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Original Contribution

Joint Associations Between Genetic Variants and Reproductive Factors in Glioma Risk Among Women

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In a pooled analysis of 4 US epidemiologic studies (1993–2001), the authors evaluated the role of 5 female reproductive factors in 357 women with glioma and 822 controls. The authors further evaluated the independent association between 5 implicated gene variants and glioma risk among the study population, as well as the joint associations of female reproductive factors (ages at menarche and menopause, menopausal status, use of oral contraceptives, and menopausal hormone therapy) and these gene variants on glioma risk. Risk estimates were calculated as odds ratios and 95% confidence intervals that were adjusted for age, race, and study. Three of the gene variants (rs4295627, a variant of *CCDC26;* rs4977756, a variant of *CDKN2A* and *CDKN2B*; and rs6010620, a variant of *RTEL1*) were statistically significantly associated with glioma risk in the present population. Compared with women who had an early age at menarche (<12 years of age), those who reported menarche at 12–13 years of age or at 14 years of age or older had a 1.7-fold higher risk and a 1.9-fold higher risk of glioma, respectively (*P* for trend = 0.009). Postmenopausal women and women who reported ever having used oral contraceptives had a decreased risk of glioma. The authors did not observe joint associations between these reproductive characteristics and the implicated glioma gene variants. These results require replication, but if confirmed, they would suggest that the gene variants that have previously been implicated in the development of glioma are unlikely to act through the same hormonal mechanisms in women.

genes; glioma; menstrual cycle; polymorphism, single nucleotide; reproduction; women

Abbreviations: CI, confidence interval; GWAS, genome-wide association study(ies); PLCO, Prostate, Lung, Colorectal, and Ovarian Cancer Screening Trial; SNP, single nucleotide polymorphism.

Gliomas represent the vast majority (>80%–90%) of adult brain cancers (1). At present, the known risk factors for gliomas (high-dose ionizing radiation and rare genetic syndromes) account for only a small proportion of cases (2). Gliomas, which are highly lethal, occur more often in men than in women (3), which suggests that there are sex-specific differences in exposures and that female hormones potentially have a protective effect. Consistent with this hypothesis, in animal studies of female and male rats implanted with glioblastoma cells, females had smaller tumors and higher survival rates (4, 5).

A number of epidemiologic studies have examined the association between female reproductive factors and exogenous hormone use and the risk for glioma (6–16). A review of these results showed that later age at menarche appeared to be consistently associated with increased glioma risk in both case-control and cohort studies (10–12, 15, 17, 18). Another study reported an inverse association between the use of oral contraceptives or hormone replacement therapy and glioma risk (9), although these results require replication.

Recently, a genome-wide association study (GWAS) by Shete et al. (19) and another by Wrensch et al. (20) reported an association between 5 common genetic variations and the risk of glioma. The mechanisms by which these variations act remain unknown, but preliminary evidence of an interaction between these 5 variants (rs2736100, a variant of the

Table 1. Descriptive Characteristics for Non-Hispanic White Female Patients With Glioma and Controls Included in an Analysis of Genes and Reproductive Factors From 4 US Studies, 1993–2001

Population Characteristic	Controls		Cases	
	No.	%	No.	%
Age, years				
<45	133	16	133	37
45–64	199	24	106	30
≥65	490	60	118	33
Educational level, years				
<12	76	9	32	10
12–15	554	68	222	67
≥16	187	23	78	23
Study				
National Cancer Institute Glioma Case-Control Study	210	26	151	45
National Institute for Occupational Safety and Health Upper Midwest Health Study	240	29	129	38
Agricultural Health Study	15	2	6	2
Prostate, Lung, Colorectal, and Ovarian Cancer Screening Trial	357	43	51	15

telomerase reverse transcriptase gene (*TERT*); rs4295627, a variant of the coiled-coil domain containing 26 gene (*CCDC26*); rs4977756, a variant of the cyclin-dependent kinase inhibitor 2A and 2B genes (*CDKN2A* and *CDKN2B*); rs498872, a variant of the pleckstrin homology-like domain, family B, member 1 gene (*PHLDB1*); and rs6010620, a variant of the regulator of telomere elongation helicase 1 gene (*RTEL1*)) and a history of allergies has been suggested; a reduced risk of glioma was reported among those with both a history of allergies and a gene variant (21). Such investigations are important in shedding light on potential mechanisms of action for these genes in the development of glioma, particularly as none of the 5 risk single nucleotide polymorphisms (SNPs) were positioned in close proximity to a SNP known to be associated with an allergy, which suggests that these genes may act through an immune mechanism directly or indirectly (21). These genes are also not currently known to play a role in hormonal carcinogenesis. *TERT* is required for maintaining telomeres and cell immortalization; *CCDC26* modulates cell differentiation and death; *CDKN2A* and *CDKN2B* are known tumor suppressor genes; *PHLDB1* has previously been implicated in neuroblastoma; and *RTEL1* is in linkage disequilibrium with another gene region that is amplified in approximately 30% of gliomas (21).

Very little is known about the etiology of gliomas, although we do know that the incidence is higher in males than in females and that multiple epidemiologic studies have suggested associations between reproductive/hormonal factors in women and glioma risk. We therefore sought to shed light on potential mechanisms for these implicated genes. The goal of our study was to evaluate the associations between selected female reproductive factors and genetic variants to determine whether any of the genes implicated in GWAS act via common hormonal mechanisms to alter the risk of glioma. To do so, we first sought to confirm the

Table 2. Association of Select Reproductive Variables With Risk of Glioma Among White Women in 4 US Studies, 1993–2001[a]

Reproductive Characteristic	Controls		Cases		Odds Ratio	95% Confidence Interval	*P* for Trend
	No.	%	No.	%			
Age at menarche, years							0.009
<12	164	20	42	13	1.00	Referent	
12–13	430	53	176	54	1.69	1.13, 2.51	
≥14	217	27	107	33	1.85	1.21, 2.84	
Oral contraceptive use							
Never	295	43	118	45	1.00	Referent	
Ever	393	57	144	55	0.71	0.51, 1.01	
Menopausal status							
Premenopausal	154	20	128	40	1.00	Referent	
Postmenopausal	635	80	190	60	0.58	0.37, 0.92	
Age at menopause, years							0.1
45–50	196	31	55	30	1.00	Referent	
<45	213	34	65	36	0.91	0.58, 1.42	
>50	217	35	63	34	1.27	0.83, 1.96	
Menopausal hormone therapy use							
Never	205	36	73	43	1.00	Referent	
Ever	359	64	95	57	0.81	0.56, 1.18	

[a] Data were adjusted for age at diagnosis, study, and educational level.

Table 3. Main Associations of 5 Single Nucleotide Polymorphisms Previously Associated With Glioma in Non-Hispanic White Women From 4 US Studies[a], 1993–2001

SNP and Genotype	Controls		Cases		Odds Ratio	95% Confidence Interval	*P* Value
	No.	%	No.	%			
rs2736100							
GG	197	24	69	21	1.00	Referent	
GT	401	49	172	52	1.27	0.90, 1.79	0.2
TT	217	27	91	27	1.22	0.83, 1.80	0.3
GT + TT	618	76	263	79	1.25	0.91, 1.73	0.2
P for trend							0.3
rs4295627							
TT	556	68	187	56	1.00	Referent	
GT	242	30	121	36	1.47	1.11, 1.97	0.008
GG	19	2	24	7	3.51	1.81, 6.82	0.0002
GT + GG	261	32	145	44	1.63	1.23, 2.14	0.0005
P for trend							0.00004
rs4977756							
AA	303	37	113	34	1.00	Referent	
AG	389	48	151	45	1.05	0.78, 1.42	0.7
GG	125	15	68	20	1.58	1.07, 2.33	0.02
AG + GG	514	63	219	66	1.17	0.89, 1.55	0.3
P for trend							0.04
rs498872							
CC	401	49	150	45	1.00	Referent	
CT	338	41	149	45	1.22	0.91, 1.62	0.2
TT	77	9	32	10	1.13	0.70, 1.82	0.6
CT + TT	415	51	181	55	1.20	0.92, 1.57	0.2
P for trend							0.3
rs6010620							
GG	472	58	218	66	1.00	Referent	
AG	296	36	99	30	0.72	0.54, 0.97	0.03
AA	49	6	15	5	0.63	0.34, 1.18	0.2
AG + AA	345	42	114	34	0.71	0.53, 0.94	0.02
P for trend							0.02

Abbreviation: SNP, single nucleotide polymorphism.
[a] Data were adjusted for age at diagnosis, study, and educational level.

independent associations of selected reproductive factors (ages at menarche and menopause, menopausal status, use of oral contraceptives, and menopausal hormone therapy) and the 5 gene variants implicated in glioma risk among women in our population drawn from 4 epidemiologic studies.

MATERIALS AND METHODS

Study population

Non-Hispanic white women with glioma and controls were selected from 2 case-control studies of adult brain tumors conducted by the National Cancer Institute (1994–1998) and the National Institute for Occupational Safety and Health (1995–1997), as well as 2 prospective cohort studies, the Prostate, Lung, Colorectal, and Ovarian Cancer Screening Trial (PLCO; 1993–2001) and the Agricultural Health Study (1993–1997). Appendix Table 1 provides details for each study that have also been published elsewhere (10, 11, 22, 23).

Reproductive risk factors

Demographic characteristics and reproductive history were ascertained from study-specific questionnaires. Reproductive history information could be harmonized for data analysis across all 4 studies for the following specific reproductive factors: age at menarche (<12, 12–13, or ≥ 14 years), oral contraceptive use (never vs. ever), menopausal

Table 4. Stratified Analysis of Reproductive Factors by Single Nucleotide Polymorphisms in Glioma Risk Among Non-Hispanic White Women in 4 US Studies, 1993–2001[a]

Reproductive Characteristic	rs2736100					rs4295627				
	GG		GT/TT		*P* for Interaction	TT		GT/GG		*P* for Interaction
	OR	95% CI	OR	95% CI		OR	95% CI	OR	95% CI	
Age at menarche, years					0.7					0.1
<12	1.0	Referent	1.0	Referent		1.0	Referent	1.0	Referent	
12–13	2.30	1.01, 5.22	1.45	0.91, 2.30		2.20	1.28, 3.78	1.12	0.60, 2.09	
≥14	2.44	1.00, 5.94	1.60	0.98, 2.63		2.00	1.12, 3.57	1.75	0.89, 3.43	
Oral contraceptive use					0.9					0.8
Never	1.0	Referent	1.0	Referent		1.0	Referent	1.0	Referent	
Ever	0.47	0.24, 0.92	0.83	0.55, 1.25		0.78	0.50, 1.20	0.63	0.35, 1.15	
Menopausal status					0.7					0.6
Premenopausal	1.0	Referent	1.0	Referent		1.0	Referent	1.0	Referent	
Postmenopausal	0.82	0.34, 2.00	0.52	0.30, 0.89		0.51	0.28, 0.92	0.86	0.39, 1.89	
Age at menopause, years					0.2					0.4
45–50	1.0	Referent	1.0	Referent		1.0	Referent	1.0	Referent	
<45	1.59	0.64, 3.92	0.78	0.46, 1.30		0.83	0.48, 1.43	1.08	0.49, 2.37	
>50	2.22	0.93, 5.33	1.02	0.62, 1.70		1.04	0.60, 1.80	1.82	0.88, 3.77	
Menopausal hormone therapy use					0.2					0.9
Never	1.0	Referent	1.0	Referent		1.0	Referent	1.0	Referent	
Ever	0.50	0.24, 1.04	1.01	0.64, 1.58		0.83	0.52, 1.33	0.79	0.41, 1.54	

Table continues

Abbreviations: CI, confidence interval; OR, odds ratio.

[a] Data were adjusted for age at diagnosis, study, and educational level.

status at diagnosis (premenopausal vs. postmenopausal), age at menopause (<45, 45–50, or >50 years), and menopausal hormone therapy use (never vs. ever).

Genotyping

Using the Illumina 660-W Human BeadChip (Illumina Corporation, San Diego, California), blood and buccal samples for the National Cancer Institute Study, the National Institute for Occupational Safety and Health Study, and the Agricultural Health Study, and the PLCO glioma cases, we conducted genotyping on the 5 gene variants (rs2736100, rs4295627, rs4977756, rs498872, and rs6010620) that were shown to be most significantly associated with glioma from 2 published GWAS conducted in the United Kingdom and the United States (19). We used available GWAS data from PLCO cohort controls who had been genotyped for previous GWAS efforts using the HumanHap300 platform (Illumina Corporation) (23–25). Because PLCO samples were genotyped using the HumanHap300 platform rather than the Illumina 660-W platform, we imputed data for the PLCO control samples using the method described by Marchini et al. (26). Three percent of the total number of samples were included as quality-control samples. For all SNP assays, greater than 99.96% concordance was obtained. On the basis of the chi-squared test, none of the SNPs showed departure from Hardy-Weinberg equilibrium at $P < 0.05$. SNP genotypes were categorized as having 0, 1, or 2 risk alleles, with the risk allele defined as the allele associated with increased risk of glioma.

Data analysis

We conducted a case-control analysis of all glioma cases and controls. We evaluated the independent associations between 1) female reproductive factors and glioma risk and 2) the 5 implicated gene variants and glioma risk by calculating odds ratios and 95% confidence intervals for all cases and controls from the 4 studies. For those reproductive factors and gene variants that demonstrated an independent association with glioma, we further evaluated their associations by conducting stratified analyses in which the risk associations between the female reproductive factors and glioma risk were evaluated by the presence or absence of the ''at-risk'' gene variant. Finally, we tested for heterogeneity (interaction) between these stratified risks; to calculate the *P* for interaction, we fitted models with the main associations of reproductive factors and genotype (either 3 variables (0 risk alleles, 1 risk allele, or 2 risk alleles) or 2 variables (0 risk alleles or 1 or 2 risk alleles)) with an interaction term between them. All models were adjusted for study-specific key design

Table 4. Continued

rs4977756					rs498872					rs6010620				
AA		AG/GG		*P* for Interaction	AA		AG/GG		*P* for Interaction	GG		AG/AA		*P* for Interaction
OR	95% CI	OR	95% CI		OR	95% CI	OR	95% CI		OR	95% CI	OR	95% CI	
				0.3					0.3					0.6
1.0	Referent	1.0	Referent		1.0	Referent	1.0	Referent		1.0	Referent	1.0	Referent	
1.54	0.80, 2.97	1.86	1.11, 3.10		1.99	1.06, 3.69	1.55	0.91, 2.66		1.85	1.08, 3.17	1.53	0.82, 2.85	
1.35	0.67, 2.74	2.34	1.35, 4.05		2.68	1.40, 5.14	1.42	0.78, 2.57		2.15	1.22, 3.79	1.41	0.71, 2.80	
				0.4					0.9					0.3
1.0	Referent	1.0	Referent		1.0	Referent	1.0	Referent		1.0	Referent	1.0	Referent	
0.83	0.45, 1.51	0.66	0.43, 1.00		0.78	0.48, 1.28	0.66	0.40, 1.08		0.69	0.45, 1.05	0.69	0.37, 1.28	
				0.2					0.4					0.1
1.0	Referent	1.0	Referent		1.0	Referent	1.0	Referent		1.0	Referent	1.0	Referent	
0.47	0.21, 1.07	0.65	0.37, 1.15		0.61	0.30, 1.22	0.56	0.30, 1.05		0.51	0.27, 0.96	0.65	0.33, 1.28	
				0.1					0.2					0.9
1.0	Referent	1.0	Referent		1.0	Referent	1.0	Referent		1.0	Referent	1.0	Referent	
0.54	0.23, 1.25	1.16	0.68, 1.98		0.72	0.38, 1.37	1.23	0.64, 2.34		1.04	0.60, 1.81	0.63	0.29, 1.38	
0.69	0.32, 1.49	1.77	1.04, 3.02		0.92	0.51, 1.68	1.91	0.99, 3.68		1.71	1.01, 2.91	0.67	0.30, 1.50	
				0.8					0.6					0.5
1.0	Referent	1.0	Referent		1.0	Referent	1.0	Referent		1.0	Referent	1.0	Referent	
0.86	0.44, 1.68	0.77	0.49, 1.21		0.90	0.52, 1.55	0.77	0.45, 1.31		0.88	0.56, 1.39	0.60	0.30, 1.21	

variables and potential confounders (age, educational level, and study). All statistical analyses were conducted using SAS, version 9.2 (SAS Institute, Inc., Cary, North Carolina).

RESULTS

In total, there were 357 female glioma cases and 822 female controls included in the present analysis. The majority of cases were derived from the National Cancer Institute and National Institute for Occupational Safety and Health case-control studies (Table 1). The substantial contribution of cohort-based controls, particularly from the PLCO cohort, resulted in a higher proportion of older controls. There were no differences in educational level by case and control status or significant differences in exposures by study controls.

We first evaluated the independent association between the 5 female reproductive characteristics and glioma risk. We found that older age at menarche was associated with a statistically significantly increased risk of glioma (Table 2). Compared with women who had an early age at menarche (<12 years of age), women who reported menarche at 12–13 years of age had a 1.7-fold higher risk (95% confidence interval (CI): 1.13, 2.51) of glioma, and women who reported menarche at 14 years of age or older had a 1.9-fold higher risk (95% CI: 1.21, 2.84; *P* for trend = 0.009). At diagnosis, postmenopausal women had a decreased risk of glioma compared with premenopausal women (odds ratio = 0.58, 95% CI: 0.37, 0.92), and women reporting ever use of oral contraceptives had a borderline significant risk compared with never users (odds ratio = 0.71, 95% CI: 0.51, 1.01). No apparent associations with risk of glioma were evident for age at menopause or use of menopausal hormone therapy.

We then evaluated the independent associations between the 5 implicated genetic variants and glioma risk. Three of the genetic variants (rs4295627, a variant of *CCDC26*; rs4977756, a variant of *CDKN2A* and *CDKN2B*; and rs6010620, a variant of *RTEL1*) were statistically significantly associated with glioma in our female population (Table 3). Although rs2736100 and rs498872 were not statistically significantly associated with glioma in our population, the risk estimates were consistent with those previously reported (19, 20).

To determine whether joint associations were evident, particularly for those reproductive characteristics (age at menarche and oral contraceptive use) and genetic variants (rs4295627, rs4977756, and rs6010620) for which independent associations were evident in our female population, we evaluated the associations between the female reproductive characteristics and glioma risk, stratified by genetic variation. We posited that if a joint association was evident, differential risks for the reproductive factors and glioma would be observed between those who did not possess a genetic variant and those who did. We found that the increased risk of glioma associated with older age at menarche was consistently observed across all gene variants, whether or not the

women possessed the implicated risk variant (Table 4). Similarly, we found that the decreased risk of glioma observed for both ever use of oral contraceptives and postmenopausal status was consistent regardless of genotype. Further, none of the *P* values for interaction was statistically significant.

DISCUSSION

In the present evaluation of glioma risk among women in 4 epidemiologic studies, we found that older age at menarche was associated with an increased risk of glioma and that both ever use of oral contraceptives and postmenopausal status were associated with a decreased risk of glioma. Of the 5 genetic variants implicated in glioma risk, 3 were found to be statistically significantly associated with glioma risk in our female population. We found no evidence of associations between older age at menarche, oral contraceptive use, or postmenopausal status and the implicated gene variants and risk for glioma. Our results require replication in larger studies (e.g., studies performed by consortia) but, if confirmed, would suggest that the genetic variants implicated in glioma are unlikely to act through the same hormonal mechanism as reproductive factors in the development of glioma.

To our knowledge, this is the first reported evaluation of joint associations between female reproductive factors and implicated gene variants from GWAS. The main association observed (that between elevated glioma and a later age at menarche) was largely consistent with the current literature (10–12, 15, 17, 18). This association provides evidence of the possibility of female hormones and estrogen having a protective effect for glioma risk, particularly as incidence rates of glioma between males and females do not differ until early adolescence, at which time incidence rates become higher in men. Further studies to identify associations between specific estrogens (e.g., androstenedione, estrone, and estradiol) and risk of glioma could shed important light on relevant mechanisms, particularly as steroid hormones are known to affect the development of the brain, which undergoes significant changes in adolescence. The lack of joint association with any of the implicated gene variants suggests that the independent gene associations may not directly interact with the role that estrogen or other steroid hormones play in early menarche in glioma risk.

The decreased glioma risk observed with oral contraceptive use was consistent with results from one other study (14), but this observation requires confirmation. Further demonstration of a dose-response relation and specificity of this association by formulation would help to confirm these suggested associations. Similarly, the elevated risk of glioma observed among postmenopausal women in the present study is consistent with that seen in the study by Schlehofer et al. (14), and further evaluation of this association in cohort studies with further details of menopausal status and other exogenous hormone use might offer additional clues about the role of female reproductive factors in glioma risk. The divergent risks observed by cancer type—decreased risk of glioma versus increased risk of breast cancer—among postmenopausal women also warrant further investigation. Although mechanisms directly related to age at menopause may be culpable, it is potentially more likely that this variable is a surrogate for exposures over a lifetime and reflects differences in interactions and other risk factor combinations.

Study strengths include the relatively large number of female cases available for evaluation of reproductive factors. The quality and completeness of the genotyping data and the exposure data for the selected reproductive characteristics from the 4 studies were high. Limitations of our study include limited power to evaluate joint associations in our population despite the inclusion of over 350 female glioma cases, the evaluation of joint associations using tag SNPs, which may serve as surrogates to the true functional polymorphism, and the derivation of our study population, the majority of whom were from case-control studies rather than cohort studies. Despite the inclusion of 2 case-control studies in which response rates were high and rapid case ascertainment was used, we cannot exclude the possibility that rapidly fatal cases were excluded and would have biased our results towards identifying associations with favorable prognosis. Finally, to pool and harmonize data across 4 studies, we limited the variables ascertained from all studies to the reproductive factors that could be evaluated. We therefore cannot exclude the possibility that other valid associations might have been identified had more precise measurements been considered for other exposures (e.g., duration or dose of menopausal hormone therapy) or of other reproductive factors that were not measured in all of the present studies (e.g., breast feeding or pregnancy). Future efforts should include further evaluation of hormone exposures, particularly life-course exposure that can only be assessed from cohort studies, and further evaluation of associations and interactions by disease grade, particularly as potential differences by grade were previously observed for some of the implicated GWAS SNPs (20).

In summary, our results support the potential basis for a hormonal pathway involved in the development of glioma. In particular, increased risk of glioma with increasing age of menarche coupled with a decreased risk associated with use of oral contraceptives suggests that early and consistent exposure to female hormones may exert a protective effect against events that lead to the development of glioma. Our results further suggest that the 5 gene variants of *TERT*, *CCDC26*, *CDKN2A* and *CDKN2B*, *PHLDB1*, and *RTEL1* act independently from these female reproductive factors in their association with glioma risk. Our results require replication, such as in international consortia in which a wide range of both exposure and genetic data is available and in which more detailed exposure assessment can be evaluated in a subset of studies.

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(Appendix follows)

Appendix Table 1. Description of 4 Studies Included in Evaluation of the Association Among Genes, Reproductive Factors, and Glioma Risk in Women, 1993–2001

Study Name	Study Design	Case Definition (Primary Glioma)	Control Selection	Study/Recruitment Period	Study State	Age Range, years	No. of Cases	No. of Controls	Reference
National Cancer Institute Study	Case-control	ICD-O-2 codes 9380–9473 and 9490–9506 (histologically confirmed)	Hospital based; frequency matched 2:1 by hospital, age, sex, race/ethnicity, and distance of residence to hospital	1994–1998	Massachusetts, Arizona, and Pennsylvania	≥18	151	210	10
National Institute for Occupational Safety and Health Study	Case-control	ICD-O-2 codes 9380–9473 (histologically confirmed)	Population based; matched 1:5:1 by age, sex, and state of residence	1995–1997	Iowa, Michigan, Minnesota, and Wisconsin	18–80	129	240	11
Prostate, Lung, Colorectal and Ovarian Cancer Screening Trial	Cohort	ICD-O-3 codes 9380–9480	Non-glioma cases for whom genome-wide association study scans had previously been completed	1993–2001	Alabama, Michigan, Colorado, Hawaii, Wisconsin, Minnesota, Pennsylvania, Utah, Missouri, and Washington, DC	55–74	51	357	23
Agricultural Health Study	Cohort	ICD-O-3 codes 9380–9480	Cohort based; frequency matched 2:1 by year of birth, sex, and race/ethnicity	1993–1997	Iowa and North Carolina	30–64	6	15	22

Abbreviations: ICD-O-2, *International Classification of Diseases for Oncology*, Second Edition; ICD-O-3, *International Classification of Diseases for Oncology*, Third Edition.