Long-Term Statin Therapy and CSF Cholesterol Levels: Implications for Alzheimer's Disease

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Key Words

Statins • Brain lipids • Alzheimer's disease • Cerebrospinal fluid cholesterol

Abstract

Background/Aims: It is not yet established whether statins (lipophilic or hydrophilic) reduce the risk of Alzheimer's disease and, if so, by differentially modifying brain lipid levels. Our aim was to assess changes in brain cholesterol metabolism as reflected in the cerebrospinal fluid (CSF) before and after treatment with either atorvastatin or simvastatin.

Methods: We carried out a longitudinal analysis of CSF cholesterol, lathosterol and 24(S)-hydroxycholesterol before and after treatment with maximum doses of statins in 10 asymptomatic subjects, 8 of whom were heterozygous for apolipoprotein E ϵ 4, and in 6 presymptomatic PS1 subjects.

Results: Statins initially reduced CSF lathosterol cholesterol and 24(S)-hydroxycholesterol in both PS1 and non-PS1 subjects reaching a nadir at 6–7 months, followed by a return to baseline at 15 months with an overshoot at 2 years, tending to return to baseline thereafter. **Conclusions:** Possible long-

term protective effects of statins are not likely largely related to the temporally-dependent biphasic effects of statin therapy upon the magnitude and direction of changes in CSF lipid levels and their subsequent return to baseline levels.

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Introduction

The risk of late-onset Alzheimer's disease is influenced by apolipoprotein E (APOE) genotype [1] and vascular risk factors [2]. Several epidemiological studies [3–5] have suggested that statins reduce the risk of late-onset Alzheimer's disease, although clinical experience to date has been mixed [6, 7]. Whether any putative benefits for reducing such risk are secondary to either the peripheral or central lipid-lowering effects of statins or alternatively

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Table 1. Age, sex, PS1 and APOE genotypes, and pre- and poststatin TC and LDL levels of PS1 and non-PS1 subjects

Subject	Age at 1st LP, years	Sex	PS1 mutation(s)	APOE genotype	Prestatin	6 months	12 months	24 months	36 months
					TC, LDL	TC, LDL, statin	TC, LDL, statin	TC, LDL, statin	TC, LDL, statin
1	44	M	C410Y	32	217, 153	128, 75, s40	140, 81, s80	121, 60, s80	N/A
2	41	M	C410Y	32	186, 133	127, 64, a20	133, 64, a40	110, 53, a40	156, 100, a40
4	34	M	C410Y	33	190, 115	153, 69, a10	109, 60, a20	104, 52, a40	151, 78, a40
3	36	M	C410Y	34	151, 105	146, 84, s40	113, 63, s80	N/A	N/A
5	53	M	P242H,R352H	44	193, 103	187, 109, s20	N/A	N/A	N/A
6	55	F	P242H,R352H	34	285, 186	177, 94, a20	188, 97, a40	175, 80, a40	N/A
7	57	F	no mutation	34	208, 112	163, 78, a20	168, 75, a40	N/A	N/A
8	68	F	no mutation	34	201, 115	164, 66, a40	135, 54, a40	138, 51, a80	141, 57, a80
9	68	F	no mutation	34	221, 117	170, 82, s40	151, 71, s40	159, 74, s80	140, 60, s80
10	45	F	no mutation	34	205, 142	142, 76, a40	162, 90, a40	N/A	N/A
11	69	F	no mutation	33	167, 108	120, 62, a20	115, 60, a20	N/A	N/A
12	56	F	no mutation	34	180, 113	148, 75, a40	173, 92, a40	N/A	N/A
13	52	M	no mutation	24	186, 122	128, 59, s40	133, 66, s40	N/A	N/A
14	52	F	no mutation	34	210, 105	187, 76, a10	167, 69, a20	N/A	N/A
15	52	F	no mutation	33	186, 118	129, 58, a20	127, 61, a20	N/A	N/A
16	61	F	no mutation	33	372, 230	s60	216, 99, s60	N/A	N/A
17	50	F	no mutation	34	230, 157	165, 90, a40	125, 66, s40	N/A	N/A

a = Atorvastatin; s = simvastatin with daily dose in milligrams after each initial; TC = total cholesterol; LDL = low-density lipoproteins; N/A = not applicable. Note: TC and LDL not available for subject 16 at 6 months.

to non-lipid-lowering 'pleotropic' effects of statins, remains an issue of much current interest.

As yet, there have not been any reports on the effects of statin therapy beyond 1 year on cerebrospinal fluid (CSF) cholesterol levels in either asymptomatic subjects who are heterozygous for APOE ϵ 4 or for presymptomatic subjects with PS1 mutations. We therefore performed a hypothesis-generating pilot study in 2 groups including 10 asymptomatic subjects, of whom 8 were heterozygous for APOE ϵ 4, and in 6 presymptomatic subjects who were PS1 mutation carriers and who were randomly selected for treatment with either simvastatin or atorvastatin.

Methods

Subject Selection

Presymptomatic PS1 mutation carriers were recruited from 2 kindreds, 1 followed since 1985 [8, 9] and the other since 1990 [10]. Each subject's prestatin CSF concentration for each analyte served as its own control for poststatin values. Only subjects who had initial prestatin abnormalities in A β ₄₂, phospho-tau and total tau levels in the CSF [11] were invited to remain in the study beyond 1 year. Eight of 10 PS1 carriers agreed to enter the study, but only 4 continued through 2 years and only 2 continued for 3 years. The reasons for dropout included a side effect to statins (1 case), desire by the subject to try a special diet (1 case) and lack of compliance with statin therapy beyond the second year (2 cases).

Non-PS1 subjects were recruited from an advertisement in the local newspaper and from notices placed with local Alzheimer Association chapters. Responders were predominantly women who had a mother or older sister who developed late-onset Alzheimer's disease before the age of 75. Fourteen subjects were originally recruited. One dropped out because of a statin side effect and 2 dropped out because of discomfort at or after the first lumbar puncture (LP), leaving 11 subjects. The CSF sample volume of one of these subjects was depleted by its use in prior studies [10, 11]. Eight of the 10 remaining non-PS1 subjects were heterozygous for APOE ϵ 4 and 9 of these 10 subjects were female (table 1).

Treatment and CSF Measurements

Studies were carried out in accordance with Institutional Review Board guidelines. Maximal statin doses were used to aggressively reduce total cholesterol and low-density lipoproteins throughout the treatment period.

CSF was obtained by LP for prestatin baselines, then at 6 and 12 months after onset of treatment (n = 16). LPs carried out in Worcester were consistently performed in the mornings for each subject throughout the study. Two subjects who had LPs carried out at Mayo Clinic, Scottsdale, had LPs performed in early to mid-afternoon. Compliance with treatment was determined by obtaining fasting lipid profiles at 3- to 6-month intervals.

The CSF was kept frozen at -80°C until analysis. Concentrations of CSF lathosterol, cholesterol and 24(S)-hydroxycholesterol as the sequential steps in cholesterol synthesis and degradation [12] were determined by gas mass spectrometry (GCMS). CSF (0.5 ml) samples were mixed with 1.0 μ g of internal standard (25,26,26,26,27,27,27-D₇-cholesterol; Cambridge Isotope Laboratories) and subjected to alkaline hydrolysis and extraction as described by Dzeletovic et al. [13] followed by conversion to the

trimethylsilyl derivatives by treatment with 50 μ l of N,O-bis(trimethylsilyl)trifluoroacetamide containing 1% trimethylchlorosilane (Pierce Chemical Co.) at 80°C for 60 min. GCMS was performed on a Waters Quattro-II triple quadrupole MS system using a 30 m $\times$ 0.25 mm ID DB-17MS capillary column (phase thickness = 0.25 μ m; J & W Scientific) with an initial hold at 250°C followed by a liner program to 275°C at 2°C per min with helium as the carrier gas at 20 kPa. Electron impact ionization (70 eV) was used with selected ion monitoring detection of m/z 458 (cholesterol), m/z 465 for D₇-cholesterol, m/z 458 (lathosterol) and m/z 413 24(S)-hydroxycholesterol. Concentrations of the analytes were calculated with reference to a 5-point external calibration curve.

Following others' examples [14–16], we make the assumption that the steady-stage levels of these CSF analytes reflect, in some manner, the production rates for lathosterol and cholesterol and the degradation rates for 24(S)-hydroxycholesterol within the brain. Similar assumptions have been made by those who have related changes in plasma 24(S)-hydroxycholesterol to corresponding changes in the brain [17, 18]. Mass spectrometrists were blinded as to the subject's genetic status, type of statin used and the treatment duration. The estimated concentrations of each analyte are independent of each other.

The lathosterol, cholesterol and 24(S)-hydroxycholesterol levels were modeled using general linear mixed models [19–21] to fit third-order polynomial growth curves [19–22] across time. The overall significance of a model was assessed using likelihood ratio χ^2 tests and the significance of individual terms of a model was evaluated using Wald tests of term coefficients. These are empirical models that are useful as they characterize the data over the range of values observed in terms of linear, quadratic and cubic components. Unlike deterministic models such as kinetic models, they do not purport to mimic the biological processes involved. These models were used to assess analyte concentrations over the duration of statin treatment. At the time these studies were carried out, none of the subjects met diagnostic criteria for mild cognitive impairment [23] or AD [24] based upon neuropsychological assessment.

Results

The demographic characteristics of these subjects with respect to age at first LP, sex, PS1 mutation location (when applicable), APOE genotype, as well as pre- and poststatin serum lipid levels are shown in table 1 below.

Lathosterol

There were no significant differences in results for PS1 and non-PS1 subjects or for those treated with simvastatin versus those treated with atorvastatin. Combined results for both non-PS1 and PS1 subjects were well characterized by third-order polynomial fits that were significantly different from a flat mean level ($p < 0.001$) with significant linear, quadratic and cubic components

Fig. 1. **a** Estimated CSF lathosterol levels (y-axis) versus time (x-axis) for PS1 subjects (solid line) and for non-PS1 subjects (broken line). **b** Estimated CSF cholesterol levels (y-axis) versus time (x-axis) for PS1 subjects (solid line) and for non-PS1 subjects (broken line). **c** Estimated CSF 24(S)-hydroxycholesterol (y-axis) versus time (x-axis) for combined PS1 and non-PS1 subjects.

(fig. 1a). Their values and standard errors are shown in table 2. The mean levels of lathosterol for the 9 subjects who left the study at 1 year did not differ significantly from the mean for the 7 continuing beyond 1 year (0.0035 ± 0.0010 vs. 0.0046 ± 0.0010 ; $p = 0.741$).

The curves in figure 1a showed peak reductions at 6–7 months after treatment with some subsequent return to

Table 2. Coefficients $\pm$ SE for fits of third-order polynomial model by analytes

Analyte	Intercept	Time	Time squared	Time cubed
ln(lathosterol)	-5.3071 ± 0.1602	-0.09637 ± 0.04502	0.008603 ± 0.003301	-0.00016 ± 0.000063
ln(cholesterol)				
Non-PS1	1.4185 ± 0.1173	-0.05755 ± 0.02709	0.007848 ± 0.002038	-0.00016 ± 0.00004
PS1	2.451 ± 0.1538	-0.05755 ± 0.02709	0.003381 ± 0.002064	-0.00006 ± 0.000044
ln(24[S]-hydroxycholesterol)	-5.7864 ± 0.07773	-0.03031 ± 0.02054	0.002624 ± 0.001479	-0.00005 ± 0.000028

The intercepts represent the extent to which each curve intercepts the y-axis at $t = 0$. These are significantly different from 0 for all analytes. The linear, quadratic and cubic terms correspond to 'time', 'time squared' and 'time cubed', respectively.

baseline at 15 months. Further increases to well above baseline reach a peak at about 2 years with a subsequent return towards baseline by 3 years.

Cholesterol

In non-PS1 subjects there was a slight decrease at 6 months also followed by a return to baseline at 15 months with a large rise above baseline that peaked at 24 months and a subsequent decline towards baseline at 36 months. The polynomial fits were also significantly different from a mean plot level ($p < 0.001$). The p values for the linear, quadratic and cubic terms were all significant (table 2). The results did not differ by statin type. The overall polynomial fit model was also significant for the PS1 subjects, although the quadratic and cubic terms did not reach significance (table 2).

The CSF cholesterol levels of PS1 subjects showed an initial decrease following statin therapy (fig. 1b). Slight upregulation was also observed peaking at 24 months as was the case for the non-PS1 subjects.

24(S)-Hydroxycholesterol

There were no significant differences between PS1 and non-PS1 subjects or for subjects treated with simvastatin or atorvastatin. Combined results demonstrated a decrease at 6–7 months followed by a return to baseline at 15 months and then further upregulation peaking at 2 years with a subsequent decline towards baseline at 3 years (fig. 1c). Whereas the overall third-order polynomial fit was highly significant ($p < 0.001$), the linear, quadratic and cubic terms only approached significance with p values of 0.147, 0.0834 and 0.084 for these terms, respectively.

The temporal changes for 24(S)-cholesterol with respect to initial minima and subsequent maxima are remarkably similar in shape to the analogous results for lathosterol (fig. 1a) and cholesterol (fig. 1b).

Discussion

Both types of statin produce an initial decline in CSF lathosterol, cholesterol and 24(S)-hydroxycholesterol for both PS1 and non-PS1 subjects. These changes are independent of statin type. The drop is greatest at 6–7 months, which corresponds to the estimated half-life of brain cholesterol synthesis [14] followed by what appears to be an upregulation for all analytes with a return baseline by 15 months and a peak at 2 years followed by a gradual return towards baseline.

Limitations and Advantages of the Present Study

The major limitation of our study is the relatively small number of subjects ($n = 16$) who completed at least the first year of the study. This issue is especially pertinent for the PS1 cohort for which only 6 of the original 8 subjects completed 1 year. The effort to recruit a presymptomatic cohort of even that size required us to contact all 30 known at-risk members of the largest kindred accessible to us (8). Of these, only roughly half agreed to presymptomatic genetic testing. Moreover, the 8 identified as mutation carriers also had to consider the long course of statin therapy and the prospects of serial LPs over several years before deciding whether to participate.

Nor could we enlarge the study for additional subjects of either PS1 or non-PS1 status once the GCMS analyses for the 3 CSF lipids had been carried out on the original set of subjects. The reason for this was that to assure standard conditions for all analyses, GCMS was carried out within a single time window under identical conditions with common reagents and standards that cannot be assured to be invariant over time. Nor was there sufficient CSF subsequently available for all subjects to simply redo their analyses together with that of an expanded group of additional subjects.

Fortunately, the statistical power of the study was not dependent solely upon the number of subjects but also upon the total number of observations for each subject over time. Thus, each curve in figure 1 represents the best fit for almost 60 data points. Moreover, once it could be established that there were no significant differences in statins upon CSF lipid metabolism in PS and non-PS1 subjects, the results from the 2 groups could be combined with an overall $n = 16$. As a result, the third-order polynomial fits for each analyte were robustly different from a flat mean with $p < 0.001$ in all cases.

Moreover, the time courses for the changes in direction and magnitude for the 3 analytes were remarkably similar (fig. 1). This result further increases confidence in these data, given that the measurements for each analyte were independent of each other.

Even so, we acknowledge that our results should be taken as hypothesis-generating pilot results to guide future studies with greater numbers of subjects especially with respect to the need to carry out such studies for at least for 3 years and to be cognizant of time-dependent changes in the direction and magnitude of CSF lipid levels following statin therapy. Even accepting such qualifications, we believe that our results for the first year of statin treatment are similar enough to those of others carried out over comparable time periods to warrant comparison with such earlier work.

Comparison of the Present Study to Previous Work

Our results are consistent with those of Simons et al. [14], who reported decreases in CSF lathosterol and 24(S)-hydroxycholesterol after 6 months of simvastatin therapy. Fassbinder et al. [15] found CSF reductions in all 3 analytes after at least 6 months of treatment with one or another of 5 different statins in normocholesterolemic subjects. Höglund et al. [16] found decreases in CSF lathosterol, but not in cholesterol or 24(S)-hydroxycholesterol, after 1 year of treatment with simvastatin.

Our results beyond 1 year are based upon studies from 7 clinically asymptomatic subjects who had abnormal prestatin CSF values for 1 or more of the Alzheimer disease markers $A\beta_{42}$, phospho-tau and total tau. Their mean lathosterol levels, as noted above, at 1 year did not differ significantly from these levels for the 8 subjects who concluded their participation in the study at 1 year. We take this result as a justification to have considered these 2 subject groups as sufficiently similar for comparing CSF sterol results beyond 1 year with those at or prior to 1 year.

How might the results reported here relate to constraints on hypotheses with respect to how statins may or may not contribute to possible risk reduction in the various forms of Alzheimer's disease? First, if our pilot results are confirmed in larger studies, then researchers who seek explanations of the effects of statins on the pathogenesis of Alzheimer disease based upon changes in CSF lipid levels should be aware of the biphasic aspect of the response of CSF lipids to statins. The direction and magnitude of these changes depend upon the duration of statin therapy. Thus, estimates of CSF lipid levels depend upon the duration of treatment at which these analytes are sampled.

Second, the decrease in CSF lipid responses to statins is only temporary. Thus, hypotheses supporting any putative beneficial effects of statins upon AD risk cannot assume a long-term decrease in CSF lipid levels.

Third, if putative beneficial effects of statins are not attributable to a consistent lowering of CSF lipids, then such effects may either be due to the effects of statins on the microvascular endothelium [4] or on the 'pleiotropic' non-lipid-lowering central effect of statins or upon both. Indeed, in the latter respect, our previous results on these same non-PS1 subjects demonstrated a substantial increase in sAPP α for both statins, albeit with a corresponding increase in sAPP β as well [10]. These effects were far more robust for simvastatin than for atorvastatin, perhaps related to the report by Wolozin et al. [5] of substantially greater reduction of Alzheimer's disease risk in subjects treated with simvastatin versus those treated with atorvastatin. Höglund et al. [15] also reported that simvastatin significantly increased sAPP α but did not find significant changes for sAPP β . Moreover, Riekse et al. [25] observed that simvastatin but not pravastatin reduced the levels of phospho-tau-181 in hypercholesterolemic subjects without dementia.

These earlier results on statin-induced increases in sAPP α coupled with the lack of any statin-induced long-term lowering of CSF lipid levels reported here take on added significance given other recent results. For example, Laurén et al. [26] have shown that cellular prion protein is a major mediator of the known synaptic dysfunction induced by amyloid- β oligomers. This new result is all the more pertinent for the potential prevention of treatment of Alzheimer's, given that α -secretase cleaves both β APP to preclude the release of an amyloidogenic fragment and cellular prion protein within the amino acid residues 95–110 otherwise crucial for $A\beta$ binding [27, 28]. Hence, the previously reported robust increase in sAPP α in human CSF indicative of an increase in α -

secretase activity in the brain with high-dose simvastatin [10] may be more significant than previously appreciated.

Finally, we consider the significance of the result that temporal changes in CSF lipid levels after statin treatment follow an entirely different time course than the linear changes over time in studies of APP metabolism [10]. Such differential findings may provide a novel constraint to assess whether modifications in neurodegeneration conform to one or another temporal signature after statin treatment or indeed after any testable therapy.

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References

- Saunders AM, Strittmatter WJ, Schmechel D, et al: Association of apolipoprotein E allele ϵ 4 with late-onset familial and sporadic Alzheimer's disease. *Neurology* 1993;43:1467-1472.
- Notkola IL, Sulkava R, Pekkanen J, et al: Serum total cholesterol, apolipoprotein E ϵ 4 allele, and Alzheimer's disease. *Neuroepidemiology* 1998;17:14-20.
- Wolozin B, Kellman W, Ruosseau P, et al: Decreased prevalence of Alzheimer disease associated with 3-hydroxy-3-methylglutaryl coenzyme A reductase inhibitors. *Arch Neurol* 2000;57:1439-1443.
- Jick H, Zornberg GL, Jick SS, et al: Statins and the risk of dementia. *Lancet* 2000;356:1627-1631.
- Wolozin B, Wang SW, Li NC, et al: Simvastatin is associated with a reduced incidence of dementia and Parkinson's disease. *BMC Med* 2007;5:20.
- Sparks DL, Kryscio RJ, Ssabbagh MN, et al: Reduced risk of incident AD with elective statin use in a clinical trial cohort. *Curr Alzheimer Res* 2008;5:416-421.
- Shepherd J, Blauw GJ, Murphy MB, et al: Pravastatin in elderly individuals at risk of vascular disease (PROSPER): a randomized controlled trial. *Lancet* 2002;360:1623-1697.
- Pollen DA: Hannah's Heirs. The Quest for the Genetic Origins of Alzheimer's Disease. Oxford, Oxford University Press, 1993, pp 1-296.
- Lippa CF, Saunders AM, Smith TW, et al: Familial and sporadic Alzheimer's disease: neuropathology cannot exclude a final common pathway. *Neurology* 1996;46:406-412.
- Hinerfeld DA, Moonis M, Swearer JM, et al: Statins differentially affect amyloid precursor protein metabolism in presymptomatic PS1 and non-PS1 subjects. *Arch Neurol* 2007;64:1672-1673.
- Moonis M, Swearer JM, Dayaw MP, et al: Familial Alzheimer disease: decreases in CSF A β 42 levels precede cognitive decline. *Neurology* 2005;65:323-325.
- Dietschy JM, Turley SD: Thematic review series: brain lipids. Cholesterol metabolism in the central nervous system during early development and in the mature animal. *J Lipid Res* 2004;45:1375-1397.
- Dzeletovic S, Breuer O, Lund E, et al: Determination of cholesterol oxidation products in human plasma by isotope dilution-mass spectrometry. *Anal Biochem* 1995;225:73-80.
- Simons M, Schwärzler F, Lütjohann D, et al: Treatment with simvastatin in normocholesterolemic patients with Alzheimer disease: a 26-week randomized, placebo-controlled, double-blind trial. *Ann Neurol* 2002;52:346-350.
- Fassbinder K, Stroick M, Bertsch T, et al: Effects of statins on human cerebral cholesterol metabolism and secretion of Alzheimer amyloid peptide. *Neurology* 2002;59:1257-1258.
- Höglund K, Thelen KM, Syversen S, et al: The effect of simvastatin treatment on the amyloid precursor protein and brain cholesterol metabolism in patients with Alzheimer's disease. *Dement Geriatr Cogn Disord* 2005;19:256-265.
- Locatelli S, Lütjohann D, Schmidt HHJ, et al: Reduction of plasma 24S-hydroxycholesterol (cerebrosterol) levels using high-dosage simvastatin in patients with hypercholesterolemia. *Arch Neurol* 2002;59:213-216.
- Vega GL, Weiner MF: Plasma 24S hydroxycholesterol response to statins in Alzheimer's disease patients: effects of gender, CYP46, and ApoE polymorphisms. *J Mol Neurosci* 2007;33:51-55.
- McLean RA, Sanders WL, Stroup WW: A unified approach to mixed linear models. *Am Stat* 1991;45:54-64.
- Singer JD, Willett JB: *Applied Longitudinal Data Analysis. Modeling Change and Event Occurrence*. Oxford, Oxford University Press, 2003.
- Verbeke G, Molenberghs G: *Linear Mixed Models for Longitudinal Data*, ed 1. New York, Springer, 2000.
- Draper NR, Smith HF: *Applied Regression Analysis*, ed 2. New York, Wiley & Sons, 1981.
- Ritchie K, Artero S, Touchon J: Classification criteria for mild cognitive impairment: a population-based validation study. *Neurology* 2001;56:37-42.
- McKhann G, Drachman D, Folstein M, et al: Clinical diagnosis of Alzheimer's disease: report of the NINCDS-ADRDA Work Group under the auspices of Department of Health and Human Services Task Force on Alzheimer's Disease. *Neurology* 1984;34:939-944.
- Riekse RG, Li G, Petrie EC, et al: Effect of statins on Alzheimer's disease biomarkers in cerebrospinal fluid. *J Alzheimers Dis* 2006;10:399-406.
- Laurén J, Gimbel DA, Nygaard HB, Gilbert JW, Strittmatter SM: Cellular prion protein mediates impairment of synaptic plasticity by amyloid- β oligomers. *Nature* 2009;457:1128-1132.
- Vincent B, Cisse MA, Sunyach C, Guillot-Sestier MW, Cheller F: Regulation of β APP and PrPc cleavage by α -secretase: mechanistic and therapeutic perspectives. *Curr Alzheimer Res* 2008;5:202-211.
- Cisse M, Mucke L: Alzheimer's disease: a prion protein connection. *Nature* 2009;457:1090-1091.