

British Journal of Cancer (2013) 108, 715–720 | doi: 10.1038/bjc.2012.536

Keywords: glioma; statins; brain tumour epidemiology

# Use of statins and risk of glioma: a nationwide case-control study in Denmark

D Gaist<sup>\*,1</sup>, L Andersen<sup>1</sup>, J Hallas<sup>2</sup>, H Toft Sørensen<sup>3</sup>, HD Schrøder<sup>4</sup> and S Friis<sup>5</sup>

<sup>1</sup>Department of Neurology, Odense University Hospital, Institute of Clinical Research, Faculty of Health Sciences, University of Southern Denmark, Sdr. Boulevard 29, 5000 Odense C, Denmark; <sup>2</sup>Clinical Pharmacology, Institute of Public Health, University of Southern Denmark, J.B. Winsløws Vej 19, 2.sal, 5000 Odense C, Denmark; <sup>3</sup>Department of Clinical Epidemiology, Aarhus University Hospital, Olof Palmes Allé 43-45, 8200 Aarhus N, Denmark; <sup>4</sup>Department of Pathology, Institute of Clinical Research, Odense University Hospital, Winsløwparken 15, 2. sal, 5000 Odense C, Denmark and <sup>5</sup>Danish Cancer Society Research Centre & Department of Public Health, University of Copenhagen, Strandboulevarden 49, 2100, Copenhagen Ø, Denmark

**Background:** Laboratory studies and a single case-control study have suggested a protective effect of statins on the risk of glioma. We wished to investigate the influence of statin use on the risk of glioma in a population-based setting.

**Methods:** We conducted a nationwide case–control study in Denmark based on population-based medical registries. We identified all patients aged 20 to 85 years with a first diagnosis of histologically verified glioma during 2000–2009. These cases were matched on birth year and sex with population controls. Prior use of statins since 1995 was classified into short-term use (<5 years) and long-term use (5 + years). We used conditional logistic regression to compute odds ratios (ORs), with 95% confidence intervals (Cls), for glioma associated with statin use, adjusted for potential confounders.

**Results:** A total of 2656 cases and 18480 controls were included in the study. The risk of glioma was reduced among long-term statin users (OR = 0.76; 95% CI: 0.59–0.98) compared with never users of statins, and was inversely related to the intensity of statin treatment among users (OR = 0.71; 95% CI: 0.44–1.15 for highest intensity). The inverse association between long-term statin treatment and glioma risk was more pronounced among men aged  $\leq 60$  years (OR = 0.40; 95% CI: 0.17–0.91) compared with men aged 60 + years (OR = 0.71; 95% CI: 0.49–1.03). An inverse association was also observed among women aged  $\leq 60$  years (OR = 0.28; 95% CI: 0.06–1.25), but not among women over age 60 years (OR = 1.23; 95% CI: 0.82–1.85).

Conclusion: Long-term statin use may reduce the risk of glioma.

Given the widespread and rapidly increasing use of statins, any association with cancer development or progression would have a substantial impact on public health. At present, statins cannot be recommended for primary cancer prevention or therapy because of conflicting evidence (Boudreau *et al*, 2010).

However, preclinical findings of antineoplastic activity of statins warrant their further evaluation as potential chemopreventive agents (Chan *et al*, 2003; Sassano and Platania, 2008; Tapia-Pérez *et al*, 2010). One line of investigation deserving particular attention is the effect of statins on gliomas, a group of central nervous system tumours of largely unknown aetiology. The most common histological subtype, glioblastoma multiforme, accounts for more

\*Correspondence: Dr D Gaist; E-mail: dgaist@health.sdu.dk

Received 6 August 2012; revised 6 November 2012; accepted 6 November 2012; published online 15 January 2013

© 2013 Cancer Research UK. All rights reserved 0007–0920/13

than 50% of gliomas, and has an incidence rate of 3.5 per 1 00 000 person-years in Nordic countries and male predominance (Lönn *et al*, 2004).

Laboratory studies of human glioma cell lines indicate that statins may exert antitumour activity through such mechanisms as inhibition of cellular proliferation, growth, migration, and by induction of apoptosis (Jones *et al*, 1994; Soma *et al*, 1994; Bouterfa *et al*, 2000; Obara *et al*, 2002; Gliemroth *et al*, 2003; Jiang *et al*, 2004; Chan *et al*, 2008; Wu *et al*, 2009; Yanae *et al*, 2011). In a phase I/II study of 18 patients with malignant gliomas, lovastatin with and without radiotherapy was well tolerated, but had minimal effect on tumour progression (Larner *et al*, 1998). To date, only one



case–control study has addressed the risk of glioma among statin users (Ferris *et al*, 2012). This study reported that  $\ge 6$  months of simvastatin use was inversely associated with glioma risk (odds ratio (OR) = 0.72; 95% confidence interval (CI): 0.52–1.00). Recall bias was a potential shortcoming of this interview-based study, with information collected from proxies in ~19% of cases.

We therefore conducted a nationwide population-based casecontrol study utilising registry data to further investigate the association between statin use and glioma risk.

## MATERIALS AND METHODS

We conducted a nested case-control study based on information from population-based Danish registries: the Danish Cancer Registry (DCR) (Storm *et al*, 1997; Gjerstorff, 2011), the Danish Civil Registration System (Pedersen, 2011), the Danish National Prescription Registry (Kildemoes *et al*, 2011), the Danish National Patient Register (DNPR) (Lynge *et al*, 2011), and Statistics Denmark. Unambiguous linkage between the registries was possible using the civil registration number assigned to all Danish residents since 1968, at birth or upon immigration to the country (Pedersen, 2011). Danish citizens, who are mainly Caucasians, have equal tax-supported access to health care provided by the Danish National Health Service.

**Case ascertainment.** The DCR has recorded incident cases of cancer on a nationwide basis since 1943 and has been shown to have an almost complete ascertainment of cancer cases (Storm *et al*, 1997; Gjerstorff, 2011). Reporting of gliomas to the DCR is mandatory for all levels of malignancy. Cancer diagnoses in the DCR are recorded according to the International Classification of Diseases, version 10 (ICD-10), and the ICD for Oncology (ICD-O-3) for topography and morphology codes.

Eligible cases were individuals with a first diagnosis of cranial or spinal glioma irrespective of level of malignancy, and no prior cancer diagnoses (except nonmelanoma skin cancer) in the DCR during the period from 1 January 2000 to 31 December 2009. A diagnosis of glioma was determined on the basis of ICD-10 diagnoses (see Appendix Table A1 for codes) that were histologically confirmed, that is, with morphology codes (ICD-O-3; see Appendix Table A1). We further classified cases by glioma subtype, that is, glioblastoma multiforme (see Appendix Table A1), astrocytoma grades II and III, oligodendroglioma grades II and III, and 'other'. The date of diagnosis recorded in the DCR was defined as the index date. We restricted the cases to individuals aged 20 to 85 years at diagnosis.

Selection of population controls. For each case, eight controls matched on birth year and sex were selected from the total Danish population through the Civil Registration System (Pedersen, 2011) using risk-set sampling (Rothman *et al*, 2008); that is, the controls had to be alive and at risk for a first diagnosis of cancer (except nonmelanoma skin cancer) at the time the corresponding case was diagnosed (index date). The Civil Registration System is continuously updated and includes information on vital status and migration. We used this information to restrict cases and controls to individuals who had resided in Denmark for at least 10 consecutive years before the index date. As the latter restriction was imposed after sampling of controls, the final ratio of cases to controls deviated slightly from 1:8.

**Statin exposure.** Information on use of statins and other drugs was obtained from the National Prescription Registry, which contains information on all prescriptions dispensed at community pharmacies in Denmark since 1995 (Kildemoes *et al*, 2011). For each prescription, the Prescription Registry records date and a full description of the dispensed product, including the anatomical therapeutic code (ATC)

(WHO, 2010) and the total number of defined daily doses (DDDs). A DDD, established by a group of experts, represents the typical daily dose required by an adult when the drug is used for its main indication (WHO, 2010). Drugs used for the same indication are in principle equipotent when measured in DDD.

We retrieved all information available from the Prescription Registry from 1995 to the index date for both cases and controls. Based on the number of statin prescriptions dispensed during the period from 1995 up to 1 year before the index date, study subjects were classified as statin ever users ( $\geq 2$  prescriptions recorded under ATC codes C10AA) and statin never users (no prescriptions for statins). Subjects with a single statin prescription were not included in the main analyses. The risk of a 'reverse causation' bias (Csizmadl et al, 2007, pp 791–810) is inherent to the study, as the first symptoms of glioma in some cases might be interpreted as a manifestation of cerebrovascular disease and result in the patient being prescribed a statin. At a later stage, it becomes evident that the patient has a tumour. Such a scenario would create a spurious excess of cancer diagnoses after statin initiation or would mask a possible genuine preventive effect. To minimise this potential bias, we disregarded statin prescriptions dispensed within 1 year before the index date.

Duration of statin use was defined as the time period between the first and last redeemed statin prescription and classified as short duration (1–5 years before index date) or long duration (5 + years before index date). We defined intensity of statin use as the cumulative number of DDDs of statins prescribed to a study subject divided by the number of days between the first and last eligible statin prescription plus 60 days. Using tertiles of intensity of statin use among controls as cutoff values, we classified intensity of use as low (lower tertile), medium (middle tertile), and high (upper tertile). In subanalyses, we classified statins as lipophilic (simvastatin, lovastatin, fluvastatin, atorvastatin, and cerivastatin) and hydrophilic (pravastatin and rosuvastatin).

**Potential confounders.** As a marker of socioeconomic status, we used the highest educational level achieved by subjects according to annually updated information from Statistics Denmark (Jensen and Rasmussen, 2011). We divided study subjects into three categories according to the number of years of schooling (7–10, 11-12, and 13 + years).

Patients suffering from a stroke are frequently prescribed statins and undergo neuroimaging. The latter might in some instances coincidentally reveal gliomas. We therefore regarded a history of stroke as a potential confounder. We defined subjects as having a history of stroke if they were recorded with ICD codes compatible with this diagnosis (see Appendix Table A1 for codes) in the DNPR, which contains data on all admissions to nonpsychiatric hospitals in Denmark since 1977 and on all outpatient contacts since 1995, including patients' civil registration number, date of admission/contact, and diagnosis codes.

Because diabetes is under intense scrutiny for its possible association with cancer (Carstensen *et al*, 2012) and is associated with statin use, we classified study subjects as diabetics if they had a history of diabetes mellitus according to the DNRP (Lynge *et al*, 2011) or had redeemed prescriptions for antidiabetic drugs before the index date (see Appendix Table A1 for codes). We also considered use of certain drugs previously reported to modify the risk of some cancers. Study subjects were classified as ever users of the following individual compounds if they had redeemed two or more prescriptions one or more years before the index date: hormone replacement therapy (HRT), low-dose aspirin, selective Cox-2 inhibitors, and other non-aspirin (NA)-NSAIDs (see Appendix Table A1 for codes).

We used parity as a proxy measure for exposure to endogenous sex hormones in women, as these may influence their glioma risk (Fisher *et al*, 2007). We calculated parity as of the index date for female cases and controls based on information available in the nationwide Fertility Database maintained by Statistics Denmark (Blenstrup and Knudsen, 2011). The women were classified into the following categories according to the number of live births: 0 (nullipara), 1, 2, 3 +, or 'missing information'.

Statistical analysis. We used conditional logistic regression to compute adjusted ORs (and 95% CI) for glioma associated with statin use, adjusting for age (birth year), sex, and time period (year of index date) and for potential confounders (years of schooling, diabetes, stroke, and use of aspirin, selective Cox2 inhibitors, and NA-NSAIDs). We tested the effect of intensity of statin treatment for trend. To explore potential effect measure modification, we performed analyses stratified by age (dichotomised according to median age of controls, i.e.,  $<60 \ vs \ge 60$  years) and gender. Information on HRT use and parity was included only in models restricted to female study subjects used in sensitivity analyses. All analyses were performed using Stata SE 12.1 (StataCorp, College

Table 1. Characteristics of study subjects in a nationwide case–control study of glioma in Denmark, 2000–2009		
Characteristic	Cases (N = 2656)	Controls ( <b>N</b> = 18 480)
Gender		
Female Male	1070 (40.3) 1586 (59.7)	7050 (38.2) 11 430 (61.9)
Age, years		
20–29 30–39 40–49 50–59 60–69 70–79 80–85	119 (4.5) 215 (8.1) 421 (15.9) 681 (25.6) 731 (27.5) 426 (16.0) 63 (2.4)	789 (4.3) 1485 (8.0) 3019 (16.3) 4905 (26.5) 5094 (27. 6) 2805 (15.2) 383 (2.1)
Parity, number of children <sup>a,</sup>	Ь	
0 1 2 3 + Missing	97 (9.1) 165 (15.4) 418 (39.1) 235 (22.0) 155 (14.5)	652 (9.3) 1100 (15. 6) 2686 (38.1) 1624 (23.0) 988 (14.0)
Schooling, number of years		
7–10 11–12 13 + Missing Diabetes Stroke	940 (35.4) 1086 (40.9) 587 (22.1) 43 (1.6) 105 (4.0) 313 (11.8)	6395 (34.6) 7608 (41.2) 4117 (22.3) 360 (2.0) 838 (4.5) 463 (2.5)
Drug use		
Statins Aspirin, low dose Selective Cox2 inhibitors NA-NSAIDs <sup>c</sup> Hormone replacement therapy <sup>a</sup>	214 (8.1) 251 (9.5) 82 (3.1) 1047 (39.4) 290 (27.1)	1601 (8.7) 1900 (10.3) 489 (2.7) 7263 (39.3) 2070 (29.4)

Abbreviations: Cox-2 = cyclooxygenase 2; NA-NSAIDs = non-aspirin-nonsteroidal antiinflammatory drugs. <sup>a</sup>Women only.

<sup>b</sup>Data incomplete for women born before 1945. No data available on births in 2009. <sup>c</sup>NA-NSAIDs other than Cox2 inhibitors.

Shown are numbers (percentages).

Station, TX, USA). The study was approved by the Danish Data Protection Agency and the Danish Medicinal Agency.

## RESULTS

Our study population comprised 2656 cases and 18 480 controls. Of these, 1586 cases (59.7%) and 11 430 controls (61.9%) were male. Cases and controls were also similar with regard to the distribution of age, parity, years of schooling, prevalence of diabetes, and use of aspirin, selective Cox2 inhibitors, NA-NSAIDs, and HRT, but not stroke (Table 1). Among subjects treated with statins ( $\geq$ 2 prescriptions), the median (interquartile range (IQR)) dose was 724 DDD (IQR: 387–1262 DDD) in cases and 720 DDD (IQR, 372–1391 DDD) in controls, and the median duration of treatment was 2.4 years (IQR, 1.1–4.7 years) in cases and 2.8 years (IQR, 1.2–5.2 years) in controls (excluding prescriptions dispensed during the year before the index date).

Long-term statin use was associated with a reduced risk of glioma (OR = 0.76; 95% CI: 0.59-0.98; Table 2) and was inversely related to treatment intensity (high-intensity treatment: OR = 0.71; 95% CI: 0.44-1.15; P-value for trend: 0.041; Table 3). The effect of long-term high-intensity use was restricted to lipophilic statins (OR = 0.69; 95% CI: 0.38-1.25). The corresponding risk estimate for hydrophilic statins exceeded unity (OR = 1.45; 95% CI: 0.31-6.69), although based on small numbers. Reduction in glioma risk varied across age and gender strata (Table 4). Risk of glioma among long-term statin users was OR = 0.37 (95% CI: 0.18-0.75) among subjects under age 60 years as compared with OR = 0.91(95% CI: 0.69-1.19) among subjects aged 60 + years. Long-term statin use was inversely related with the risk of glioma among men (OR = 0.61; 95% CI: 0.44-0.86), but not among women (OR = 1.01; 95% CI: 0.69-1.49; Table 4). However, among female long-term statin users under age 60 years, the risk of glioma was OR = 0.28 (95% CI: 0.06-1.25). Among men, the risk reduction was also more pronounced among those under age 60 years (OR = 0.40; 95% CI: 0.17-0.91), but was also substantial among men aged 60 + years (OR = 0.71; 95% CI: 0.49-1.03). Ever use of statins reduced the risk of glioblastoma multiforme (OR = 0.90; 95% CI: 0.73-1.12), the most frequent type of glioma (57.9% of cases; Supplementary eTable 1). The risk reduction for glioblastoma multiforme was more pronounced among long-term statin users (OR = 0.79; 95% CI: 0.59–1.06), in particular among subjects with high intensity statin use (OR = 0.67; 95% CI: 0.37–1.20).

We performed a number of sensitivity analyses. We first repeated all analyses including those with one prescription only in the non-use reference group, then with long-term use defined as 7 + years of statin use, and lastly excluding NSAIDs as a covariate. Analyses with women in separate strata were repeated with HRT and parity included as confounder variables. The results of the sensitivity analyses were very similar to those of the main analyses (data not presented).

Table 2. Ever use of statins and risk of glioma				
Use of statin	Cases	Controls	Crude OR (95% Cl)	Adjusted OR <sup>a</sup> (95% CI)
Never	2442	16879	1 (ref.)	1 (ref.)
Ever	214	1601	0.89 (0.76–1.05)	0.88 (0.73–1.05)
<5 years	118	770	1.02 (0.83–1.26)	0.96 (0.76–1.20)
5+ years	96	831	0.77 (0.61–0.96)	0.76 (0.59–0.98)

Abbreviations: CI = confidence interval; OR = odds ratio; ref. = reference. <sup>a</sup>Adjusted for years of schooling, diabetes, stroke, and use of aspirin, selective cyclooxygenase 2 (Cox2) inhibitors, and non-aspirin-nonsteroidal anti-inflammatory drugs (NA-NSAIDs).

Table 3. Duration and intensity of statin use and glioma risk				
Statin use	Cases	Controls	Crude odds ratio (95% confidence interval (CI))	Adjusted odds ratio <sup>a</sup> (95% Cl)
Never use	2442	16 879	1 (reference)	1 (reference)
Short-term use <sup>b,c</sup>				
Low intensity	24	179	0.87 (0.57–1.34)	0.94 (0.60–1.47)
Medium intensity	41	250	1.11 (0.79–1.57)	1.10 (0.77–1.56)
High intensity	53	341	1.04 (0.77–1.41)	1.01 (0.73–1.39)
Long-term use <sup>b,c</sup>				
Low intensity	42	352	0.81 (0.58–1.12)	0.81 (0.57–1.14)
Medium intensity	33	273	0.78 (0.54–1.14)	0.86 (0.58–1.26)
High intensity	21	206	0.67 (0.42–1.06)	0.71 (0.44–1.15)

<sup>a</sup>Adjusted for years of schooling, diabetes, stroke, and use of aspirin, selective cyclooxygenase 2 (Cox2) inhibitors, and non-aspirin-nonsteroidal anti-inflammatory drugs (NA-NSAIDs). <sup>b</sup>Short term: <5 years of use; long term: 5 + years of use.

<sup>c</sup>Cutoff values for low-, medium-, and high-intensity statin use defined by tertiles of intensity of use among controls.

#### DISCUSSION

We found that long-term use of statins was associated with a reduced risk of glioma. Although based on limited statistical precision, the potential chemopreventive effect was limited to users of lipophilic statins. This may be explained by the physiological properties of lipophilic statins, that is, their better ability to cross the blood-brain barrier compared with hydrophilic statins (Botti *et al*, 1991; Vuletic *et al*, 2006). Furthermore, the effect of long-term statin use may be more pronounced among men and, for both genders, among those <60 years of age. We found it particularly intriguing that the point estimates for our main findings remained unchanged when we limited to cases with histologically verified glioblastoma multiforme, the most aggressive form of glioma, with only 3.3% of patients surviving for 5 years (Bondy *et al*, 2008).

In the only other epidemiological study that has addressed the relation between statin use and glioma risk, a case–control study conducted in the United States, statin use among 458 cases was compared with that among 353 controls (Ferris *et al*, 2012). Statin use for  $\geq 6$  months was associated with a reduced risk of glioma (OR = 0.72; 95% CI: 0.52–1.00) and the risk was further reduced in subjects with a long treatment duration (>120 months of use, OR = 0.44; 95% CI: 0.15–0.97). The OR of glioma associated with statin use was 0.59 (95% CI: 0.38–0.92) among men and 0.85 (95% CI: 0.51–1.43) among women. Our findings are compatible with this study, including the gender difference in the risk estimates for glioma, although direct comparisons between the two studies are hampered by differences in design and setting.

Our study has a number of strengths. Our study was performed in a setting with free access to health services independent of income. We used nationwide registries with complete coverage and continuously collected data on all Danish residents. Our approach thus eliminated recall bias and minimised selection bias. The DCR enabled us to identify incident cases of glioma and cancer-free controls with minimal misclassification. We also restricted our sample to histologically verified cases, which enhanced case validity and allowed us to perform analyses on subtypes of glioma. Our study also has some potential weaknesses that should be considered. Because the National Prescription Registry has collected nationwide data only since 1995, the medication histories of our subjects spanned 5 to 15 years, depending on their index dates. This left-truncation of Prescription Registry data may give rise to two potential problems. First, subjects who stopped using statins before 1995 were misclassified as never users in our study. Second, the duration of current use may be underestimated in subjects with statin use before 1995. However, such misclassifications most likely would produce a conservative misclassification bias, that is, reduce the association between statin use and glioma risk. Furthermore, as statins were only used sparsely in Denmark before the publication of the Scandinavian Simvastatin Survival Study (4S) (Scandinavian Simvastatin Survival Study Group, 1994) in 1994 (Riahi *et al*, 2001), we believe that such misclassification is minor. Our study was also limited by our inability to evaluate compliance with statin prescriptions; however, a drug utilisation study of statin use in the Danish population indicates that degree of statin persistence is high (Larsen *et al*, 2002).

It is conceivable that before their detection, brain tumours could exert influences on adherence to medication use, for example, by influencing cognitive skills. By ignoring the use of statins in the year before the index date, we believe that we have severely reduced the impact of such effects, particularly in our analyses restricted to cases of glioblastoma multiforme, a glioma subtype with wellestablished rapid development.

Exposure to ionising radiation is the only established environmental cause of glioma (Fisher et al, 2007; Bondy et al, 2008). Although we were not able to adjust for this covariate, the attributable risk proportion of radiation is small and unlikely to be associated with statin use. More importantly, we only included study subjects with an initial primary cancer, so that individuals with previous cancers who may have been exposed to ionising radiation were excluded by design. As well, lifestyle factors indirectly could influence our study findings if such factors were both related to the likelihood of being prescribed statins and risk of glioma. According to a recent Danish study linking prescription data on statin use to data from a survey of 13996 subjects (including 1641 current statin users), there was no indication of a particularly healthy lifestyle associated with statin use (Thomsen et al, 2011). However, another Danish study based primarily on statin use in the initial years following the launch of these drugs showed a clear socioeconomic gradient in statin use among men but not women (Thomsen et al, 2005). Another concern is that we only accounted for highest achieved level of schooling, which may have resulted in residual confounding of socioeconomic indicators. Importantly, however, a Danish study based on DCR data found no association between incidence of central nervous system tumours and socioeconomic status (Schmidt et al, 2008).

Table 4. Effect of duration of statin use on glioma risk by gender and age strata				
	Cases (exposed/ unexposed)	Controls (exposed/ unexposed)	Crude odds ratio (95% CI)	Adjusted odds ratio <sup>a</sup> (95% Cl)
Age				
<60 years Short term Long term 60 + years Short term Long term	28/1398 10/1398 90/1044 86/1044	205/9834 159/9834 565/7045 672/7045	0.97 (0.65–1.46) 0.46 (0.24–0.89) 1.04 (0.82–1.32) 0.84 (0.66–1.07)	0.78 (0.48–1.27) 0.37 (0.18–0.75) 1.02 (0.79–1.32) 0.91 (0.69–1.19)
Gender		L		
Female Short term Long term	48/980 42/980	260/6520 270/6520	1.20 (0.87–1.67) 1.00 (0.71–1.42)	1.12 (0.79–1.61) 1.01 (0.69–1.49)
Short term Long term	70/1462 54/1462	510/10 359 561/10 359	0.93 (0.71–1.21) 0.65 (0.48–0.87)	0.86 (0.64–1.16) 0.61 (0.44–0.86)
Gender and age				
Female, <60 years Short term Long term	7/550 2/550	59/3654 37/3654	0.79 (0.35–1.75) 0.37 (0.09–1.54)	0.73 (0.30–1.77) 0.28 (0.06–1.25)
Female, 60+ years Short term Long term	41/430 40/430	201/2866 233/2866	1.33 (0.93–1.92) 1.11 (0.77–1.59)	1.26 (0.85–1.87) 1.23 (0.82–1.85)
Male, <60 years Short term Long term	21/848 8/848	146/6180 122/6180	1.06 (0.66–1.69) 0.49 (0.24–1.03)	0.80 (0.44–1.44) 0.40 (0.17–0.91)
Male, 60+ years Short term Long term	49/614 46/614	364/4179 439/4179	0.88 (0.64–1.21) 0.69 (0.50–0.96)	0.87 (0.61–1.23) 0.71 (0.49–1.03)

Abbreviation: CI=Confidnce interval. Short term: <5 years of use; long term: 5+ years of use.

<sup>a</sup>Adjusted for years of schooling, diabetes, stroke, and use of aspirin, selective cyclooxygenase 2 (Cox2) inhibitors, and non-aspirin-nonsteroidal anti-inflammatory drugs (NA-NSAIDs).

Therefore, lifestyle factors and socioeconomic status are not likely to have substantially affected our findings.

# REFERENCES

Because of the observational design of our study, we cannot exclude the possibility that inadequately measured confounders influenced our results, although at present only a limited number of risk factors have been established for glioma (Fisher *et al*, 2007; Bondy *et al*, 2008).

Therefore, our finding of a reduced risk of glioma associated with long-term statin use may be causal. The possibility of genderand age-specific effects, indicated by our study, if replicated in other settings, could potentially provide guidance to targeted therapeutic intervention trials.

# ACKNOWLEDGEMENTS

This study was supported by grants from the Danish Cancer Society (grant no. R56-A2879) and University of Southern Denmark. The funding sources had no role in the design, analysis, and interpretation of the results, and thus the authors were independent from the funding sources.

## CONFLICT OF INTEREST

The authors declare no conflict of interest.

Blenstrup LT, Knudsen LB. Danish registers on aspects of reproduction. Scand J Public Health (2011) 39(Suppl 7): 79–82.

- Bondy ML, Scheurer ME, Malmer B, Barnholtz-Sloan JS, Davis FG, Il'yasova D, Kruchko C, McCarthy BJ, Rajaraman P, Schwartzbaum JA, Sadetzki S, Schlehofer B, Tihan T, Wiemels JL, Wrensch M, Buffler PA (2008) Brain tumor epidemiology: consensus from the brain tumor epidemiology consortium. *Cancer* 113(Suppl 7): 1953–1968.
- Botti RE, Triscari J, Pan HY, Zayat J (1991) Concentrations of pravastatin and lovastatin in cerebrospinal fluid in healthy subjects. *Clin Neuropharmacol* 14: 256–261.
- Boudreau DM, Yu O, Johnson J (2010) Statin use and cancer risk: a comprehensive review. *Expert Opin Drug Saf* **9**: 603–621.
- Bouterfa HL, Sattelmeyer V, Czub S, Vordermark D, Roosen K, Tonn JC (2000) Inhibition of Ras farnesylation by lovastatin leads to downregulation of proliferation and migration in primary cultured human glioblastoma cells. *Anticancer Res* 20: 2761–2771.
- Carstensen B, Witte DR, Friis S (2012) Cancer occurrence in Danish diabetic patients: duration and insulin effects. *Diabetologia* 55: 948–958.
- Chan KK, Oza AM, Siu LL (2003) The statins as anticancer agents. *Clin Cancer Res* **9**: 10–19.
- Chan DY, Chen GG, Poon WS, Liu PC (2008) Lovastatin sensitized human glioblastoma cells to TRAIL-induced apoptosis. J Neurooncol 86: 273–283.
- Csizmadl I, Collet J-P, Boivin J (2007) Bias and confounding in pharmacoepidemiology. In: *Pharmacoepidemiology*, Strom BL (ed) (Wilay and Sons: West Sussex (4th edn), pp, 791–810.
- Ferris J, McCoy L, Neugut A, Wrensch M, Lai RHMG (2012) CoA reductase inhibitors, NSAIDs and risk of glioma. *Int J Cancer* 131(6): E1031–E1037.

- Fisher JL, Schwartzbaum JA, Wrensch M, Wiemels JL (2007) Epidemiology of brain tumors. *Neurol Clin* 25: 867–890.
- Gjerstorff ML. The Danish Cancer Registry. Scand J Public Health (2011) 39 (7 Suppl): 42–45.
- Gliemroth J, Zulewski H, Arnold H, Terzis AJ (2003) Migration, proliferation, and invasion of human glioma cells following treatment with simvastatin. *Neurosurg Rev* 26: 117–124.
- Jensen VM, Rasmussen AW (2011) Danish education registers. *Scand J Public Health* **39**(Suppl 7): 91–94.
- Jiang Z, Zheng X, Lytle RA, Higashikubo R, Rich KM (2004) Lovastatininduced up-regulation of the BH3-only protein, Bim, and cell death in glioblastoma cells. J Neurochem 89: 168–178.
- Jones KD, Coldwell WT, Hinton DR, Su Y, He S, Anker L, Law RE (1994) Lovastatin induces growth inhibition and apoptosis in human malignant glioma cells. *Biochem Biophys Res Commun* 205: 1681–1687.
- Kildemoes HW, Toft Sørensen H, Hallas J (2011) The Danish National Prescription registry. *Scand J Public Health* **39**(7 Suppl): 38–41.
- Larner J, Lawe J, Laws E, Packer R, Myers C, Shaffrey M (1998) A phase I-II trial of lovastatin for anaplastic astrocytoma and glioblastoma multiforme. *Am J Clin Oncol* 21: 579–583.
- Larsen J, Andersen M, Kragstrup J, Gram LF (2002) High persistence of statin use in a Danish population: compliance study 1993-1998. Br J Clin Pharmacol 53: 375–378.
- Lynge E, Sandegaard JL, Rebolj M (2011) The Danish National Patient Register. *Scand J Public Health* **39**(Suppl 7): 30–33.
- Lönn S, Klaeboe L, Hall P, Mathiesen T, Auvinen A, Christensen H, Johansen C, Salminen T, Tynes T, Feychting M (2004) Incidence trends of adult primary intracerebral tumors in four Nordic countries. *Int J Cancer* 108: 450–455.
- Obara S, Nakata M, Takeshima H, Kuratsu J, Maruyama I, Kitajima I (2002) Inhibition of migration of human glioblastoma cells by cerivastatin in association with focal adhesion kinase (FAK). *Cancer Lett* 185: 153–161.
- Pedersen CB (2011) The Danish Civil Registration System. Scand J Public Health **39**(Suppl 7): 22–25.
- Riahi S, Fonager K, Toft E, Hvilsted-Rasmussen L, Bendsen J, Paaske Johnsen S, Sørensen HT (2001) Use of lipid-lowering drugs in 1991-98 in Northern Jutland, Denmark. Br J Clin Pharmacol 52: 307–311.
- Rothman K, Greenland S, Lash TL (2008) Modern Epidemiology. *Wolters Kluwer Health*. Lippincott Williams & Wilkins: Philadelphia, 3rd edn.

Scandinavian Simvastatin Survival Study Group (1994) Randomised trial of cholesterol lowering in 4444 patients with coronary heart disease: the Scandinavian Simvastatin Survival Study (4S). *Lancet* **344**: 1383–1389.

Sassano A, Platanias LC (2008) Statins in tumor suppression. *Cancer Lett* **260**: 11–19.

- Schmidt LS, Nielsen H, Schmiedel S, Johansen C (2008) Social inequality and incidence of and survival from tumours of the central nervous system in a population-based study in Denmark, 1994-2003. Eur J Cancer 44: 2050–2057.
- Soma MR, Baetta R, Bergamaschi S, De Renzis MR, Davegna C, Battaini F, Fumagalli R, Govoni S (1994) PKC activity in rat C6 glioma cells: changes associated with cell cycle and simvastatin treatment. *Biochem Bioph Res Commun* 200: 1143–1149.
- Storm HH, Michelsen EV, Clemmensen IH, Pihl J (1997) The Danish Cancer Registry-history, content, quality and use. Dan Med Bull 44: 535–539.
- Tapia-Pérez JH, Sanchez-Aguilar M, Schneider T (2010) The role of statins in neurosurgery. Neurosurg Rev 33: 259–270.
- Thomsen RW, Johnsen SP, Olesen AV, Mortensen JT, Bøggild H, Olsen J, Sørensen HT (2005) Socioeconomic gradient in use of statins among Danish patients: population-based cross-sectional study. Br J Clin Pharmacol 60: 534–542.
- Thomsen RW, Nielsen RB, Nørgaard M, Horsdal H, Larsen FB, Sørensen HT (2011) Lifestyle profile among statin users [Abstract]. *Pharmacoepidemiol Drug Saf* **20**(Suppl 1): S111.
- Vuletic S, Riekse RG, Marcovina SM, Peskind ER, Hazzard WR, Albers JJ (2006) Statins of different brain penetrability differentially affect CSF PLTP activity. *Dement Geriatr Cogn Disord* 22: 392–398.
- WHO (2010) Collaborating Centre for Drug Statistics Methodology. Guidelines for ATC Classification and DDD Assignment 2011: Oslo.
- Wu H, Jiang H, Dunuy L, Xiong L, Changsheng Q, Zhou D, Mahmood A, Chop M (2009) Effect of simvastatin on glioma cell proliferation, migration, and apoptosis. *Neurosurgery* 65: 1087–1097.
- Yanae M, Tsubaki K, Satou T, Itoh T, Imano M, Yamazoe Y, Nishida S (2011) Statin-induced apoptosis via the suppression of ERK1/2 and Akt activation by inhibition of the genanylgeranyl-pyrophosphotase biosyntesis in glioblastoma. J Exp Clin Cancer Res 30: 74.

This work is licensed under the Creative Commons Attribution-NonCommercial-Share Alike 3.0 Unported License. To view a copy of this license, visit http://creativecommons. org/licenses/by-nc-sa/3.0/

Supplementary Information accompanies the paper on British Journal of Cancer website (http://www.nature.com/bjc)

#### APPENDIX

#### Table A1. List of codes used in the analysis

#### Cancer codes

Glioma
ICD-10
C71.0-C71.9, C72.0, D33.0-D33.4, D43.0-D43.4
ICD-0-3
94403 – glioblastoma multiforme
94003, 94013, 94103, 94113 – astrocytoma grade II and III
94503, 94513, 94603 – oligodendroglioma grade II and III
93801, 93803, 93813, 93823, 93831, 93900-94001, 94121-94401,
94413–94501 – 'other'
Anatomical therapeutic classification codes
Statins
C104 A01 Simon statis

C10AA01 – Simvastatin
C10AA02 - Lovastatin
C10AA03 – Pravastatin*
C10AA04 - Fluvastatin
C10AA05 - Atorvastatin
C10AA06 - Cerivastatin
C10AA07 - Rosuvastatin*

#### Table A1. (Continued) Other drugs (covariates) Antidiabetics A10A - insulin A10B - oral antidiabetics Hormone replacement therapy G03C, G03D, G03F, G03HB01 Aspirin, low dose (tablet size 75, 100, or 150 mg) B01AC06 Selective Cox-2 inhibitors M01AH Non-aspirin NSAIDs M01A, except M01AH and M01AX Hospital discharge codes Diabetes ICD-8: 249, 250 ICD-10: E10-E14 Stroke ICD-8: 431, 433, 434 ICD-10: I60, I61, I63

Abbreviations: ICD = International Classification of Diseases; Cox-2 = cyclooxygenase 2; NSAIDs = nonsteroidal anti-inflammatory drugs. \*Classified as hydrophilic; other statins classified as lipophilic.