Concentrations of IGF-I and IGFBP-3 and brain tumor risk in the European Prospective Investigation into Cancer and Nutrition

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Abstract: Background: Insulin-like growth factor-1 (IGF-I) is important in normal brain development but in the adult brain, IGF-I overexpression may be a risk factor for tumor development. Methods: We examined the association between circulating concentrations of IGF-I and IGFBP-3 in relation to risk of gliomas (74 low-grade, 206 high-grade gliomas), meningiomas (n = 174) and acoustic neuromas (n = 49) by using a case–control design nested in the European Prospective Investigation into Cancer and Nutrition. IGF-I and IGFBP-3 were measured by ELISAs.Conditional logistic regression was used to compute ORs and corresponding 95% CIs. Results: The risk of low-grade gliomas was elevated with increased IGF-I (OR = 3.60, 95% CI: 1.11–11.7; top vs. bottom quartile) and decreased with elevated IGFBP-3 concentrations (OR = 0.28, 95% CI: 0.09–0.84) after mutual adjustment of these two factors; these results became nonsignificant after exclusion of the first year of follow-up. No association was observed for high-grade gliomas or meningiomas. Both high IGF-I and IGFBP-3 concentrations were associated with risk of acoustic neuromas (IGF-I: OR = 6.63, 95% CI: 2.27–19.4, top vs. bottom tertile; IGFBP-3: OR = 7.07, 95% CI: 2.32–21.6), even after excluding the first year of follow-up. Conclusion: High concentrations of IGF-I might be positively associated with risk of low-grade gliomas and acoustic neuromas, although we cannot exclude reverse causation, in particular for low-grade gliomas.

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Concentrations of IGF-I and IGFBP3 and brain tumor risk in the European Prospective Investigation into Cancer and Nutrition (EPIC)

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Abstract

Background: Insulin-like growth factor-1 (IGF-I) is important in normal brain development, but in the adult brain IGF-I overexpression is a signal for neoplastic processes.

Methods: We examined the association between circulating concentrations of IGF-I and IGFBP3 in relation to risk of gliomas (74 low-grade, 206 high-grade gliomas), meningiomas (n=174) and acoustic neuromas (n=49) using a case-control design nested in the European Prospective Investigation into Cancer and Nutrition. IGF-I and IGFBP3 were measured by enzyme-linked immunosorbent assays.Conditional logistic regression was used to compute odds ratios (OR) and corresponding 95% confidence intervals (CI).

Results: The risk of low-grade gliomas was elevated with increased IGF-I (OR=3.60, 95% CI 1.11-11.7; top vs. bottom quartile) and decreased with elevated IGFBP-3 concentrations (OR=0.28, 95% CI 0.09-0.84) after mutual adjustment of these two factors; these results became non-significant after exclusion of the first year of follow-up. No association was observed for high-grade gliomas or meningiomas. Both high IGF-I and IGFBP-3 concentrations were associated with risk of acoustic neuromas (IGF-I: OR=6.63, 95% CI 2.27-19.4, top vs. bottom tertile; IGFBP-3: OR=7.07, 95% CI 2.32-21.6), even after excluding the first year of follow-up.

Conclusion: High concentrations of IGF-I might be positively associated with risk of low-grade gliomas and acoustic neuromas, although we cannot exclude reverse causation for low-grade gliomas.

Impact: Factors of the IGF axis might be involved in the etiology of some types of brain tumors.

Introduction

Tumors of the brain vary widely by degree of malignancy; high-grade gliomas tend to produce more severe and rapidly progressive symptoms whereas low-grade gliomas, meningiomas and acoustic neuromas typically produce less severe, intermittent or slowly progressive symptoms. Besides increasing age, high-dose radiation, and some hereditary syndromes, no risk factors for brain tumors have been established and the etiology of brain tumors remains largely unknown (1).

Insulin-like growth factors (IGFs) are multifunctional peptides that regulate cell proliferation, differentiation, and apoptosis (2). IGF-I is important in normal brain development to promote the growth process, and deletion of the IGF-I gene is associated with reduced brain growth and mental retardation (3). In the adult brain, by contrast, IGF-I over-expression is a signal for neoplastic processes (4), and IGF-I and -II genes are regularly found to be over-expressed in gliomas and meningiomas (5). In vitro, IGF-I receptor and IGF binding proteins promote mitogenesis and differentiation in glial cells, oligodendrocytes, and neural cells (5). While most of IGF-I and its binding proteins in the circulation is produced by the liver (6), these peptides are also synthesized locally in most other organs, including the brain and other neural tissues (7). The actions of IGF-I on neural tissues are influenced by the endocrine effects of circulating IGF-I, which can enter the central nervous system (CNS) (5), and paracrine or autocrine effects of IGF-I produced within the tissue itself.

The hepatic production of IGF-I and of its major plasmatic binding protein IGFBP-3 is stimulated by growth hormone (GH) (8). Adult height relates to longitudinal growth rates during childhood and adolescence, which are strongly influenced by levels of IGF-I, although the association of final body stature with

circulating IGF-I levels during adulthood is less strong (10). Recently, body height was found to be positively associated with glioma risk in a US cohort study (9). In EPIC, body height was associated with IGF-I levels in men, but not in women (11). Acromegaly, a condition resulting from excessive growth hormone secretion, has also been found to be associated with an increased risk of CNS tumors (12), although this may partly be due to pituitary irradiation for treating the adenomas responsible for the condition.

To date, only one small nested case-control study with 22 glioma cases and 400 controls examined the associations between serum concentrations of IGF-I and IGF binding protein (BP)-3, the most important binding protein of circulating IGF-I (8), and glioma risk (13). The authors reported inverse associations of gliomas with both IGF-I and IGFBP-3 concentrations, although only the association between circulating IGF-I and gliomas was statistically significant. This observation is not consistent with findings from many studies that observed direct associations between circulating IGF-I and the risks of several other types of cancer (14), and contrasts with observations from experimental and histo-pathological studies indicating that IGF-I may promote tumor development in the central nervous system (4, 15).

The aim of this study was to examine the association of IGF-I and IGFBP-3 concentrations with the risk of gliomas, meningiomas, and acoustic neuromas in a case-control study nested within the European Prospective Investigation into Cancer and Nutrition (EPIC).

Material and Methods

Study description.

EPIC is a prospective cohort study designed to examine the association of diet and lifestyle with the risk of cancer and other chronic diseases. The cohort comprising a total of more than 500.000 male and female participants was recruited in 23 centers in 10 European countries. Participants were recruited between 1992 and 2000; most centers recruited subjects from the general population, but in Utrecht and Florence only women from breast cancer screening programs were recruited. Additionally, the Spanish and Italian centers include blood donors and the French cohort consists of members of a health insurance for state school employees. A high proportion of participants of the Oxford cohort are vegetarians or health-conscious volunteers. The cohorts of France, Utrecht, Florence, and Norway include women only.

Information on lifestyle and diet was collected during baseline examination. Diet was assessed using country-specific validated dietary assessment instruments (16, 17). Information on lifestyle factors such as smoking, alcohol consumption, or physical activity, but also on education, occupation, or medical and reproductive history has been collected using questionnaires and personal interviews. Anthropometric measurements have been conducted during baseline examination (18).

Following a standardized protocol, a blood sample of 30 mL was collected in all participating EPIC countries. In most centers, except Oxford, blood samples were stored protected from light at 5-10°C until further processing and aliquoting. In the Oxford center, blood samples were collected throughout the United Kingdom and transported to the laboratory in Norfolk by mail at ambient temperature. In all centers except Denmark and Sweden, 0.5 ml aliquots of serum, plasma, red blood cells, and buffy coat were filled into plastic straws and stored in liquid nitrogen at -196°C. In the

Danish centers, 1 ml aliquots were filled into tubes and stored in the vapor phase of liquid nitrogen containers (-150°C). In Sweden, the samples were stored at -80°C.

Case assessment and matching.

The follow-up in EPIC is based on population cancer registries in Denmark, Italy, Netherlands, Spain, Sweden, United Kingdom and Norway. In Germany and Greece a combination of methods including health insurance records, cancer and pathology registries, and active follow-up through study subjects and their next-of-kin was used. Mortality data are also collected from either the cancer registry or mortality registries at the regional or national level. For each EPIC center, closure dates of the study period were defined as the latest dates of complete follow-up for both cancer incidence and vital status (dates varied between centers, from December 2002 to December 2006).

By December 2006, 799 histologically confirmed primary brain tumor cases have been recorded in the EPIC database. Of these, we excluded 102 cases because they did not provide a blood sample at recruitment and a further 103 cases because the diagnosis was unspecified beyond that of primary brain tumor (ICD-O M8000). Cases from the center from Malmö were not available for our analyses (n=68); France was excluded from the analysis as self-reported brain tumors had not been histologically confirmed when the study was initiated. Nineteen cases were excluded because of technical problems or insufficient serum volume. The final analytical sample included 505 primary brain tumors, namely 282 gliomas [International classification of diseases for oncology (ICD-O) codes M9380 – M9473], 176 meningiomas (ICD-O M9530-M9539), and 49 acoustic neuromas (ICD-O M9540). In addition, glioma cases were divided into high grade-glioma (ICD-O codes: M9440/3, M9441/3, M9442/3, M9401/3, M9380/3, M9451/3) and low grade-glioma

(ICD-O codes: M9382/3, M9383/1, M9384/1, M9390/0, M9394/1, M9400/3, M9411/3, M9420/3, M9421/3, M9450/3, M9505/1, M9391/3).

Two controls per case were selected, matched for gender, study (recruitment) centre, age at recruitment, date at blood donation, and fasting status at blood donation (time since last consumption of foods or drinks; last meal < 6, 3-6, or <3 hours before blood draw).

Hormone assays.

Serum IGF-I and IGFBP-3 concentrations were measured in the Immunoassay Laboratory at the German Cancer Research Center, Heidelberg, Germany. Both peptides were analyzed by enzyme-linked immunosorbent assays (ELISAs) purchased from Beckman Coulter. Prior to total IGF-I analysis, IGF-I was separated from IGF-I binding proteins by an acid-ethanol extraction step. Cases and matched controls were measured in singleton within the same batch. Each analytical batch further included three different serum quality control samples. Laboratory staff was blinded to the case/control status of the study samples. Mean intra- and inter-batch coefficients of variation were 2.9% and 14.4% respectively for IGF-I and 1.7% and 15.5% respectively for IGFBP-3.

Statistical analysis.

To examine the association of IGF-I, IGFBP-3, and molar IGF-I/IGFBP-3 ratio with BMI, Spearman's partial coefficients of correlation adjusted for age, sex and EPIC recruitment center were computed.

Conditional logistic regression was used to examine the association of IGF-I and IGFBP-3 concentrations with brain tumor risk. We also computed the molar ratio of IGF-I to IGFBP-3 (IGF-I/IGFBP-3 ratio) as a marker of the estimated IGF-I level

biologically available to bind to its receptor. Concentrations of IGF-I and IGFBP-3 as well as IGF-I/IGFBP-3 ratio were categorized into quartiles based on the distribution among all controls. Due to the small number of acoustic neuromas, we used tertiles instead of quartiles to examine the respective associations.

Crude models took into account matching criteria; multivariate models additionally adjusted for body mass index (BMI) (in sex-specific quartiles) or waist-hip ratio (WHR) (alternatively; in sex-specific quartiles), education (primary school or less; secondary school; vocational training; university; missing), smoking history (never, former, current, missing), and physical activity (active, moderately active, moderately inactive, inactive). Adjustments for these factors did not appreciably change any of the results and therefore were excluded from the final models. Thus, only the matching criteria (age, gender, center, follow-up time, time of the day of blood draw, and fasting status) remained as implicit adjustments controlled for through the conditional regression models. Further analyses were conducted with mutual adjustments between IGF-I and IGFBP-3 concentrations.

In sub-analyses, in which we stratified by sex, age at diagnosis (</≥ 60 years at diagnosis), BMI (</≥ median BMI), and length of follow-up (</≥ median follow-up time in cases = 4.4yrs), odds ratios (OR) were estimated for continuous measurements IGF-1, IGFBP-3, and IGF-I/IGFBP-3 ratio transformed on the log2 scale. In this scale, a unit increase corresponds to a doubling of concentration. Statistical tests for heterogeneity by sex, age at diagnosis, BMI, and length of follow-up were based on chi-square statistics, calculated as the deviations of logistic beta-coefficients observed in each of the subgroups, relative to the overall beta-coefficient. Finally, we performed sub-analyses excluding cases diagnosed within the first year of

follow-up to examine potential 'reverse causation' bias. All analyses were conducted with SAS version 9.1.

Results

The median time between date of study recruitment and diagnosis of tumor among cases was 4.4 years (glioma 4.5, meningioma 4.2, acoustic neuromas 4.1). Baseline characteristics of cases and controls are shown in Table 1. Cases and controls did not differ by selected baseline characteristics except that meningioma cases had a slightly higher BMI than controls. In controls, circulating levels of IGF-I were not statistically significantly correlated with BMI (Spearman r = 0.01; p-value 0.77), but there were significant correlations of IGFBP-3 (Spearman r = 0.15; p-value <0.01) and molar IGF-I/IGFBP-3 ratio (Spearman r = -0.09; p-value <0.01) with BMI.

Gliomas. High IGF-I or high IGFBP-3 concentrations were not statistically significantly associated with glioma risk (Table 2). The strength of the relationship of IGF-I and IGFBP-3 with gliomas was slightly attenuated after mutually adjusting for the two factors. Likewise, there was no significant association between molar IGF-I/IGFBP3 ratio and glioma risk. Excluding the first year of follow-up to examine a potential reverse causation bias did not change the associations between the measured biomarkers and glioma risk (IGF-I: 1.11, 95% CI 0.68-1.81, top vs. bottom quartile; IGFBP-3: 0.92, 0.58, 1.46; IGF-I/IGFBP-3: 1.13, 0.67, 1.91).

We conducted sub-group analyses to examine whether the associations between factors of the IGF-system differed by sex, BMI, age at tumor diagnosis, or length of follow-up. Besides a statistically significant effect modification of the association between IGFBP-3 concentration and glioma risk by BMI, we did not observe major

differences between these sub-groups (data not shown). Per doubling in IGFBP-3 concentration, glioma risk decreased by more than 50% (OR=0.48, 95% CI 0.23-0.99) in subjects with normal BMI, but no statistically significant association was seen in obese subjects (OR=1.26, 95% CI 0.67-2.36; p-heterogeneity=0.048).

High- vs. low-grade gliomas. After stratifying by tumor grade, neither IGF-I nor IGFBP-3 concentrations were associated with the risk of high-grade gliomas (Table 3). However, although IGF-I and IGFBP-3 were not associated with low-grade gliomas in the basic model, a high IGF-I concentration was related to an increased risk of low-grade gliomas after additionally adjusting for IGFBP-3 concentration (OR=3.60, 95% CI 1.11-11.7; top vs. bottom quartile). In contrast, IGFBP-3 concentration was inversely associated with low-grade gliomas after adjusting for IGF-I (OR=0.28, 95% CI 0.09-0.84). A high IGF-I/IGFBP-3 ratio was associated with a non-statistically significant increase in low-grade glioma risk in the categorical model; a two-fold increase in the ratio yielded an OR=2.80 (95% CI 0.98-7.98). Excluding the first year of follow-up resulted in an attenuated association of IGF-I (OR=1.23, 95% CI 0.45-3.36 [2.05, 0.59-7.13 adjusted for IGFBP-3]; top versus bottom quartile), IGFBP-3 (OR=0.54, 95% CI 0.21-1.38 [0.34, 0.11-1.07 adjusted for IGF-I]; top versus bottom quartile), and IGF-I/IGFBP-3 (OR=2.01, 95% CI 0.68-5.92; top versus bottom quartile) with low-grade gliomas.

Meningiomas. As for gliomas, circulating levels of IGF-I and IGFBP-3 as well as the IGF-I/IGFBP-3 ratio were not associated with meningioma risk (Table 2). Excluding the first year of follow-up did not materially alter the observed associations for IGF-I or IGFBP-3, respectively (IGF-1: OR 1.13, 95% CI 0.61, 2.09, top vs. bottom quartile; IGFBP-3: 1.71, 0.90, 3.27; IGF-I/IGFBP-3: 0.87, 0.47, 1.64).

In subgroup analyses, we observed statistically significant heterogeneity in the association between IGF-I/IGFBP-3 ratio and meningioma risk by sex and age (data not shown). The IGF-I/IGFBP-3 ratio was significantly inversely associated with mengioma risk in men (OR=0.21, 95% CI 0.06-0.75 per doubling in ratio), but not in women (OR=1.23, 95% CI 0.65-2.33, p-heterogeneity=0.02). Furthermore, the IGF-I/IGFBP-3 ratio was significantly inversely associated with meningioma risk in young (< 60 years of age at diagnosis; OR=0.43, 95% CI 0.19-0.98 per doubling in ratio), but not in older cases (OR=1.72, 95% CI 0.74-4.01, p-heterogeneity=0.02).

Acoustic neuromas. We observed positive associations of circulating IGF-1 and IGFBP-3 concentrations and acoustic neuromas (Table 2). These associations were attenuated after mutual adjustments between the two peptides, although the ORs in the highest tertiles remained significantly increased compared with the bottom tertiles. As expected, the IGF-I/IGFBP-3 ratio showed no association with risk of acoustic neuroma. The results did not change strongly after we excluded cases that occurred during the first year of follow-up (IGF-1: OR 6.93, 95% CI 2.24-21.5, top vs. bottom tertile; IGFBP-3: 6.09, 1.94-19.1; IGF-I/IGFBP-3: 2.66, 0.82-8.67). No statistically significant heterogeneity by age at diagnosis, length of follow-up, BMI, and sex were observed (data not shown).

Discussion

This is the first large nested case-control study to examine the association of circulating concentrations of IGF-I and its most important binding protein IGFBP-3 with the risk of different types of brain tumor, namely high- and low-grade gliomas, meningiomas and acoustic neuromas. We did not observe a general association

between IGF-I or IGFBP-3 concentrations or the molar ratio of IGF-I to IGFBP3 with risk of gliomas and mengiomas. However, subjects with high circulating levels of IGF-I had an increased risk and those with a high level of IGFBP-3 had a decreased risk of low-grade gliomas after mutual adjustments between IGF-I and IGFBP-3. Additionally, we observed that both concentrations of IGF-I and IGFBP3 were positively associated with the risk of acoustic neuromas.

High circulating concentrations of IGF-I and IGF binding proteins have been found to be associated with increased risks of several types of cancers, including cancers of the colon (19), prostate (20), or breast cancer (21). There is increasing evidence that IGFs play an important role in the growth and differentiation of the central nervous system and specific receptors for IGF-I and IGF-II have been identified in normal human brain (15). IGF pathways show similar expression and functional features during central nervous system development and tumorgenesis (8, 22, 23). IGFs have been reported to be over-expressed to varying degrees in central nervous system tumors, such as glioma (4, 15), and in vitro studies have shown that IGF-I promotes mitogenesis and differentiation in glial cells (24, 25). IGF binding proteins prolong the half-life of IGF-I by competing with IGF-I receptor for binding IGF-I. In addition, IGFBP-3 may have intrinsic growth inhibiting and pro-apoptotic effects independent of sequestering IGF-I (26, 27), although in some experimental studies it has also been observed to exert growth-promoting effects (28). IGFBP expression can increase neoplastic processes, for example in gliomas (29). IGF-I is produced by the liver, but also in the brain and the spinal cord (22). It has been shown that circulating IGF-I enters the brain and the spinal cord via a saturable transport system at the brain-blood barrier (7). The half-life of IGF-I and the rate of entry of IGF-I into the central nervous system are likely to be related to the concentration of IGFBPs in the circulation (7). Vice versa, it might be possible that

IGF-I produced by tumor cells in the brain crosses the brain-blood barrier and enters the circulation. However, the contribution of locally produces IGF-I leaking into systemic circulation is likely to be small compared with liver production. Based on the attenuation of the association between IGF-I concentration and risk of gliomas, we cannot exclude potential reverse causation.

We did not observe strong and consistent associations of circulating levels of IGF-I, IGFBP-3, or the molar ratio of IGF-I to IGFBP-3 with the risk of gliomas in general. However, high IGF-I concentrations were positively and high IGFBP-3 concentrations were inversely associated with the risk of low-grade gliomas after mutual adjustment, whereas no associations were observed for high-grade gliomas. So far, only one previous epidemiological study examined the association of IGF-I or IGFBP-3 with gliomas. Lönn et al. observed an inverse association of IGF-I concentration with glioma risk, but no association with IGFBP-3 concentration in a case-control study nested within the prospective ATBC trial in Finland (13). Adjustment for age, BMI, smoking, self-reported history of allergies, education, intervention group assignment, time period of diagnostic did not change the results. This study was too small to stratify by glioma grade. In a case-control study, polymorphisms in IGF-I, IGF-II, IGF-I receptor, IGF-II receptor, and IGFBP-3 genes were examined in relation to glioma and meningioma risk (28). The majority of SNPs were not related to glioma risk. Interestingly, however, one SNP in IGF-I and two SNPs in the IGF-I receptor gene were associated with the risk of low- but not high-grade gliomas. The functionality of these polymorphisms, however, appears to be unclear and, thus, an interpretation with respect to our results is difficult. However, one might speculate that there are different genetic pathways that lead to high- and low-grade glioma. IGF-I receptors are overexpressed in gliomas and meningiomas and *in vitro*, IGF-I receptor has been shown to promote mitogenesis and

differentiation in glial cells, oligodendrocytes, and neural cells (5). The associations of IGF-I and IGFBP-3 concentrations with low-grade gliomas were attenuated and not statistically significant anymore after excluding the first year of follow-up. This might be due to the even lower number of cases and, thus, more imprecision, but we also cannot exclude a certain extend of reverse causation such that the association is caused by cases diagnosed in the first year of follow-up. However, the number of cases with low-grade glioma diagnosed in the first or even the first two years is too small for a meaningful statistical analysis.

Circulating concentrations of IGF-I or IGFBP-3 were generally not associated with the risk of *meningiomas*. The only associations observed in our study were a decreasing meningioma risk with increasing IGF-I/IGFBP-3 ratio in males and in cases that were diagnosed at an earlier age. However, the implications are unclear, especially because the number of meningioma cases among men is small (n=45) and chance might be the reason for this statistically significant finding. This is the first study to examine this association. Previously, Lönn et al. (28) also did not report any association of polymorphisms in IGF-I, IGF-II, IGF-I receptor, IGF-II receptor, and IGFBP-3 genes with meningioma risk.

Acoustic neuromas (vestibular schwannoma) are benign and slowly growing nerve sheath tumors of the vestibulocochlear nerve. The incidence of this tumor is growing in recent years although this increase could simply be due to improved diagnostic technologies (30). The etiology of this tumor is largely unknown besides an association with moderate to high doses of ionizing radiation, which is a risk factor for all brain tumors. However, ionizing radiation can explain only a small fraction of the tumors. To the best of our knowledge, our finding of a direct association of

circulating IGF-I and IGFBP-3 concentrations with acoustic neuromas has not previously been reported.

The reason for an inverse association of IGFBP-3 with low-grade gliomas after adjusting for circulating IGF-I and a direct association of IGFBP-3 with acoustic neuromas is unclear. A positive association of IGF-I with acoustic neuromas is consistent with IGF-I's mitogenic effects. The association of IGFBP-3 concentration with cancer risk has first been thought to be inverse as IGFBP-3 is thought to determine the concentration of free, i.e., biologically active, IGF-I in the circulation. However, the results of previous studies on different types of cancer have been inconsistent with some studies indeed showing inverse associations, but some also showing no or even a positive association (14). It has been discussed that different assays measuring concentrations of total or intact IGFBP-3 could cause differences between studies (14), but this does not explain differences in our study between two different types of brain tumors, i.e., different effects of IGFBP-3 on low-grade gliomas and acoustic neuromas. We measured intact IGFBP-3, not total IGFBP-3, which also includes IGFBP-3 fragments that are less biologically active.

Our study is so far the second study on IGF-I and IGFBP-3 concentration and risk of brain tumor. Its advantages are its size (505 histologically confirmed primary brain tumors), the prospective design, and the ability to adjust for a number of potential confounders. As in most epidemiological studies, we cannot exclude chance as a possible explanation of our findings, especially as the number of cases in the groups in which we observed statistically significant associations are small. The estimates observed in our study may, thus, be an overestimation of the true effect. Also, the attenuation of the associations observed for low-grade gliomas after excluding the first year of follow-up supports that our results have to be interpreted

with care. In addition, we were not able to adjust environmental risk factors such as ionizing radiation. While there is rationale for an inverse association of IGFBP-3 and low-grade glioma, the positive association between IGFBP-3 and risk of acoustic neuromas is difficult to interpret due to the small number of cases.

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References

1. Bondy, ML, Scheurer, ME, Malmer, B, et al. Brain tumor epidemiology: consensus from the Brain Tumor Epidemiology Consortium. Cancer 2008;113*:* 1953-68.
2. Khandwala, HM, McCutcheon, IE, Flyvbjerg, A, et al. The effects of insulin-like growth factors on tumorigenesis and neoplastic growth. Endocr Rev 2000;21*:* 215-244.
3. Joseph D'Ercole, A, and Ye, P. Expanding the Mind: Insulin-Like Growth Factor I and Brain Development. Endocrinology 2008;149*:* 5958-5962.
4. Zumkeller, W, and Westphal, M. The IGF/IGFBP system in CNS malignancy. Mol Pathol 2001;54*:* 227-9.
5. Russo, VC, Gluckman, PD, Feldman, EL, et al. The insulin-like growth factor system and its pleiotropic functions in brain. Endocr Rev 2005;26*:* 916-43.
6. Pollak, MN, Schernhammer, ES, and Hankinson, SE. Insulin-like growth factors and neoplasia. Nat Rev Cancer 2004;4*:* 505-18.
7. Pan, W, and Kastin, AJ. Interactions of IGF-1 with the blood-brain barrier in vivo and in situ. Neuroendocrinology 2000;72*:* 171-8.
8. Jones, JI, and Clemmons, DR. Insulin-like growth factors and their binding proteins: biological actions. Endocr Rev 1995;16*:* 3-34.
9. Moore, SC, Rajaraman, P, Dubrow, R, et al. Height, body mass index, and physical activity in relation to glioma risk. Cancer Res 2009;69*:* 8349-55.
10. Gunnell, D, Oliver, SE, Donovan, JL, et al. Do Height-Related Variations in Insulin-Like Growth Factors Underlie the Associations of Stature with Adult Chronic Disease? J Clin Endocrinol Metab 2004;89*:* 213-218.
11. Crowe, FL, Key, TJ, Allen, NE, et al. A cross-sectional analysis of the associations between adult height, BMI and serum concentrations of IGF-I and IGFBP-1 -2 and -3 in the European Prospective Investigation into Cancer and Nutrition (EPIC). Ann Hum Biol 2010.
12. Baris, D, Gridley, G, Ron, E, et al. Acromegaly and cancer risk: a cohort study in Sweden and Denmark. Cancer Causes Control 2002;13*:* 395-400.
13. Lönn, S, Inskip, PD, Pollak, MN, et al. Glioma Risk in Relation to Serum Levels of Insulin-Like Growth Factors. Cancer Epidemiol Biomarkers Prev 2007;16*:* 844-846.
14. Renehan, AG, Zwahlen, M, Minder, C, et al. Insulin-like growth factor (IGF)-I, IGF binding protein-3, and cancer risk: systematic review and meta-regression analysis. Lancet 2004;363*:* 1346-53.
15. Glick, RP, Lichtor, T, and Unterman, TG. Insulin-like growth factors in central nervous system tumors. J Neurooncol 1997;35*:* 315-25.
16. Riboli, E, and Kaaks, R. The EPIC Project: rationale and study design. European Prospective Investigation into Cancer and Nutrition. Int J Epidemiol 1997;26 Suppl 1*:* S6-14.
17. Kaaks, R, Slimani, N, and Riboli, E. Pilot phase studies on the accuracy of dietary intake measurements in the EPIC project: overall evaluation of results. European Prospective Investigation into Cancer and Nutrition. Int J Epidemiol 1997;26 Suppl 1*:* S26-36.
18. Haftenberger, M, Lahmann, PH, Panico, S, et al. Overweight, obesity and fat distribution in 50- to 64-year-old participants in the European Prospective Investigation into Cancer and Nutrition (EPIC). Public Health Nutr 2002;5*:* 1147-62.
19. Rinaldi, S, Cleveland, R, Norat, T, et al. Serum levels of IGF-I, IGFBP-3 and colorectal cancer risk: results from the EPIC cohort, plus a meta-analysis of prospective studies. Int J Cancer 2010;126*:* 1702-15.

20. Roddam, AW, Allen, NE, Appleby, P, et al. Insulin-like growth factors, their binding proteins, and prostate cancer risk: analysis of individual patient data from 12 prospective studies. Ann Intern Med 2008;149*:* 461-71, W83-8.
21. Key, TJ, Appleby, PN, Reeves, GK, et al. Insulin-like growth factor 1 (IGF1), IGF binding protein 3 (IGFBP3), and breast cancer risk: pooled individual data analysis of 17 prospective studies. Lancet Oncol 2010;11*:* 530-42.
22. Daftary, SS, and Gore, AC. IGF-1 in the brain as a regulator of reproductive neuroendocrine function. Exp Biol Med (Maywood) 2005;230*:* 292-306.
23. Trojan, J, Cloix, JF, Ardourel, MY, et al. Insulin-like growth factor type I biology and targeting in malignant gliomas. Neuroscience 2007;145*:* 795-811.
24. Tranque, PA, Calle, R, Naftolin, F, et al. Involvement of protein kinase-C in the mitogenic effect of insulin-like growth factor-I on rat astrocytes. Endocrinology 1992;131*:* 1948-1954.
25. Åberg, ND, Blomstrand, F, Åberg, MAI, et al. Insulin-like growth factor-I increases astrocyte intercellular gap junctional communication and connexin43 expression in vitro. J Neuroscience Res 2003;74*:* 12-22.
26. Rajah, R, Valentinis, B, and Cohen, P. Insulin-like growth factor (IGF)-binding protein-3 induces apoptosis and mediates the effects of transforming growth factor-beta1 on programmed cell death through a p53- and IGF-independent mechanism. J Biol Chem 1997;272*:* 12181-8.
27. Rajah, R, Lee, KW, and Cohen, P. Insulin-like growth factor binding protein-3 mediates tumor necrosis factor-alpha-induced apoptosis: role of Bcl-2 phosphorylation. Cell Growth Differ 2002;13*:* 163-71.
28. Lonn, S, Rothman, N, Shapiro, WR, et al. Genetic variation in insulin-like growth factors and brain tumor risk. Neuro Oncol 2008;10*:* 553-559.
29. Zumkeller, W. IGFs and IGF-binding proteins as diagnostic markers and biological modulators in brain tumors. Expert Rev Mol Diagn 2002;2*:* 473-7.
30. Propp, JM, McCarthy, BJ, Davis, FG, et al. Descriptive epidemiology of vestibular schwannomas. Neuro-oncol 2006;8*:* 1-11.