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 comorbidity 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°C) 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,

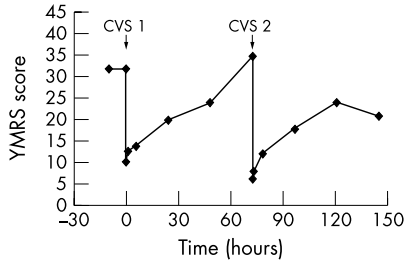

Figure 1 Severity of manic symptoms, represented by score on the Young mania rating scale (YMRS) as a function of time. Left caloric vestibular stimulation was given at 0 hours and again at 72 hours.