)	NS		
GT1b	1 (3%)	14 (3%)	NS		
GQ1b	1 (3%)	31 (7%)	NS		

Differences between groups were examined with the χ^2 or Fisher's exact test.
CI, confidence interval; F, female; M, male; NS, not significant (p value $>$0.05); OR, odds ratio.

To determine the frequency and clinical features of hand onset GBS, we reviewed the medical records of 464 consecutive patients with the disease. Eleven had been treated at our hospital, the others were referred to our laboratory from other hospitals for antiganglioside antibody tests. Hand onset GBS was diagnosed when the first symptom that a GBS patient recognised was hand weakness. Paraesthesiae and other sensory symptoms may have preceded hand weakness, but patients who developed weakness in both the hands and legs on the first day of illness were excluded.

We found that 33 (7%) of the patients reviewed had hand onset GBS. Frequent initial symptoms were weak hand grip and clumsy fingers. Paraesthesiae in the hands or all four limbs had preceded hand weakness in eight of them. Three patients presented with facial palsy, diplopia, or blurred vision on the day of hand weakness onset. Weakness was limited to the hands and arms throughout the acute phase of illness in four patients (12%), while it spread to the legs in the others. Assisted ventilation was required for four patients (12%). Compared with the other patients, those with hand onset GBS more often had a history of preceding diarrhoea, had antiganglioside IgG antibodies, and, less frequently, had sensory disturbance (table 1). Of the autoantibodies present, anti-GM1, anti-GM1b, and anti-GD1a IgG were significantly associated with hand onset GBS. Antecedent *C jejuni* infection was proven by serological assay (n = 19) or stool culture (n = 2) in 20 (61%) of the patients with hand onset GBS.

Hand onset GBS patients (n = 25) who had antiganglioside IgG, evidence of preceding *C jejuni* infection, or both often had a history of previous gastrointestinal symptoms but rarely cranial nerve involvement or sensory disturbance. Although most patients had received plasmapheresis or intravenous immunoglobulin within two weeks of onset, seven showed irreversible neurological damage at the last consultation. Moderate or mild weakness remained in the arms and legs of four patients (from four to 22 months after onset), and weakness mainly remained in the upper limbs of three (from two to four months after onset). Results of nerve conduction studies done within two weeks of weakness onset were available for 10 patients: five had predominant axonal disturbance and the others showed unclassified findings. In contrast, the eight patients who had neither antiganglioside IgG nor evidence of a preceding *C jejuni* infection often had a history of previous respiratory infection and sensory disturbance. All had received plasmapheresis or intravenous immunoglobulin and, except for one, had no or only mild weakness 12 months after onset. The exception required assisted ventilation at nadir and still had moderate distal weakness and amyotrophy in the legs six months after onset. Results of nerve conduction studies were available for four patients who had neither antiganglioside IgG nor evidence of a preceding *C jejuni* infection; all of these had a primary demyelinating disturbance.

In the larger population, we confirmed previous findings that hand onset GBS is related to *C jejuni* enteritis and anti-GM1 antibody,[3] although the cases reported had either *C jejuni* or anti-GM1 antibody. Furthermore, we found that hand onset GBS is characterised by pure motor symptoms and the presence of IgG antibodies against GM1b and GD1a, as well as those against GM1. Residual symptoms were frequent in the *C jejuni* or autoantibody related populations, but no statistical analysis was made of outcome. In contrast, one quarter of the patients with hand onset GBS had no evidence of previous *C jejuni* infection or antiganglioside IgG. Antecedent respiratory infection symptoms and sensory involvement were characteristic, and those patients tended to have better outcomes than the others.

It is noteworthy that motor deficit remained only in the arms during the course of the illness in the four hand onset GBS patients, two of whom were positive for *C jejuni* serology and antiganglioside IgG. Another patient who developed acute pure motor neuropathy following *C jejuni* enteritis was reported to have localised weakness in his hands and anti-GM1 IgG.[4] Although that patient had preserved tendon reflexes in the four limbs, a serial electrophysiological study confirmed the diagnosis of an axonal variant of GBS, indicating that anti-GM1 IgG and *C jejuni* infection are related to hand-predominant weakness in GBS. It also is noteworthy that the six patients who had hand onset GBS had an initial diagnosis of cervical spondylosis (n = 4), lacunae infarction (n = 1), or brachial plexus neuritis (n = 1) on hospital admission. Frequent hand function problems have been reported even in mildly affected GBS patients who could walk unaided at nadir.[5] Early treatment has been suggested in such cases. Recognition of the clinical characteristics of hand onset GBS may lead to a good prognosis because individuals can be given specific treatment as early as possible.

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Lack of association between interleukin-1β polymorphism (–511) and ischaemic stroke

A growing body of evidence suggests an important role for interleukin 1 (IL-1) in the pathogenesis of brain damage following cerebral ischaemia. Central administration of IL-1 exacerbates brain damage, and over-expression of the IL-1 receptor antagonist (IL-1Ra) or blockade of IL-1 converting enzyme activity reduces infarct size dramatically (reviewed by Touzani *et al*[1]). Clinical studies suggest there is intrathecal IL-1 production early during stroke.[2]

Table 1 Distribution of risk factors and IL-1β genotypes in patients and controls

	Stroke patients (n = 183)	Controls (n = 180)	p Value
Age (years) (mean (SD))	65.2 (14.7)	64.8 (14.8)	0.80
Male	81 (44.3)	69 (38.3)	0.25
Hypertension	139 (76)	94 (52.2)	$<$0.01
Diabetes mellitus	33 (18)	32(17.8)	0.59
History of myocardial infarction	15 (8.21)	9 (5)	0.22
Current smoker	47 (25.7)	21 (11.7)	$<$0.01
IL-1β genotypes			0.37
CC	94 (51.4)	87 (48.3)	
CT	69 (37.7)	79 (43.9)	
TT	20 (10.9)	14 (7.8)	
T allele frequency (%)	29.8	29.7	0.97

Values are n (%) unless stated otherwise.

A single nucleotide polymorphism in the promoter region of IL-1β at position −511 resulting in C–T transition influences the protein production, and IL-1β-511T carriers are reported to be higher producers of IL-1β than IL-1β-511C carriers.[3]

In the study described here we investigated whether IL-1β polymorphism (−511) can be involved in the genetic susceptibility to ischaemic stroke. We studied 183 consecutive patients with ischaemic stroke presenting to our stroke unit and 180 control subjects without a history of stroke. Control subjects were recruited from spouses of the patients, from individuals admitted to the university hospital for any reason other than neurological diseases, and from persons randomly selected from the community of our town. All patients, controls, and their parents had to be of white extraction.

Cerebral infarction was defined as a focal neurological deficit of sudden onset that persisted beyond 24 hours, documented by brain computed tomography or magnetic resonance imaging, indicating the presence of infarction or the absence of haemorrhage.

Stroke aetiology was defined according to the TOAST criteria[4]: 66 patients had large vessel disease, 50 had small vessel disease, 49 had cardioembolic stroke, and 18 had stroke of undetermined aetiology.

Arterial hypertension was diagnosed when its presence was documented in the medical records or if two or more readings of blood pressure were ⩾160 mm Hg (systolic) or ⩾95 mm Hg (diastolic) before the onset of stroke or three months later. Diabetes mellitus was diagnosed if the patient gave a history of diabetes that was confirmed by their medical records or was taking insulin or an oral hypoglycaemic agent. A patient was defined as a current smoker if there was a history of cigarette smoking during the last five years.

Genomic DNA was extracted from peripheral blood using a commercially available kit from Qiagen. Interleukin-1β polymorphism (−511) was detected using the polymerase chain reaction and restriction enzyme digestion as described elsewhere.[5]

All subjects gave informed consent and the local ethics committee approved the study protocol.

The sample size was calculated with a power of 80% at the 0.05 significance level. The sample size would allow detection of a relative risk by allele of 2.2. Differences between groups were examined using the χ^2 test or the unpaired Student *t* test as appropriate. Probability (p) values of less than 0.05 were considered statistically significant.

The characteristics of study subjects and distribution of IL-1 genotype are shown in table 1.

There was no significant difference between stroke patients and controls in age and sex.

Allele frequency in both controls and patients was in Hardy-Weinberg equilibrium (p = 0.32 for controls, p = 0.40 for stroke patients).

There was no significant difference between stroke patients and controls in IL-1β genotype distribution. There was also no relation between IL-1β polymorphism and any particular stroke subtype: large vessel disease, for TT, 7/66 (10.6%); small vessel disease, 6/50 (12.0%); cardioembolic stroke, 7/49 (14.3%) (p = 0.24, χ^2 test).

Comment

We failed to find a relation between IL-1β polymorphism (−511) and ischaemic stroke in this Polish population. Recently Seripa *et al* investigated the same polymorphism in an Italian population of 110 stroke survivors and 101 healthy controls[6] and also did not find any significant association between IL-1β polymorphism (−511) and stroke, although they showed a significantly higher frequency of the IL-1Ra 1/1 genotype in stroke survivors than in controls.

Several issues should be taken in account in interpreting the results of our study.

First, cytokines do not work alone, but in a network. Therefore a genetic predisposition to produce anti-inflammatory cytokines (for example, IL-10 or IL-1Ra) could interfere with the biological effects of IL-1.

Second, we did not examine another IL-1β polymorphism in exon 5 at position +3953 which could determine IL-1β synthesis.

Third, we cannot exclude the possibility that IL-1β polymorphism (−511) is associated with one particular stroke subtype; however, in our study we found no relation between IL-1 polymorphism and large vessel disease, small vessel disease, or cardioembolic stroke. From our point of view, there is currently a lack of strong evidence indicating a functional association between IL-1 and any particular stroke subtype.

Fourth, IL-1 may be linked to inflammatory mechanisms of atherogenesis. Hypertension and smoking play an important role in the pathogenesis of atherosclerosis. In our study the incidence of hypertension and smoking was higher in stroke patients than in controls, and the frequency of the TT allele was higher in smokers than in non-smokers (15.4% *v* 7.9%, p = 0.18) and in subjects with hypertension than in those without (10.3% *v* 7.7%, p = 0.48). Atherosclerosis is related to large vessel disease; however, in our study the IL-1 genotype distribution did not differ significantly between patients with large vessel disease and controls.

Fifth, further studies are needed that are focused on achieving sufficient power to detect a possible smaller allelic relative risk (<2).

In conclusion, our results do not support the hypothesis that IL-1β polymorphism (−511) is associated with ischaemic stroke.

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