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## Sensitivity of malaria parasites to nitric oxide at low oxygen tensions

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A role in protection against malaria for nitric oxide (NO) and its derivatives, including peroxynitrite, has been suggested.<sup>1</sup> The difficulty is to reconcile the known antimicrobial activity of NO with the immunologically privileged position of *Plasmodium* inside erythrocytes. Whereas other protozoan parasites free in the blood (*Trypanosoma*) or in macrophages (*Leishmania* and *Toxoplasma*) are accessible to NO, the intimacy of *Plasmodium* with haemoglobin (Hb), a molecule with a high affinity for NO, it has been argued, makes NO-mediated immunity an improbable defence against blood-stage malaria.<sup>2</sup>

The affinity of Hb for NO raises the question of how NO exerts any biological activity. Hb may in fact have a dual role, as both scavenger and donor of NO.<sup>3,4</sup> Binding of O<sub>2</sub> to haem irons in Hb promotes the binding of NO to cysteine $\beta$ 93, forming S-nitrosoHb. Deoxygenation is accompanied by an allosteric transition in this molecule from high to low O<sub>2</sub> affinity, and the consequent release of the NO group. Hence, whether Hb becomes nitrosylated depends on how oxygenated the atmosphere of the microenvironment is. Tight control of the vasorelaxant properties of NO thereby functions to regulate blood flow and to facilitate efficient delivery of O<sub>2</sub> to tissues.

These observations led us to propose that the antimalarial effect of NO may vary with the prevailing O<sub>2</sub> tension: the

lower this is, the more susceptible malaria parasites become. We examined the effects of chemical generators of NO on the growth in vitro of synchronised ring stage *P falciparum* when incubated over a range of physiologically relevant O<sub>2</sub> tensions at 37°C (table). Parasite growth was optimal at 5% O<sub>2</sub>, so results were normalised to compare growth modulation by NO in each gaseous atmosphere (provided by BOC, London, or Oxoid, Basingstoke, Hants, UK). After incubation with NO donors, a drop in proliferation of parasitised erythrocytes was observed, which became consistently more pronounced with reducing concentrations of O<sub>2</sub>. At 1% O<sub>2</sub>, parasite viability was low, as reincubation in the absence of NO caused minimal resumption of growth. When co-cultured with *P falciparum*, J774 macrophages activated to produce 60–90  $\mu$ mol/L NO by 4 h pre-exposure to 400 U/mL interferon- $\gamma$  and 10 ng/mL lipopolysaccharide were largely cytostatic at 5% O<sub>2</sub>, but cytotoxic at 1% O<sub>2</sub>, to the malaria parasites.

Our findings show that intraerythrocytic malaria parasites are sensitive to NO in vitro and that the effect of NO increases in direct proportion to the decrease in O<sub>2</sub> tension. An equilibrium between binding (and therefore scavenging) and unbinding (and therefore donating) NO is reached at a given variable O<sub>2</sub> tension. Under certain conditions in vivo, usually met when erythrocytes are saturated in O<sub>2</sub>, Hb binds to NO with strong affinity. However, with decreasing O<sub>2</sub> tension at arterial-venous transit, Hb readily releases NO.<sup>4</sup> It is therefore in the non-scavenging environment of the capillaries and post-capillary venules that release of NO from erythrocytes would mostly occur. Given the relatively short half life of NO, these vessels are precisely those where the intimate cell to cell contact that their small bore dictates provides an ideal environment for NO, derived from macrophages or endothelial cells, to be a host defence. This dynamic circuit for exchange of NO within the vasculature explains how NO and its derivatives can be proactive against malaria. It also lends support for the hypothesis that NO plays a part in the aetiology of cerebral malaria;<sup>5</sup> in these circumstances excessive localised production of NO leads to immunopathology rather than protection.

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Gaseous atmosphere (%)			Proliferation in absence of NO	p*	Proliferation in presence of NO	p*	Change in proliferation	P†
O <sub>2</sub>	CO <sub>2</sub>	N <sub>2</sub>	cpm (SD) (n=4)		cpm (SD) (n=4)			
20	5	75	5720 (611) (-11.0%)	<0.05	5514 (607) (+65.1%)	<0.001	-3.6%	>0.05
12	5	83	6146 (492) (-4.4%)	>0.05	4243 (412) (+26.9%)	<0.005	-30.8%	<0.003
5	5	90	6428 (573) (0)	—	3341 (354) (0)	—	-48.0%	<0.001
1	9	90	5335 (587) (-17.0%)	<0.01	722 (101) (-78.4%)	<0.0002	-86.5%	<0.0001

Data are for growth of parasites in the absence or presence of a predetermined IC<sub>50</sub> concentration of 85  $\mu$ mol/L of the NO donor spermine NONOate. Cultures were initiated at 0.5% parasitaemia, 1.5% haematocrit. Values in parentheses represent percentage changes from baseline of parasite growth in standard atmospheric conditions of 5% O<sub>2</sub>, 5% CO<sub>2</sub>, and 90% N<sub>2</sub>. \*p-value of Student's t-test used to compare changes from baseline within each treatment group. †p-value of Mann-Whitney test used to compare changes from baseline between treated and untreated groups. Similar results were attained with other NO donors

### Effect of varying O<sub>2</sub> tension on the growth of *P falciparum* in absence or presence of NO

## Parkinsonian signs and mortality from Alzheimer's disease

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Level of cognitive impairment has been associated with mortality in Alzheimer's disease (AD)<sup>1</sup> although few studies have examined risk of death associated with other clinical manifestations. Parkinsonian signs are common in Alzheimer's disease,<sup>2</sup> are associated with cognitive impairment, and are associated with mortality in the population.<sup>3</sup> We prospectively examined whether parkinsonian signs predicted death from AD when controlling for age, sex, and level of cognitive function

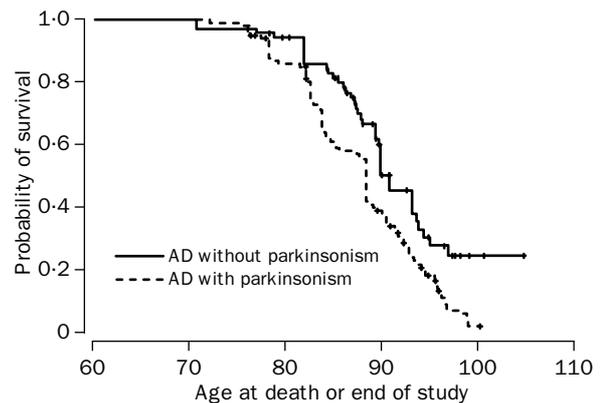
In 1982–84, of 4485 residents in the geographically-defined community of East Boston, MA, USA aged 65 years and older, 3622 (80.8%) underwent a structured interview to assess their medical and social problems, and also a brief test of cognitive function. A stratified random sample of 467 had a structured neurological evaluation which included eight cognitive function tests, an assessment of 12 parkinsonian signs, and diagnostic classification for AD.<sup>4</sup> Cognitive tests were converted to Z-scores (with the population weighted mean and standard deviations) and averaged to yield a global measure of cognitive function with each integer representing one standard deviation. 12 parkinsonian signs were converted to parkinsonian domain scores, and a dichotomous measure of parkinsonism.<sup>3</sup> Complete vital status follow-up was available up to Dec 31, 1992. The average length of follow-up was 9.2 years.

Underlying and immediate causes of death were coded according to the ICD-9-CM system. Cox proportional hazards models were used to estimate mortality risk by level of cognitive function and parkinsonism status, with adjustments for age and sex. Because mortality, prevalence of Alzheimer's disease, and prevalence of parkinsonism are all strongly linked with age, all models were validated using graphical and analytical techniques to check for possible non-linearity and interactions. They were also adjusted for stratified sampling (SUDAAN), thereby reflecting mortality among those with Alzheimer's disease in the entire population.

Seven people were not classified for parkinsonism; of the remaining 460, 131 had Alzheimer's disease, corresponding to about 10% of the whole community older than 65 years after adjusting for the sampling design.<sup>4</sup> Of these, 76 had parkinsonism (mean age 84.2 [SD=7.0] and mean cognitive function score=-1.4 [SD=0.76]). 55 did not have parkinsonism (mean age=83.2 [6.6] and mean cognitive function score -1.1 [0.53]).

Overall, 99 of the 131 (76%) people with Alzheimer's disease died; 64 (84%) with parkinsonism and 35 (64%) without parkinsonism. In a proportional-hazards model, level of cognitive impairment was associated with increased risk of death (RR 1.49; 95% CI 1.12–2.00); and parkinsonism was also associated with increased risk of death (RR 2.55; 1.56–4.17) (figure). When the effects of the global cognitive-function score and parkinsonism were examined simultaneously, the risk associated with cognitive impairment was 1.35 (1.01–1.82) per standard unit, and the risk associated with parkinsonism was 2.34 (1.41–3.88), controlling for age and sex.

One previous clinic-based study found a relation between parkinsonian signs and mortality from Alzheimer's disease.<sup>2</sup> A necropsy study found that neurofibrillary tangles in the substantia nigra of persons who died from Alzheimer's disease were associated with parkinsonian signs,<sup>5</sup> suggesting that among persons with Alzheimer's disease, parkinsonian signs may be a marker of more widespread Alzheimer's



**Probability of survival by age for persons who had Alzheimer's disease with parkinsonism, and who had Alzheimer's disease without parkinsonism at the baseline evaluation**

From a sample of persons over age 65 in a community population, weighted to reflect the stratified sampling.

disease pathology. Overall, these data suggest that parkinsonian signs are a predictor of mortality from Alzheimer's disease.

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## Failure of co-trimoxazole in *Pneumocystis carinii* infection and mutations in dihydropteroate synthase gene

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For HIV-infected patients with CD4 counts under 200/ $\mu$ L, life-long co-trimoxazole (trimethoprim-sulphamethoxazole) is standard to prevent *Pneumocystis carinii* pneumonia (PCP).<sup>1</sup> This has raised concerns that *P carinii* might develop resistance to this useful drug. Sulphamethoxazole targets dihydropteroate synthase (DHPS) and trimethoprim targets dihydrofolate reductase, sequential enzymes in folate metabolism; sulphamethoxazole accounts for nearly all the activity in animal models. Recently Kazanjian et al identified mutations in the human *P carinii* DHPS gene which were found mostly in patients previously receiving prophylaxis with a sulphonamide or with dapsone.<sup>2</sup> However, they did not determine whether patients had actually had prophylaxis broken through. We report two HIV-infected patients in whom prophylaxis or treatment with co-trimoxazole failed, and in whom we identified DHPS mutations.

hPC-wt	A	T	I	D	I	G	G	Q	S	T	R	P	G	S	-	-	H	V	S	I	E	E	E	I	S	R	V	I	P			
hPC-mut	A	T	I	D	I	G	G	Q	S	A	R	S	G	S	-	-	H	V	S	I	E	E	E	I	S	R	V	I	P			
rPC	A	T	I	D	I	G	G	Q	S	T	R	P	G	S	-	-	Y	I	I	P	L	E	E	I	F	R	V	I	P			
<i>S. cerevisiae</i>	S	V	I	D	V	G	G	C	S	T	R	P	N	S	-	-	I	Q	A	S	E	E	E	E	I	R	S	R	I	P		
<i>E. coli</i>	A	T	I	D	V	G	G	E	S	T	R	P	G	A	-	-	A	E	V	S	V	E	E	E	L	Q	R	V	I	P		
<i>B. subtilis</i>	A	H	I	D	I	G	G	E	S	T	R	P	G	A	-	-	E	C	V	S	E	D	E	E	M	S	R	V	I	P		
<i>M. tuberculosis</i>	A	G	I	V	D	V	G	G	E	S	T	R	P	G	A	-	-	T	R	V	D	P	A	V	E	T	S	R	V	I	P	
<i>P. sativum</i>	A	D	I	I	D	I	G	A	Q	S	T	R	P	M	A	-	-	S	R	I	S	A	E	E	E	L	G	R	I	I	P	
<i>S. pneumoniae</i>	A	S	M	L	D	I	G	G	E	S	T	R	P	G	S	-	-	S	V	E	I	E	E	I	Q	R	V	V	I	P		
<i>T. gondii</i>	A	D	V	V	D	V	G	G	E	A	T	N	P	F	R	V	A	G	E	V	P	L	A	V	E	R	E	R	V	V	I	P
<i>P. falciparum</i>	A	S	V	L	D	I	G	G	E	S	S	A	P	F	V	-	-	I	P	N	P	K	I	S	E	R	D	L	V	V	I	P

### Partial alignment of DHPS of different organisms

High conservation of residues indicated by arrows, corresponding to 55 and 57 of human *P. carinii*, and 62 and 64 of *E. coli*. hPC-wt, wild type human *P. carinii*; hPC-mut, mutant human *P. carinii*; rPC, rat *P. carinii*. Genbank accession numbers for the sequences are: rPC, M86602; *Saccharomyces cerevisiae* X96722; *E. coli*, L06494; *Bacillus subtilis*, D26185; *Mycobacterium tuberculosis*, Z95557; *Pisum sativum*, Y08611; *Streptococcus pneumoniae*, U16156; *Toxoplasma gondii*, U81497; and *Plasmodium falciparum*, U07706.

A 31-year-old man with a CD4 count of  $5/\mu\text{L}$ ,<sup>3</sup> who had taken dapsone or co-trimoxazole prophylaxis for 2 years, developed PCP. He received intravenous co-trimoxazole (serum level, 122 mg/dL) and corticosteroids. After 3 weeks he still had symptoms and was hypoxaemic. A transbronchial biopsy specimen showed alveoli filled with an acellular eosinophilic exudate and many organisms, characteristics of untreated *P. carinii* pneumonia. Treatment was changed to pentamidine and within 1 week he was substantially improved.

A 39-year-old man with a CD4 count of 9 cells/ $\mu\text{L}$ , who had taken co-trimoxazole prophylaxis for 3 years, developed PCP which responded to high-dose co-trimoxazole. Prophylactic co-trimoxazole was resumed but over the next 13 months he had two recurrences of PCP, both of which responded to high-dose co-trimoxazole.

*P. carinii* DHPS gene from two samples (days 0 and 21) for the first patient, and two samples (day 0 of episodes 1 and 2) for the second patient, as well as two necropsy samples from 1985, was amplified by PCR, subcloned, and sequenced (2–6 clones/sample). While both necropsy samples showed wild-type sequences, all clones from both samples of both patients showed two mutations identical to those identified by Kazanjian et al, which resulted in a change from Thr-Arg-Pro to Ala-Arg-Ser at positions 55 to 57 of the predicted DHPS sequence.<sup>2</sup> This corresponds to aminoacids 62 to 64 of the *E. coli* DHPS, a region recently shown to be an active site of this enzyme, involved in binding of both substrate and sulphanilamide.<sup>3</sup> This region is highly conserved in all DHPS sequences that have been identified to date (figure).

Because human *P. carinii* cannot be cultured and because the entire human *P. carinii* multifunctional *fas* gene, which includes DHPS, has not yet been cloned, we cannot say whether these mutations confer drug resistance. However, the high aminoacid conservation at these positions among disparate species, the localisation of the mutations to a binding site for sulpha drugs, and the occurrence of these mutations under selective drug pressure strongly support this conclusion. In *Plasmodium*, sulpha resistance has been associated with a mutation at a homologous position.<sup>4</sup> In a report of 82 "breakthrough" cases of *P. carinii* pneumonia developing during prophylaxis, 9% occurred in apparently compliant patients with CD4 counts of more than 50 cells/ $\mu\text{L}$  who were receiving co-trimoxazole prophylaxis, suggesting that these cases may represent resistant *P. carinii* isolates.<sup>5</sup>

Our observations and those of others<sup>2</sup> suggest that drug-discovery programmes to identify and develop agents with different mechanisms of action may need to be accelerated so that the advances of the past two decades are not lost.

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## CCR2-64I allele and genotype association with delayed AIDS progression in African women

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Variation in the rate of progression of HIV-1-infected people to AIDS is partly due to host genetic factors. In European and North American cohorts, delayed progression is associated with polymorphisms in the chemokine/receptor loci CCR5, CCR2, and SDF-1.<sup>1–3</sup> The CCR5 mutation ( $\Delta 32$ ) prevents expression of the principal co-receptor for entry of non-syncytium-inducing (NSI) HIV-1 isolates, but how the point mutation in the CCR2b gene (CCR2-64I, encoding an isoleucine-valine substitution at position 64 in the receptor's first transmembrane domain) mediates protection is unknown. CCR2b is a co-receptor for only a few HIV-1 strains, so protection may be indirect, through intracellular interactions of variant CCR2 proteins with other chemokine receptors<sup>1</sup> or through linkage with unidentified variants within the CCR gene cluster on chromosome 3, such as the recently described CCR5 promoter polymorphism which is tightly linked to the 64I mutation.<sup>3</sup> The protective effect of the CCR2-64I was initially questioned,<sup>4</sup> but it is now clear that the effect is masked unless seroincident (rather than seroprevalent) cohorts are studied.<sup>1,3</sup> The powerful recessive protective effect of a variant in the 3' untranslated region (UTR) of the SDF-1 chemokine may be related to the role of SDF-1 as the ligand for CXCR-4, the co-receptor used by late-stage SI variants of HIV-2.<sup>2</sup> The CCR5 and SDF-1 allelic variants are rare in African-Americans and virtually absent in native Africans,<sup>1,2</sup> but the CCR2-64I variant is common in most ethnic groups studied, including Africans.<sup>1</sup>

We studied these polymorphisms in a cohort of African commercial sex workers (CSWs) in Nairobi, Kenya; more than 90% of the women are infected with HIV-1, and progression to AIDS is more rapid than in European and North American cohorts, with a median time of 3.5 years.<sup>5</sup> Genotyping used ARMS-PCR with sequence-specific primers for CCR2 in all donors, and in the slow progressor (SP) group for SDF-1 by sequence-specific amplification followed by MSP-1 digestion, as described;<sup>2</sup> the CCR5- $\Delta 32$  mutation was not studied since it is absent in Africans and was not found in this cohort.

CCR2-64I allele frequency was 23% in CSWs (n=235), and 20.5% in 105 seropositive Kenyans enrolled in a study

Disease category	Nairobi cohort			Whites (previously reported)		
	Frequency			Frequency		
	n	Allele	Genotype	n	Allele	Genotype
Rapid progressors (AIDS -87 <4 years)	22	0.11	0.18	80	0.06	0.11
Slow progressors (AIDS -87 >12 years)	52	0.33	0.48	397	0.11	0.21
Control HIV +ves (not SP/RP or undetermined)	161	0.22	0.40	915	0.10	0.19
Combined	235	0.23	0.40	1392	0.10	0.19
FET p value	235	0.005	0.014	1392	0.04	0.032
Relative risk (95% CI)		4.17 (1.13-18.9)			2.33 (0.97-4.87)	
Attributable risk						
AR-1		21%			9%	
AR-2		46%			42%	

\*FET p value designates Fisher's Exact Test comparing protective (CCR2+/64I and 64I/64I) vs non-protective genotypes (CCR2+/+) in SPs vs RPs. RR is computed comparing RP vs SP genotype frequencies, with 95% CI in parenthesis. (Allele frequencies show the presence of the 64I mutation on either chromosome, so the denominator is n×2). Attributable risk is computed in two ways: AR 1-SP vs RP + controls, AR 2-SP vs RP alone (ie extremes of progression only).

#### Frequency distribution of the CCR2-64I protective alleles and genotypes (CCR2+/64I and CCR2-64I/64I) in African and white cohorts

of mother-to-child HIV-transmission, over twice that reported in whites ( $F=0.10$ ).<sup>1</sup> Survival analysis was not done because many CSWs were seropositive on study entry: therefore the results are presented as a defined-disease category analysis, where the cohort is arbitrarily partitioned into rapid progressors (RP, who develop AIDS within 4 years of seroconversion), slow progressors (SP, who avoid AIDS for at least 12 years from study entry), and seropositive controls from the rest of the cohort outside the RP/SP categories or of undetermined status (AIDS here is the development of an AIDS defining-illness according to the 1987 CDC definition). The protective CCR2 allele (CCR2-64I) and genotype (CCR2+/64I and 64I/64I) frequencies were significantly elevated among SPs compared to RPs and controls ( $p=0.005$  and  $p=0.14$ , respectively) (table). The CCR2 protective genotypes were three times more frequent among SPs, with a relative risk (RR) of 4.17: the degree of genotypic discordance between slow and rapid progressors is twice as large as that previously reported in white cohorts, divided into the same disease categories (TT 2.05).<sup>1</sup> An estimated 21% of SPs (attributable risk, AR) are accounted for by CCR2 genotype-associated protection, compared with 9% in Caucasians: these figures rise to 46% and 42% respectively if the patients who do not fall into either slow or rapid progression categories are removed.

Seven SP women had CD4+ T-cell counts in the normal range ( $>500/\mu\text{L}$ ); three are CCR2-64I/64I homozygotes. In contrast, no homozygotes for the SDF-1 $\alpha$  3' UTR mutation were detected amongst the SP group. Four women were heterozygotes for SDF-1+/3'A, but this genotype does not have any protective effect.<sup>2</sup>

Rapid progression to AIDS is a striking feature of the Nairobi CSW cohort, with a median time to AIDS less than a third that seen in European and North American cohorts. Between 21% and 46% of the survival of women who have not developed AIDS more than 12 years after HIV-1 infection can be attributed to possession of one or two protective CCR2-64I alleles. Although additional undiscovered factors may contribute to this rapid disease progression, it is plausible that the absence of the CCR5 and SDF-1 protective alleles found in white people is partly responsible. As further host-gene polymorphisms which influence HIV-1 susceptibility and disease progression are identified, it will be important to examine their impact in

other racial groups to understand fully the interaction between different host and viral genotypes.

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## Low frequency of lymph-node metastasis in *BRCA1*-associated breast cancer

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*BRCA1*-associated breast cancers harbour morphoclinical patterns that are thought to be linked to a natural history that is different from sporadic cases.<sup>1</sup> *BRCA1* breast cancers are more often histoprognosis grade 3, highly proliferating, poorly differentiated tumours, with a high frequency of p53 over-expression and oestrogen-receptor negativity.<sup>1-3</sup> Surprisingly, despite these indicators of bad prognosis, the overall survival in *BRCA1* breast cancers is equivalent to sporadic cases, as reported by Verhoog and colleagues.<sup>2</sup> To explore this apparent discrepancy, we investigated an important prognostic factor, axillary lymph-node involvement, in a series of *BRCA1* breast cancers and controls. Since more than 90% of the variance of the lymph-node metastasis is explained by tumour size in population-based studies and the probability of lymph-node involvement increases with tumour diameter,<sup>4</sup> stratification by size was done. This stratification allows avoidance of an obvious bias of selection such as increased awareness and/or selective screening of family members at risk.<sup>2</sup>

54 invasive primary breast cancer cases from 39 French

Size (mm)	Controls (n=24 740)	<i>BRCA1</i> (n=54)
≤9	275/1335 (21%)	0/6
10-19	2316/6984 (33%)	3/17 (18%)
20-29	3272/7282 (45%)	5/16 (31%)
30-39	2257/4329 (52%)	3/9 (33%)
40-49	1267/2112 (60%)	1/2 (50%)
≥50	1889/2698 (70%)	2/4 (50%)

#### Frequency of axillary lymph-node metastasis according to tumour size in *BRCA1*-associated breast cancer cases and controls

*BRCA1* families were selected on the availability of the operative/pathological primary tumour diameter and the axillary lymph-node status (46 tumours with a *BRCA1* mutation, and eight cases from families with a posterior probability of linkage of at least 90%). In our series, *BRCA1* breast cancers were mainly of histoprognostic grade 3 (76%; 41 out of 54 tumours), oestrogen-receptor negative (84%; 26 out of 31 tumours), and progesterone negative (74%; 23 out of 31 tumours). These data are similar to previous reports.<sup>1,2</sup> As controls, we used 24 740 invasive and non-metastatic tumours from the SEER Program of the National Cancer Institute.<sup>4</sup> A two-sided Mantel-Haenszel test was computed to compare the differences between the two populations.

According to stratification by tumour size (table), *BRCA1* breast cancers had a weaker frequency of axillary lymph-node involvement than controls ( $p=0.013$ ). Since lymph-node involvement reflects the metastatic capacity of the tumour, our preliminary results imply that the relation between these two elements is different in sporadic and hereditary cases. *BRCA1* breast tumours may be a subset of tumours with a lower ability to spread than controls, or their metastatic potential may be expressed later in the course of the disease. Other factors such as protease activity, heat-shock proteins, invasion or formation of lymphatic and blood vessels may explain this phenomenon, and should be analysed in this context.

If confirmed, these findings may clarify, at least partly, why no decrease in the overall survival is observed in *BRCA1* breast cancer despite indicators of bad prognosis. An important consequence is that early clinically detected *BRCA1* tumours have a low rate of nodal metastasis. Therefore, breast self-examination and/or clinician's examination, in addition to the other preventive procedures according to women's preference,<sup>5</sup> may be of great value to identify curable disease in *BRCA1* breast-cancer-prone women.

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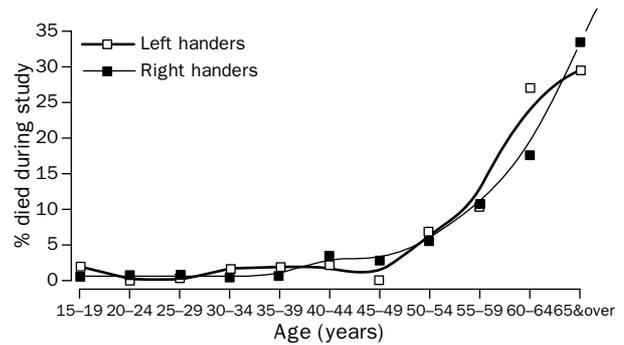
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## Left-handedness and premature death

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Many studies have found less left-handedness in older age groups. This has been attributed to a more relaxed attitude to left-handedness in more recent times, to gradual adaptation to a dextral world, or to premature mortality.<sup>1</sup>

We did a longitudinal study of left-handedness and



Mortality in relation to handedness in different age groups

mortality in a family practice. People aged between 15 and 70 were mailed the Edinburgh Inventory,<sup>2</sup> which assesses handedness. A laterality quotient (LQ) was calculated ranging from -100 (strongly left-handed) to +100 (strongly right-handed). Of the questionnaires which arrived at the correct address 82.17% were returned correctly providing 6097 responses. The mean age was 41.4 years and 46% were male.<sup>2</sup> 9 years later, the practice register was used to trace the patients. Those who were no longer registered were traced via death certification, through the local Family Health Service Authority, and through the Office of Population Census and Surveys register. 387 had died, 48 (0.8%) were lost to follow-up, but the remainder were known to be alive. An analysis of variance was performed with LQ as the main variable; age, gender, and whether the subject had died were built into the analysis. Age (1 df,  $F=84.424$ ,  $p<0.001$ ), and gender (1 df,  $F=11.505$ ,  $p=0.001$ ), were important variables. However, death was not (1 df,  $F=0.074$ ,  $p=0.786$ ) (figure).

As the majority of patients were right-handed it is possible that we missed significant premature mortality within the left-handed minority. We reanalysed using two groups, right-handed (positive LQ) and left-handed (negative LQ). The prevalence of left-handedness was 8.00%. The 14 cases who scored LQ=0 were excluded (0.23%). 6.48% of right-handers and 4.98% of left-handers died. As a proportion, fewer left-handers died, but this was not statistically significant (difference in mortality 1.5% [95% CI 1.63%];  $Z$  score 1.29;  $p<0.1$ , two-tail). As left-handers were younger than right-handers (means 37.4 and 41.6 years respectively) this may have contributed to the survival of the left-handers and so an analysis of variance was performed with gender and age as covariables. Handedness did not make a significant contribution to the outcome of death ( $F=0.17$ ;  $p=0.317$ ).

Whilst this study does not preclude the possibility of a small effect of left-handedness on longevity it is clearly not of the order of magnitude seen in cross-sectional or longitudinal studies.<sup>3</sup>

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