SHORT COMMUNICATION

BJC

British Journal of Cancer (2015) 113, 1254–1258 | doi: 10.1038/bjc.2015.364

Keywords: ovarian cancer; low-grade serous carcinoma; KRAS; BRAF

Impact of mutational status on survival in low-grade serous carcinoma of the ovary or peritoneum

David M Gershenson*[,1], Charlotte C Sun[1] and Kwong-Kwok Wong[1]

[1]*Department of Gynecologic Oncology and Reproductive Medicine, The University of Texas MD Anderson Cancer Center, Houston, TX 77030, USA*

Background: Low-grade serous carcinoma of the ovary or peritoneum is a distinct, well- recognized histologic subtype characterized by young age at diagnosis, relative chemoresistance, and prolonged overall survival. Common mutations reported to be found within this subtype include KRAS and BRAF.

Methods: Using clinical information of patients from our IRB-approved registry and tissue from a subset of these patients, we performed mutational analysis for KRAS and BRAF using the direct Sanger sequencing technique and correlated findings with the clinical outcome, overall survival (OS).

Results: In 79 cases, patients with KRAS or BRAF mutations ($n=21$) had a significantly better OS than those with wild-type KRAS or BRAF ($n=58$) (106.7 months (95% CI, 50.6, 162.9) *vs* 66.8 months (95% CI, 43.6, 90.0)), respectively ($P=0.018$).

Conclusions: Mutational status appears to be a potential prognostic factor in low-grade serous carcinoma of the ovary or peritoneum.

Low-grade serous carcinoma of the ovary or peritoneum is a distinct histologic subtype that may arise either *de novo* or following a diagnosis of serous tumour of low malignant potential (STLMP) (Crispens *et al*, 2002; Malpica *et al*, 2004; Gershenson *et al*, 2006; Shvartsman *et al*, 2007; Gershenson *et al*, 2015). Its clinical behaviour is characterized by young age at diagnosis, relative chemoresistance, and prolonged overall survival (OS) relative to high-grade subtypes of ovarian cancer (Gershenson *et al*, 2006; Gershenson *et al*, 2015). In addition, the diagnosis of the *de novo* presentation most commonly is made in the advanced stages.

In 2003, Singer and colleagues reported that KRAS and BRAF mutations occurred with a frequency of 35% and 33%, respectively, in low-grade serous carcinoma of the ovary, or what they termed, 'invasive micropapillary serous carcinoma' (Singer *et al*, 2003). However, subsequent reports indicated a much lower frequency of BRAF mutation in low-grade serous carcinoma (Wong *et al*, 2010; Grisham *et al*, 2012; Farley *et al*, 2013; Tsang *et al*, 2013). The frequency of KRAS mutations ranged from 16 to 41% (Wong *et al*, 2010; Grisham *et al*, 2012; Farley *et al*, 2013). These results confirm that low-grade serous carcinoma has a distinct molecular pathway, and that, specifically, the mitogen-activated protein kinase pathway appears to have a major role in the pathogenesis of this subtype. Although the initial clinical trial of a MEK inhibitor, selumetinib, demonstrated promising activity in recurrent low-grade serous carcinoma of the ovary or peritoneum, with an objective response rate of 15%, there was no correlation between response and mutational status (Farley *et al*, 2013). Subsequent phase III clinical trials studying the activity and toxicity of different MEK inhibitors in recurrent low-grade serous carcinoma are ongoing; these trials include translational research objectives intended to re-test the hypothesis that response to this targeted therapy approach is correlated with activation of the mitogen-activated protein kinase pathway. The purpose of this study was to investigate OS based on KRAS or BRAF mutational status in low-grade serous carcinoma.

*Correspondence: Dr DM Gershenson; E-mail: dgershen@mdanderson.org

Received 29 May 2015; revised 11 September 2015; accepted 20 September 2015; published online 20 October 2015

MATERIALS AND METHODS

This study was approved by the institutional review board at the University of Texas M.D. Anderson Cancer Center. The Low-Grade Serous Tumor Database is a longitudinal database that contains clinico-demographic information from patients who have provided written informed consent in accordance with protocol guidelines. We identified patients with low-grade serous carcinoma of the ovary or peritoneum for whom tumour tissue was available for study. All patients provided written informed consent for use of their tumour specimens. Eligibility criteria for inclusion in this study were: (i) Original diagnosis of advanced stage STLMP with recurrence as metastatic low-grade serous carcinoma or *de novo* diagnosis of stage II–IV low-grade serous carcinoma; and (ii) adequate clinical information based on completeness of follow-up, date of last contact, and current status. Patients with STLMP without recurrence as low-grade serous carcinoma or those with stage I low-grade serous carcinoma were excluded. Pathology slides of all patients were reviewed by MD Anderson gynaecologic pathologists and documented as low-grade serous carcinoma using criteria that have been previously reported by our group (Malpica *et al*, 2004; Schmeler *et al*, 2011). Formalin-fixed, paraffin-embedded or frozen tissue blocks were retrieved from the Department of Pathology or the Gynecologic Oncology Tumor Repository, respectively, and MD Anderson gynaecologic pathologists confirmed tissue sections selected for mutational analysis for KRAS and BRAF to contain low-grade serous carcinoma with more than 40% tumour cells. Following extraction of DNA, direct Sanger sequencing of PCR products was performed as described previously (Wong *et al*, 2010). Statistical analyses were performed using IBM SPSS version 21 (Armonk, NY, USA). Chi-square test was used to compare the differences in categorical variables between groups. The Fisher's exact test was used when appropriate. Overall survival (OS) times were calculated from the date of confirmed tissue diagnosis for *de novo* low-grade serous carcinoma patients and from the date of recurrence as low-grade serous carcinoma for the patients with STLMP to the date of last contact or death, respectively. The cumulative distribution of OS was estimated using the method of Kaplan and Meier (Kaplan and Meier, 1958). The log-rank test was used to compare differences between survival curves. The individual effects of age, race, primary site, surgery type, KRAS/BRAF mutation status, residual disease at the completion of surgery, disease status at completion of primary therapy, low-grade serous carcinoma type (recurrent low malignant potential or *de novo* low-grade), and stage on OS were assessed using Cox proportional hazards regression. Variables with *P*-values <0.25 on univariable analysis were included in the multivariable analysis. A *P*-value of <0.05 was considered statistically significant.

Table 1. Patient characteristics ($N=79$)

	Wild type ($n=58$)	BRAF/KRAS mutation ($n=21$)	*P*-value
Median age, years (range)	46.3 (21.1, 79.0)	44.3 (26.4, 71.5)	0.59
Race			0.11
White	45 (77.6%)	14 (66.7%)	
Black	4 (6.9%)	5 (23.8%)	
Other	9 (15.5%)	2 (9.5%)	
Year of diagnosis of LGSC			0.21
1975–1992	12 (20.7%)	2 (9.5%)	
1993–2009	46 (79.3%)	19 (90.5%)	
Site			0.08
Ovary	46 (79.3%)	20 (95.2%)	
Peritoneum	12 (20.7%)	1 (4.8%)	
Stage			0.89
II	3 (5.2%)	1 (4.8%)	
III	39 (67.2%)	13 (61.9%)	
IV	7 (12.1%)	2 (9.5%)	
STLMP→LGSC	9 (15.5%)	5 (23.8%)	
Initial surgery			0.91
Primary CRS	47 (81.0%)	17 (81.0%)	
NACT followed by IDS	9 (15.5%)	3 (14.3%)	
No surgery	2 (3.4%)	1 (4.8%)	
Residual disease at the completion of surgery[a]			0.16
No gross residual disease	12 (20.7%)	7 (33.3%)	
Gross residual disease	40 (69.0%)	11 (52.4%)	
No surgery	2 (3.4%)	1 (4.8%)	
Unknown	4 (6.9%)	2 (9.5%)	
Initial systemic treatment			0.34
Non-platinum chemotherapy	4 (6.9%)	0 (0.0%)	
Platinum-based chemotherapy	52 (89.7%)	17 (81.0%)	
No chemotherapy	1 (1.7%)	2 (9.5%)	
Hormonal treatment	1 (1.7%)	2 (9.5%)	
Disease status at completion of primary treatment			0.79
No disease	26 (44.8%)	9 (50.0%)	
Disease present	30 (51.7%)	9 (50.0%)	
No chemotherapy	1 (1.7%)	2 (9.5%)	
Unknown	1 (1.7%)	1 (4.8%)	

Abbreviations: CRS = cytoreductive surgery; IDS = interval debulking surgery; LGSC = low-grade serous carcinoma; NACT = neoadjuvant chemotherapy; STLMP = serous tumour of low malignant potential.
[a]Cases of primary cytoreductive surgery and interval debulking surgery were combined for this analysis.

RESULTS

Seventy-nine cases that met the eligibility criteria and for whom either formalin-fixed, paraffin-embedded ($n=75$) or frozen ($n=4$) tissue were available for mutational analysis were identified. Patients were diagnosed with low-grade serous carcinoma between 1975 and 2009. Patient characteristics are detailed in Table 1. Sixty-five (82.3%) patients had stage II–IV de novo tumour, and 14 (17.7%) had metastatic tumour following an original diagnosis of advanced stage STLMP. The primary site of disease was ovary for 66 (83.5%) patients and peritoneum for 13 (16.5%) patients. Median age at diagnosis was 46 years (range, 21–79 years). Most patients underwent surgery at some point during their clinical course, and most had multiple lines of systemic therapies. The majority of patients underwent primary cytoreductive surgery, had gross residual disease at completion of surgery, and initially received platinum-based chemotherapy. There were no significant differences in characteristics between wild-type and BRAF/KRAS mutation cases except for site (ovary *vs* peritoneum).

Mutational analysis revealed KRAS mutation (12 G12D, 2 G12V, 2 G12A, 1 G12S, and 1 G12R) in 18 (22.8%) cases and BRAF V600E mutation in 3 (3.8%) cases, for a total of 21 mutations (26.6%). No detectable mutations (wild-type) of KRAS or BRAF were identified in 58 (73.4%) cases. The median OS for the entire cohort of 79 patients was 81.3 months (95% CI, 66.1, 96.4 months). The median OS for women whose tumours contained a KRAS or BRAF mutation was 106.8 months (95% CI, 50.6, 162.9) compared with 66.8 months (95% CI, 43.6, 90.0)

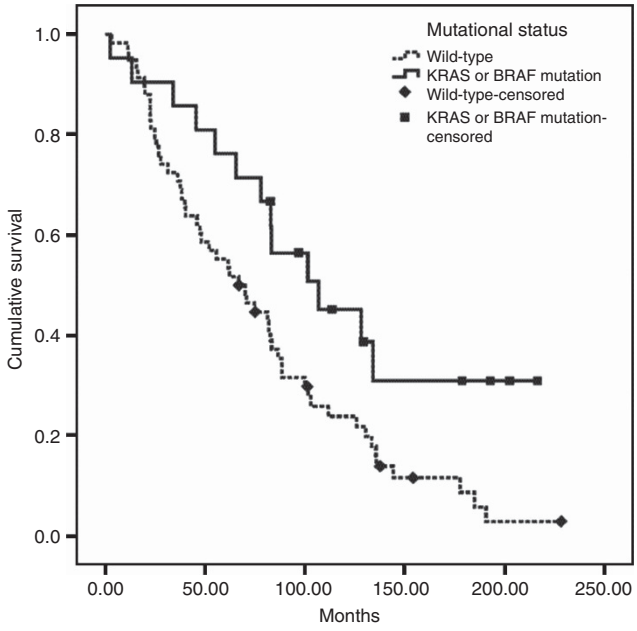

Figure 1. Overall survival. The median OS for women with KRAS or BRAF mutation was 106.8 months (95% CI, 50.6, 162.9) compared with 66.8 months (95% CI, 43.6, 90.0) for women whose tumours contained no KRAS or BRAF mutations ($P = 0.018$).

for women whose tumours contained no KRAS or BRAF mutations ($P = 0.018$) (Figure 1).

The results of univariable and multivariable Cox proportional hazards regression are shown in Table 2. Only KRAS/BRAF mutation status, residual disease at the completion of surgery, and disease status at the completion of primary therapy were included in the multivariable analysis. Compared with the wild type, the presence of a KRAS/BRAF mutation conferred a protective effect on OS (HR = 0.49; 95% CI (0.26, 0.95); $P = 0.03$). Conversely, compared with no disease at the completion of primary therapy, the presence of persistent disease resulted in compromised OS (HR = 2.17; 95% CI (1.23, 3.83); $P = 0.007$).

DISCUSSION

Our preliminary results suggest that a KRAS or BRAF mutation may serve as a favourable prognostic factor and have a significant impact on outcome in women with metastatic low-grade serous carcinoma of the ovary or peritoneum. Furthermore, after adjusting for the effects of other variables, the influence of KRAS/BRAF mutational status on OS remained statistically significant. Potential limitations of this study include the traditional method of genomic sequencing and potential selection bias associated with patients seen in a tertiary care centre. In addition, we have combined cases of BRAF and KRAS mutations, not definitely understanding whether the effect of these mutations on

Table 2. Univariable and multivariable Cox proportional hazards for overall survival

	Univariable			Multivariable		
Variable	**HR**	**95% CI**	***P***	**HR**	**95% CI**	***P***
Primary site						
Ovary (reference) Peritoneal	— 0.98	— 0.51, 1.88	— 0.95			
Age, years	1.00	0.98, 1.02	0.81			
Race						
White (reference) Non-white	— 1.14	— 0.59, 2.26	— 0.69			
Surgery type			0.37			
Primary CRS (reference) NACT followed by IDS No surgery	— 0.84 0.38	— 0.41, 1.71 0.09, 1.55	— 0.64 0.18			
KRAS/BRAF mutation status						
Wild type (reference) KRAS/BRAF mutation	— 0.48	— 0.26, 0.89	— 0.02	— 0.49	— 0.26, 0.95	— 0.03
Residual disease at completion of surgery			0.03			0.26
No gross residual disease (reference) Gross residual disease No surgery Unknown	— 2.41 0.71 1.57	— 1.26, 4.60 0.16, 3.20 0.50, 4.96	— 0.008 0.65 0.44	— 1.53 0.46 1.06	— 0.74, 3.16 0.10, 2.15 0.32, 3.51	— 0.25 0.32 0.92
Disease status at completion of primary therapy			0.002			0.03
No disease (reference) Disease present No chemotherapy	— 2.46 0.68	— 1.44, 4.22 0.16, 2.89	— 0.001 0.60	— 2.17 1.03	— 1.23, 3.83 0.23, 4.63	— 0.007 0.97
Stage			0.88			
II (reference) III/IV STLMP→LGSC	— 1.31 1.21	— 0.41, 4.25 0.39, 4.35	— 0.65 0.77			
LGSC type						
Recurrent LMP (reference) *de novo* LGSC	— 1.07	— 0.57, 2.00	— 0.84			

Abbreviations: CI = confidence interval; CRS = cytoreductive surgery; HR = hazard ratio; IDS = interval debulking surgery; LGSC = low-grade serous carcinoma; NACT = neoadjuvant chemotherapy; STLMP = serous tumour of low malignant potential; STLMP = serous tumour of low malignant potential; LGSC = low-grade serous carcinoma; LMP = low malignant potential.

outcome is similar. We have done so because there are only three cases with BRAF mutation, but future studies of larger cohorts will hopefully further illuminate this issue. Future investigations need to include more sensitive next-generation sequencing techniques, interrogation of other gene mutations, such as NRAS, and a larger number of patients with comparable long follow-up times. For example, Emmanuel *et al* (2014) found NRAS mutations in 9% of invasive serous carcinomas with adjacent STLMP. In addition, if our findings are confirmed, combined with future data on the activity of targeted therapies in low-grade serous carcinoma in the context of their molecular profile, this information may allow greater individualization of treatment.

In contrast to the findings of this study, several reports have suggested the association of KRAS or BRAF mutations with poorer outcome compared with wild-type KRAS or BRAF in a variety of malignancies (Andreyev *et al*, 2001; Souglakos *et al*, 2009; Johnson *et al*, 2012). The explanation for this potential discordance is unclear. The ability of oncogenes to induce senescence in normal cells and premalignant tumours is well established (Dhomen *et al*, 2009; Collado and Serrano, 2010; Vicent *et al*, 2010). In addition, when wild-type p53 is reactivated in a mouse hepatocellular carcinoma induced by oncogenic ras and knockdown of p53, tumours cells undergo senescence and activation of the immune system. In one report, immune cells rapidly cleared senescent tumour cells to prevent further progression or even resulted in regression (Xue *et al*, 2007). As most low-grade ovarian serous cancer cells have wild-type p53, it is possible that this subtype with a KRAS mutation may have senescent tumour cells that are cleared by immune cells, thereby inhibiting tumour progression. However, further investigation to elucidate this potential mechanism is required.

Although low-grade serous carcinoma is associated with superior survival outcomes compared with high-grade serous carcinoma and other high-grade ovarian cancers, such as clear cell and high-grade endometrioid subtypes, nevertheless, over 70% of women with low-grade serous carcinoma relapse and ultimately succumb to their cancer. Thus, it is important that we continue to concentrate on better understanding the biology of this rare subtype while concomitantly working toward improving treatment.

ACKNOWLEDGEMENTS

Funding: This work was supported in part by The National Cancer Institute at the National Institutes of Health (P50 CA83639 to DMG and KKW), National Institutes of Health through Cancer Center Support Grant (P30 CA016672 to DMG, CCS, and KKW), and The Sara Brown Musselman Fund for Serous Ovarian Cancer Research (DMG, CCS, and KKW).

CONFLICT OF INTEREST

The authors declare no conflict of interest.

REFERENCES

Andreyev HJ, Norman AR, Cunningham D, Oates J, Dix BR, Iacopetta BJ, Young J, Walsh T, Ward R, Hawkins N, Beranek M, Jandik P, Benamouzig R, Jullian E, Laurent-Puig P, Olschwang S, Muller O, Hoffmann I, Rabes HM, Zietz C, Troungos C, Valavanis C, Yuen ST, Ho JW, Croke CT, O'Donoghue DP, Giaretti W, Rapallo A, Russo A, Bazan V, Tanaka M, Omura K, Azuma T, Ohkusa T, Fujimori T, Ono Y, Pauly M, Faber C, Glaesener R, de Goeij AF, Arends JW, Andersen SN, Lövig T, Breivik J, Gaudernack G, Clausen OP, De Angelis PD, Meling GI, Rognum TO, Smith R, Goh HS, Font A, Rosell R, Sun XF, Zhang H, Benhattar J, Losi L, Lee JQ, Wang ST, Clarke PA, Bell S, Quirke P, Bubb VJ, Piris J, Cruickshank NR, Morton D, Fox JC, Al-Mulla F, Lees N, Hall CN, Snary D, Wilkinson K, Dillon D, Costa J, Pricolo VE, Finkelstein SD, Thebo JS, Senagore AJ, Halter SA, Wadler S, Malik S, Krtolica K, Urosevic N (2001) Kirsten ras mutations in patients with colorectal cancer: the 'RASCAL II' study. *Br J Cancer* **85**: 692–696.

Collado M, Serrano M (2010) Senescence in tumours: evidence from mice and humans. *Nat Rev Cancer* **10**: 51–57.

Crispens MA, Bodurka D, Deavers M, Lu K, Silva EG, Gershenson DM (2002) Response and survival in patients with progressive or recurrent serous ovarian tumors of low malignant potential. *Obstet Gynecol* **99**: 3–10.

Dhomen N, Reis-Filho JS, da Rocha Dias S, Hayward R, Savage K, Delmas V, Larue L, Pritchard C, Marais R (2009) Oncogenic Braf induces melanocyte senescence and melanoma in mice. *Cancer Cell* **15**: 294–303.

Emmanuel C, Chiew Y-E, George J, Etemadmoghadam D, Sharma R, Russell P, Kennedy C, Fereday S, Hung J, Galletta L, Hogg R, Wain GV, Brand A, Balleine R, Anglesio MS, MacConaill LE, Palescandolo E, Hunter SM, Campbell I, Dobrovic A, Wong SQ, Do H, Clarke CL, Harnett PR, Bowtell DD, deFazio A. Australian Ovarian Cancer Study (AOCS) (2014) Genomic classification of serous ovarian cancer with adjacent borderline differentiates RAS-pathway and TP53-mutant tumors and identifies NRAS as an oncogenic driver. *Clin Cancer Res* **20**(24): 6618–6630.

Farley J, Brady WE, Vathipadiekal V, Lankes HA, Coleman R, Morgan MA, Mannel R, Yamada SD, Mutch D, Rodgers WH, Birrer M, Gershenson DM (2013) Selumetinib in women with recurrent low-grade serous carcinoma of the ovary or peritoneum: an open-label, single-arm, phase 2 study. *Lancet Oncol* **14**: 134–140.

Gershenson DM, Sun CC, Lu KH, Coleman RL, Sood AK, Malpica A, Deavers MT, Silva EG, Bodurka DC (2006) Clinical behavior of stage II-IV low-grade serous carcinoma of the ovary. *Obstet Gynecol* **108**: 361–368.

Gershenson DM, Bodurka D, Lu K, Nathan LC, Milojevic L, Wong KK, Malpica A, Sun CC (2015) The impact of age and primary disease site on outcome of women with low-grade serous carcinoma of the ovary or peritoneum: Results of a large single-institution registry of a rare tumor. *J Clin Oncol* **33**: 2675–2682.

Grisham RN, Iyer G, Garg K, DeLair D, Hyman DM, Zhou Q, Iasonos A, Berger MF, Dao F, Spriggs DR, Levine DA, Aghajanian C, Solit DB (2012) BRAF Mutation is associated with early stage disease and improved outcome in patients with low-grade serous ovarian cancer. *Cancer* **119**: 548–554.

Johnson ML, Sima CS, Chaft J, Paik PK, Pao W, Kris MG, Ladanyi M, Riely GJ (2012) Association of KRAS and EGFR mutations with survival in patients with advanced lung adenocarcinomas. *Cancer* **119**: 356–362.

Kaplan EL, Meier P (1958) Nonparametric estimation from incomplete observations. *J Am Stat Assoc* **53**: 457–481.

Malpica A, Deavers MT, Lu K, Bodurka DC, Atkinson EN, Gershenson DM, Silva EG (2004) Grading ovarian serous carcinoma using a two-tier system. *Am J Surg Pathol* **28**: 496–504.

Schmeler KM, Sun CC, Malpica A, Deavers MT, Bodurka DC, Gershenson DM (2011) Low-grade serous primary peritoneal carcinoma. *Gynecol Oncol* **121**: 482–486.

Shvartsman HS, Sun CC, Bodurka DC, Mahajan V, Crispens M, Lu KH, Deavers MT, Malpica A, Silva EG, Gershenson DM (2007) Comparison of the clinical behavior of newly diagnosed stages II-IV low-grade serous carcinoma of the ovary with that of serous ovarian tumors of low malignant potential that recur as low-grade serous carcinoma. *Gynecol Oncol* **105**: 625–629.

Singer G, Oldt R, Cohen Y, Wang BG, Sidransky D, Kurman RJ, Shih I-M (2003) Mutations in BRAF and KRAS characterize the development of low-grade ovarian serous carcinoma. *J Natl Cancer Inst* **95**: 484–486.

Souglakos J, Philips J, Wang R, Marwah S, Silver M, Tzardi M, Silver J, Ogino S, Hooshmand S, Kwak E, Freed E, Meyerhardt JA, Saridaki Z, Georgoulias V, Finkelstein D, Fuchs CS, Kulke MH, Shivdasani RA (2009) Prognostic and predictive value of common mutations for treatment response and survival in patients with metastatic colorectal cancer. *Br J Cancer* **101**: 465–472.

Tsang YT, Deavers MT, Sun CC, Kwan S-Y, Kuo E, Malpica A, Mok SC, Gershenson DM, Wong K-K (2013) KRAS (but not BRAF) mutations in

ovarian serous borderline tumour are associated with recurrent low-grade serous carcinoma. *J Pathol* **231**: 449–456.

Vicent S, Chen R, Sayles LC, Lin C, Walker RG, Gillespie AK, Subramanian A, Hinkle G, Yang X, Saif S, Root DE, Huff V, Hahn WC, Sweet-Cordero EA (2010) Wilms tumor 1 (WT1) regulates KRAS-driven oncogenesis and senescence in mouse and human models. *J Clin Invest* **120**: 3940–3952.

Wong K-K, Tsang YT, Deavers MT, Mok SC, Zu Z, Sun C, Malpica A, Wolf JK, Lu KH, Gershenson DM (2010) BRAF mutation is rare in advanced-stage low-grade ovarian serous carcinomas. *Am J Pathol* **177**: 1611–1617.

Xue W, Zender L, Miething C, Dickins RA, Hernando E, Krizhanovsky V, Cordon-Cardo C, Lowe SW (2007) Senescence and tumour clearance is triggered by p53 restoration in murine liver carcinomas. *Nature* **445**: 656–660.