

Although the ROSA26 promoter has been shown to have activity on its own and might lead to broad gene expression in transgenic mice⁸, the high rate of homologous recombination and generalized *lacZ* expression observed here suggest that targeting of genes to the ROSA 26 locus may be a desirable method to achieve ubiquitous expression during development or in the adult.

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Philippe Soriano

Program in Developmental Biology, Division of Basic Sciences, Fred Hutchinson Cancer Research Center, 1100 Fairview Avenue North, Seattle, Washington 98109, USA. e-mail: psoriano@fhcrc.org

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Variation in *DCP1*, encoding ACE, is associated with susceptibility to Alzheimer disease

The $\epsilon 4$ allele of the gene encoding apolipoprotein E (*APOE*) is the only well-replicated genetic risk factor for non-autosomal dominant forms of Alzheimer disease¹ (AD). However, the *APOE* genotype is estimated to account for less than one-half of the genetic variance². Following reports that the *DCP1***D* allele of a common insertion (*I*)/deletion (*D*) polymorphism in the gene encoding angiotensin converting enzyme³ (dipeptidyl carboxypeptidase 1; *DCP1*) is associated with increased longevity⁴, we

hypothesized that *DCP1***D* might protect against the development of AD and that, similarly, the *DCP1***I* allele may confer increased risk. We tested this hypothesis in a case-control sample from Cardiff and independent replication samples from London and Belfast (Table 1).

Our analysis of the Cardiff data showed a significant association between AD and *DCP1* genotypes ($\chi^2=8.98$, d.f.=2, $P=0.011$) and alleles ($\chi^2=5.34$, d.f.=1, $P=0.02$). The variance in genotypic distribution appeared attributable to an excess

of *DCP1***I*/*I* and *DCP1***I*/*D* genotypes in AD cases ($P=0.006$; odds ratio (OR)=2.43, 95% CI=1.35–4.39, after Bonferroni correction for multiple testing in the present study; $P=0.046$ after correction for 8 genes studied previously in this sample). We subsequently tested this finding in the London and Belfast samples and found similar significant associations (London, $P=0.0008$, OR=2.71, 95% CI=1.5–4.9; Belfast, $P=0.017$, OR=1.82, 95% CI=1.11–2.98).

We combined the samples in a stratified analysis⁵, and found that AD cases had a highly significant excess of *DCP1***I*/*I* and *DCP1***I*/*D* genotypes ($n=542$) compared with controls ($n=386$; $\chi^2=24.22$, d.f.=1, $P=0.000001$). This yielded an OR of 2.22 (95% CI=1.62–3.1) with no evidence of heterogeneity between samples ($P=0.6$).

We then used logistic regression to test for the presence of the *DCP1* association after accounting for the effects of *APOE* (Table 2). After we stratified the total data set for *APOE*, the case-control differences remained highly significant and confirmed that a dominant model provides a best fit for the data (*DCP1***I*/*I* and *DCP1***I*/*D* versus *DCP1***D*/*D*, $\chi^2=16.10$, d.f.=1, $P=0.00006$). We observed no evidence of an interaction between alleles of *DCP1* and *APOE* ($P=0.89$). In addition, there was no evidence of association with age of onset, gender or family history (data not shown).

These findings suggest that genetic variation at the *DCP1* locus predisposes to AD in a manner that is independent of *APOE* variation. They might also help explain the unexpected association of *DCP1***D*/*D* with longevity⁴. However, we must consider the possibility that the low frequency of the *DCP1***D*/*D* genotype in AD may have been due to the exclusion of cases with cardiovascular disease, for which some evidence exists of an association with the *DCP1***D*/*D* genotype⁶. This appears unlikely for a number of reasons. First, the impact of the *DCP1***D*/*D* genotype on cardiovascular disease is controversial, relatively small and restricted to specific geographical areas and to patient subgroups with highly heterogeneous clinical mani-

Table 1 • *DCP1* genotype and allele distributions

	Genotypes			Alleles		OR _{I,I/ID/DD} (95% CI)
	II	ID	DD	I	D	
Cardiff cases ^a (n=198)	41 (0.21)	121 (0.61)	36 (0.18)	203 (0.51)	193 (0.49)	2.43 (1.4–4.4)
Cardiff controls (n=77)	12 (0.16)	38 (0.49)	27 (0.35)	62 (0.40)	92 (0.60)	
London cases ^b (n=135)	23 (0.17)	88 (0.65)	24 (0.18)	134 (0.50)	136 (0.50)	2.71 (1.5–4.9)
London controls (n=111)	22 (0.20)	48 (0.43)	41 (0.37)	92 (0.41)	130 (0.59)	
Belfast cases (n=209)	63 (0.30)	114 (0.55)	32 (0.15)	240 (0.57)	178 (0.43)	1.82 (1.1–3)
Belfast controls (n=198)	55 (0.28)	94 (0.47)	49 (0.25)	204 (0.51)	192 (0.49)	
Total cases ^c (n=542)	127 (0.23)	323 (0.60)	92 (0.17)	577 (0.53)	507 (0.47)	2.22 (1.6–3.1)
Total controls (n=386)	89 (0.23)	180 (0.47)	117 (0.30)	358 (0.46)	414 (0.54)	

I, *DCP1***I*; D, *DCP1***D*; II, *DCP1***I*/*I*; DD, *DCP1***D*/*D*; ID, *DCP1***I*/*D*; n, total number of individuals genotyped; CI, confidence interval. The ascertainment, diagnosis and collection of case and control groups for the three centres are detailed elsewhere^{9–11}. Mean age of onset for the cases from Cardiff, London and Belfast were 70.25 y (s.d.±9.35), 82.3 y (s.d.±6.7) and 76.60 y (s.d.±6.26), respectively. Mean age of collection for the control samples were 73.46 y (s.d.±6.2), 80.8 y (s.d.±4.5) and 77.09 y (s.d.±6.42), respectively. Cases and controls in Cardiff and London were of UK origin, whereas patients and controls for the Belfast sample consisted of individuals with parents and grandparents born in Northern Ireland. All controls had a minimum mini-mental state examination score¹² of 25. *APOE* genotypes were produced as described¹³. *DCP1* genotypes were produced using established methods¹⁴ followed by a quality control amplification step necessary in detecting under-amplified *DCP1***I* alleles¹⁵. ^{a,b,c}Significant deviation from Hardy-Weinberg equilibrium (HWE; $P_a=0.002$; $P_b=0.0004$; $P_c=0.000005$), as would be expected with a genotypic association with disease. Genotypes for control groups were all within HWE (Cardiff, $P=0.78$; London, $P=0.26$; Belfast, $P=0.5$; total controls, $P=0.47$). Odds ratios for each centre and for the total samples were calculated on the risk conferred by the presence of one or more copies of the *DCP1***I* allele. Standard χ^2 test was used to test for genotypic and allelic association and logistic regression to test for *DCP1* and *APOE* interactions. Combined analysis of data from the two centres used Woolf's method⁵.

Table 2 • DCP1 and APOE genotype data

DCP1	—/—	APOE	
		—/ε4	ε4/ε4
Total cases (n=542)			
II	59 (58.6)	53 (53.6)	15 (12.5)
ID	151 (152.8)	144 (139.8)	28 (32.5)
DD	47 (43.3)	34 (39.6)	11 (9.2)
Total controls (n=386)			
II	71 (69.2)	17 (17.8)	1 (2.3)
ID	140 (141.5)	37 (36.3)	3 (3.6)
DD	92 (90.3)	23 (23.2)	2 (2.3)

Compound genotypes for DCP1 and APOE in cases and controls. The values expected, assuming independence between DCP1 and APOE genotypes, are shown in parentheses. II, DCP1*II*; DD, DCP1*D/*D; ID, DCP1*I/*D.

festations⁶. Second, cases with vascular symptoms were only excluded from our patient groups if they had histories of obvious stepwise cognitive deterioration consistent with vascular dementia. Third, vascular dementia cases were also excluded from our screened age-matched control groups. Fourth, our control allele and genotype frequencies were similar to those reported for the general population by a number of studies, including one from a very similar geographical location⁶. Finally, analysis of DCP1 genotypes in additional vascular dementia cases (n=15), and in those dementia cases with a history of stroke (n=21) excluded from the London sample, showed an excess of the DCP1*I allele (vascular, 0.50; other excluded cases, 0.57) rather than an excess of the DCP1*D/*D genotype.

Given that our findings have been replicated in two independent case-control samples and the high level of statistical sig-

nificance, these results are unlikely to be false positives arising from ethnic stratification or multiple testing.

The mechanisms by which DCP1 influences susceptibility to AD is unknown, however, several lines of evidence suggest that ACE may have a role in AD by modulating inflammation⁷ and implicate the brain renin-angiotensin system in cognitive processes⁸. Whatever the mechanism, and in spite of an OR of little more than 2, the presence of the DCP1*I allele in over 70% of the elderly population warrants further study of this gene and its role as a significant risk factor for AD.

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Patrick G. Kehoe¹, Carsten Russ², Stephen McIlroy³, Hywel Williams¹, Peter Holmans¹, Clive Holmes², Danae Liolitsa², Djamil Vahidass³, John Powell², Bronagh McGleenon³, Malcolm Liddell¹, Robert Plomin², Kevin Dynan³, Nigel Williams¹, Jim Neal⁴, Nigel J. Cairns⁵, Gordon Wilcock⁶, Peter Passmore³, Simon Lovestone², Julie Williams¹ & Michael J. Owen

¹Neuropsychiatric Genetics Unit, Divisions of Psychological Medicine and Medical Genetics, University of Wales College of Medicine, Heath Park, Cardiff, CF4 4XN, Wales, UK. ²Institute of Psychiatry, De Crespigny Park, Denmark Hill, London, SE5 8AF, UK. ³Department of Geriatric Medicine, Whitla Medical Building, The Queen's University Belfast, 97 Lisburn Road, Belfast, BT9 7BL, Northern Ireland.

⁴Department of Pathology, University of Wales College of Medicine, Heath Park, Cardiff, CF4 4XN, UK. ⁵MRC Brain Bank, Institute of Psychiatry, De Crespigny Park, Denmark Hill, London, SE5 8AF, UK. ⁶Department of Care of the Elderly, University of Bristol, Frenchay Hospital, Bristol, BS16 1LE, UK.

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