KATARIINA KORHONEN

Pathological and Epidemiological Aspects of Meningioma

With special emphasis to sex hormones

ACADEMIC DISSERTATION
To be presented, with the permission of the board of the School of Medicine of the University of Tampere, for public discussion in the Small Auditorium of Building B, School of Medicine of the University of Tampere, Medisiinarinkatu 3, Tampere, on April 27th, 2012, at 12 o’clock.

UNIVERSITY OF TAMPERE
Dedicated to my dad and my family
ABSTRACT

**Background:** There is mounting evidence that sex hormones play an important role in development of meningiomas but the tumorigenesis of meningiomas is still largely unknown. The results of previous studies on hormonal treatments and meningioma risk have been equivocal, which makes it difficult to draw any definitive conclusions. Carbonic anhydrases (CA) are enzymes that participate in numerous biological processes in humans including maintenance of the acid-base balance. CA II and IX have recently been shown to be expressed in several cancers but there is little information on their expression in meningiomas. The purpose of this study was to report the expression of sex hormone receptors and carbonic anhydrases II and IX in meningiomas, and evaluate the effect of reproductive factors and exogenous sex hormone exposure on meningioma risk in a population-based case-control study and a cohort study.

**Material and methods:** The tumor samples for the analysis of sex hormone receptors and carbonic anhydrases were obtained from 447 patients, who underwent surgery for intracranial meningioma at the Tampere University Hospital during 1989-1999. Both sex hormone receptors and carbonic anhydrases were analysed immunohistochemically. All women diagnosed with meningioma in Finland at ages 20-69 years old in five university hospitals during the study period were eligible for the case-control study. Information on use of hormonal treatments for birth control or menopausal symptoms was collected by interviews. Control subjects were randomly identified from the population register using frequency matching by area and age. For the cohort study we identified from the medical reimbursement register 266,518 women, who had received reimbursement for estradiol-only or estradiol-progestin combination therapy in the age of 50 years or higher in 1994-2009 and used it at least for six months. The meningioma
cases within the cohort were identified from the Finnish Cancer Registry and linked to the medical imbursement register for defining the hormone use.

**Results:** Most meningiomas expressed progesterone receptors but less than half of them were positive for androgren or estrogen receptors. The expression of all sex hormone receptors was similar in both men and women. Estrogen receptor positive meningiomas had a higher proliferation index than estrogen receptor negative ones. Unlike sex hormone receptors, CA II and IX were rare in meningiomas. CA II expression was more common in atypical and malignant meningiomas than in benign meningiomas. Indicators of endogenous sex hormone exposure were not associated with a meningioma risk, except for an increasing number of children being directly related to meningioma risk. A non-significant positive association was found between tumors expressing progesterone receptors and use of oral contraceptives. In the cohort study, estradiol-only therapy was associated with an increased meningioma incidence.

**Conclusions:** Estrogen receptors may have more involvement in pathogenesis of meningiomas than is earlier thought. Expression of CA II is higher in atypical and malignant meningiomas compared to benign tumors suggesting CA II may be a target molecule for antitumor therapy when treating of grade II-III meningiomas. Reproductive factors do not seem to have major importance in tumorigenesis of meningiomas. Regarding meningioma risk, combination therapy for postmenopausal symptoms seems to be safer than use of estradiol-only regimen.
TIIVISTELMÄ


vähintään puolen vuoden ajan. Tiedot tutkimusväestössä todetusta meningeooma-tapauksista saatiin Suomen Syöpärekisteristä.


**Yhteenveto:** Aiemmat tutkimukset ovat keskittyneet PR:eihin meningeoomissa mutta tämän tutkimuksen perusteella estrogeenin vaikutus meningeoomien kehittymiseen saattaakin olla aiempaa luultua suurempi. CA II saattaa tulevaisuudessa olla kohde-entsyymi kehitetäessä uusia hoitoja pahanlaatuisille meningeoomille. Vaihdevuosioireiden yhdistelmähoito on meningeoomariskin suhteen turvallisempi kuin pelkkä estradioli.
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<th>Abbreviation</th>
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<tr>
<td>AR</td>
<td>Androgen receptor</td>
</tr>
<tr>
<td>CA II</td>
<td>Carbonic anhydrase II</td>
</tr>
<tr>
<td>CA IX</td>
<td>Carbonic anhydrase IX</td>
</tr>
<tr>
<td>CBTRUS</td>
<td>The Central Brain Tumor Registry of the United States</td>
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<tr>
<td>CNS</td>
<td>Central nervous system</td>
</tr>
<tr>
<td>COX-2</td>
<td>Cyclo-oxygenase-2</td>
</tr>
<tr>
<td>CPA</td>
<td>Cyproterone acetate</td>
</tr>
<tr>
<td>CT</td>
<td>Computed tomography</td>
</tr>
<tr>
<td>EGFR</td>
<td>Epidermal growth factor receptor</td>
</tr>
<tr>
<td>EPIC</td>
<td>The European Prospective Investigation into Cancer and Nutrition</td>
</tr>
<tr>
<td>EPT</td>
<td>Estrogen-progestin therapy</td>
</tr>
<tr>
<td>ER</td>
<td>Estrogen receptor</td>
</tr>
<tr>
<td>ET</td>
<td>Estrogen-only therapy</td>
</tr>
<tr>
<td>GABA</td>
<td>Gamma-Amino Butyric acid</td>
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<tr>
<td>GnRH</td>
<td>Gonadotropin releasing hormone</td>
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<tr>
<td>Gy</td>
<td>Gray</td>
</tr>
<tr>
<td>HR</td>
<td>Hazard ratio</td>
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<tr>
<td>LI</td>
<td>Labeling index</td>
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<tr>
<td>MF</td>
<td>Magnetic field</td>
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<tr>
<td>MHT</td>
<td>Menopausal hormone therapy</td>
</tr>
<tr>
<td>MPA</td>
<td>Medroxyprogesterone acetate</td>
</tr>
<tr>
<td>MRI</td>
<td>Magnetic resonance imaging</td>
</tr>
<tr>
<td>NETA</td>
<td>Norethisterone acetate</td>
</tr>
<tr>
<td>NF2</td>
<td>Neurofibromatosis type II</td>
</tr>
<tr>
<td>OC</td>
<td>Oral contraceptive</td>
</tr>
<tr>
<td>OR</td>
<td>Odds ratio</td>
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<tr>
<td>PDGF</td>
<td>Platelet-derived growth factor</td>
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<td>Abbreviation</td>
<td>Full Form</td>
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<tr>
<td>PFS</td>
<td>Progression free survival</td>
</tr>
<tr>
<td>PI</td>
<td>Proliferation index</td>
</tr>
<tr>
<td>PR</td>
<td>Progesterone receptor</td>
</tr>
<tr>
<td>PVA</td>
<td>Polyvinyl alcohol</td>
</tr>
<tr>
<td>SD</td>
<td>Standard deviation</td>
</tr>
<tr>
<td>SIR</td>
<td>Standardized incidence ratio</td>
</tr>
<tr>
<td>SRS</td>
<td>Stereotactic radiosurgery</td>
</tr>
<tr>
<td>TGF-alpha</td>
<td>Transforming growth factor alpha</td>
</tr>
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LIST OF ORIGINAL PUBLICATIONS

This thesis is based on the following original publications, Roman numerals I-IV.


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1. INTRODUCTION

Even though meningioma is the most common primary brain tumor, its etiology is largely unknown. In addition to a few rare hereditary conditions, ionizing radiation is the only well established risk factor. Sex hormones have long been suspected of playing a role in the development of meningiomas but the pathways are still unknown. Results of expression of progesterone receptors are quite uniform, but there is still debate concerning the frequency of meningiomas expressing estrogen receptors. If sex hormone receptors prove to be important for development of meningiomas, hormonal treatments may be associated with a risk of meningioma. A few case-control studies have evaluated meningioma risk in relation to use of menopausal hormone therapy, but their results have not been consistent. Recently, increased meningioma risk associated with menopausal treatments was reported by two large cohort studies.

CAs are under vigorous investigation. The findings suggest that certain CAs including CA IX and XII can be used as biomarkers for cancer (Wykoff et al., 2000). Several drugs have already been developed, that are based on inhibition of carbonic anhydrases. There is little information on expression of CAs in meningiomas but they are found in several different types of malignant brain tumors.

The main objective of this thesis was to evaluate risk factors for meningiomas. The specific aims included 1) expression of sex hormones, 2) expression of carbonic anhydrases II and IX in meningiomas and, 3) the association of meningioma risk and menopausal hormonal therapy.
2. REVIEW OF THE LITERATURE

2.1 Etiology of meningiomas

2.1.1 Occurrence

Meningiomas constitute 38% of all primary intracranial tumors in women and 20% in men (Claus et al., 2005). They arise from leptomeningeal arachnoidal cap cells, their precursor cells or even stem cells. It is assumed that meningiomas can occur anywhere where arachnoid cells are found. Even though the majority of meningiomas occur intracranially, they can also be found intraspinally, within the bone of the skull, rarely in the orbit and in the subcutaneous tissue. About 90% of meningiomas are intracranial and most of the remainder occur in the spine. Spinal meningiomas and schwannomas comprise 90% of all intradural tumors (Van Goethem et al., 2004). Meningioma is the most common spinal tumor in women. In spinal meningiomas, the difference in female: male ratio is even higher, being 3-4:1 (Gottfried et al., 2003).

Incidence increases with age and the median age at diagnosis is 64 years. From the age of 35, meningioma is the most common primary brain and CNS tumor. In contrast, in children, meningioma accounts only for 1.9% of primary brain and CNS tumors. The age-specific incidence of meningioma is less than 8/100 000 person-years in the ages of people between 45-54 years old and almost 40/100 000 person-years in the ages of people 85 years
and older. Meningiomas are more often diagnosed in women than in men, occurring with a sex ratio of 2.2:1 (CBTRUS, 2011). Their age-standardized incidence rate (world standard population as the reference) in 2001 was reported by the Finnish Cancer Registry as being 1.6/100 000 men and 5.5 for women. In the other Nordic countries the incidence rates are comparable: 1.9 for men and 4.5 for women (Klaeboe et al., 2005). An approximate age-standardized (to the year 2000 U.S. standard population) annual incidence of meningioma was 6.6 per 100 000 person-years in the 2011 CBTRUS Statistical Report. In a smaller study, consisting of first primary CNS tumors in California, age-adjusted incidence of meningioma was 6.1/100 000 for women and 2.7/100 000 for men (Brown et al., 2009). These estimates may be biased downward, because a significant proportion of meningiomas are not treated surgically. In autopsy studies, an estimate of 2.8% prevalence of subclinical meningiomas in women has been suggested (Krampla et al., 2004). Incidence of meningiomas has gradually increased in the developed countries (Dobes et al., 2011; Klaeboe et al., 2005). This may be attributable to better imaging strategies but exogenous risk factors may also play a role.

2.1.2 Risk factors

2.1.2.1 Radiation and mobile phones

There is strong epidemiologic evidence linking ionizing radiation and meningioma. In a cohort of 10,834 children treated for tinea capitis by low-dose (mean 1.5 Gy) radiation therapy in Israel, a relative risk of 9.5 (95% CI: 3.5-25.7) for developing a meningioma was found compared to their non-irradiated siblings (Ron et al. 1988). On the other hand, the study conducted among survivors of atomic bombs in Nagasaki and Hiroshima
showed only a slightly increased meningioma risk (excess relative risk per Sievert 0.6, 95% CI -0.01-1.8). The average radiation doses in this study were lower (approximately less than 0.2 Gy) and more than half of the subjects were adults at the time of the radiation exposure (Preston et al., 2002). High-dose radiation treatment to the head or neck for cancer has also been shown to increase the risk of meningioma (Al-Mefty, 2004). Especially in children, the central nervous system is the most often irradiated site and meningioma is the most common secondary tumor among children treated with radiation therapy (Neglia et al., 2006; Galloway, 2011). These children require long-term follow-up because the meningioma risk starts to increase 10 years after irradiation (Galloway et al., 2011). Clinically, radiation induced meningiomas differ from primary intracranial meningiomas. They present at younger age, they are more often multiple, have a higher tendency to recur, and are more often atypical or anaplastic (Strojan, 2000; Wilson, 1994).

Low doses of ionizing radiation in medical and occupational settings (diagnostic x-rays, radiation at work) have not been shown to increase meningioma risk (Phillips et al., 2005; Flint-Richter, 2011; Samkange-Zeeb, 2010), but a small contribution cannot be excluded (Blettner et al., 2007, Longstreth et al., 2004; Preston-Martin et al., 1995).

Exposure to electromagnetic fields (MF) has been suspected as one of the risk factors for brain tumors. In a case-control study carried out in France, MF exposure was assessed at occupational settings and at home. A total of 67 meningiomas cases were included in the study. A significantly increased meningioma risk was found following exposure to extremely low frequency MF (OR 3.02 95% CI 1.10-8.25) (Baldi et al., 2011). In a larger hospital based case-control study conducted in the USA, occupational exposure to MF was not associated with the risk of meningioma or glioma (Coble et al., 2009).

The measurements made on phantoms show that almost all of the
radiofrequencies of electromagnetic field exposure from the mobile phone is absorbed in the brain hemisphere on the side of phone use (Cardis et al., 2008). The meningeal tissue is close to the skin and hence at risk from the exposure. There have been multiple studies on the use of cell phones and meningioma risk but with the exception of one, no significant association has been found in any of these studies (Schüz et al., 2006; Cardis et al., 2010). An increased risk with ever-use of mobile phones was reported by Hardell et al. (2005) with OR of 1.7 (95% CI 1.0-3.0). Use of mobile phones for more than ten years resulted in OR of 2.1 (95% CI 1.1-4.3). The most recent study considered the brain tumor risk in relation to estimated radio frequency dose absorbed in the brain (Cardis et al., 2011). They found an increase in glioma risk with higher levels of radio frequency dose in people whose brains had absorbed high levels of radio frequency energy from mobile phone use of 10 or more years. Smaller increase was found in meningioma risk (OR 1.34, 95% CI 0.55-3.25). The problem in all of these studies is a relatively short follow-up as development of meningioma after exposure can be decades and for case-control studies there is also the potential for recall bias.

2.1.2.2 Head trauma

Head trauma has been suggested to have an influence on meningioma risk but the subject is still controversial. It has been suggested that chronic inflammation or granuloma formation of the injured area can lead to meningeal irritation and development of a tumor (Barnett et al., 1986; François et al., 2010). Several small case-control studies have reported elevated odds ratios ranging from 1.2 to 6.4. In a population-based case-control study with 200 cases and 400 control subjects, head trauma was associated with an increased risk of meningioma, especially when head
injury took place 10 to 19 years before the reference date (Phillips et al., 2002). The severity of head trauma did not have a dose-response relationship. In Preston-Martin’s (1998) case-control study of 1500 patients, an elevated meningioma risk was suggested for men. However, Annegers et al. (1979) ended up with an opposite finding. In their large series of almost 3000 patients, only three patients developed meningiomas after head trauma. Later, a large Danish cohort study which consisted of 228,055 Danish residents hospitalized for head trauma between 1977 and 1992 found an excess of meningiomas during the first year of the injury but no clear excess remained when the first year was excluded (SIR 1.2, 95% CI 0.8-1.7). They concluded that most of the excess of post traumatic tumors was likely caused by detection of tumors already present before the injury (Inskip et al., 1998).

2.1.2.3  *Occupational and medical risk factors*

The role of occupational exposures in the etiology of meningioma has been investigated in numerous epidemiological studies. Attempts to link exposure to industrial chemicals and meningioma risk have been inconsistent. Exposure to lead is associated with an increased meningioma risk in men (Navas-Acién et al., 2002). Women with an occupational exposure to herbicides have a higher meningioma risk than women with no exposure (OR 2.4, 95%CI 1.4, 4.3). Also, there was a significant trend of increasing risk with increasing years of exposure. Meningioma risk was not increased among men but this may be explained by a small number of meningioma cases among men (Samanic et al., 2008). Earlier, a significantly increased meningioma risk was reported among male agricultural consultants (SIR 5.4, 95% CI 1.11-15.8) (Pukkala 1995).
Women with previous incidence of breast cancer have an increased meningioma risk compared to women without breast cancer. In addition, female meningioma patients experience a higher breast cancer risk (Custer et al., 2002). In a large population-based study, the cumulative observed rate of meningioma in women with a previous breast cancer diagnosis was 58 times the expected rate. No such association was found for men (Rao et al., 2009). Also, other sex hormone related conditions, such as uterine fibroids and endometriosis, are more common among women with meningioma than among controls. A slightly increased risk of ovarian and endometrial cancer is reported (Claus et al., 2011).

Immunological factors may be involved in development of meningioma. A small inverse association between allergic conditions and meningioma has been described (Berg-Beckhoff et al., 2009) and a significant protective effect of eczema has been found (OR 0.74, 95% CI 0.60-0.91) (Wigertz et al., 2007). It has been suggested that this hyper-responsiveness to antigens may also lead to more effective tumor immunosurveillance in the brain (Claus et al., 2011). In a meta-analysis of eight case-control and cohort studies examining the association between atopic diseases and meningioma, no statistically significant associations were found (Linos et al., 2007).

2.1.2.4 Genetic risk factors

Some rare inherited genetic syndromes predispose to meningioma. Neurofibromatosis type 2 (NF2), a rare autosomal dominant disorder, is the most important. It is caused by inactivating mutations of the NF2 tumor suppressor gene. The penetrance is almost 100% by the age of 60 years. About 50% of patients with NF2 present with meningioma (Evans, 1992). Even though NF2 affects both sexes equally, female predominance in meningioma incidence is still observed in NF2 (Antinheimo et al., 1997). Of
the meningiomas associated with NF2, 8-10% are intraspinal. Meningiomas in NF2 patients tend to arise earlier in life than sporadic meningiomas and are more often fibroblastic subtype and multiple (McLendon, 1998). Without NF2, only 4% of meningiomas are multiple (Antinheimo et al., 2000). NF2 associated meningiomas may also be more aggressive than sporadic ones. They occur more frequently in unusual locations such as ventricles or the optic nerve sheath. In pediatric patients, meningioma often is the first sign of NF2.

Family history of meningioma may increase meningioma risk in first-degree relatives. A two-fold risk was found by Malmer et al. (2003). Hemminki et al. (2009) used data from the Swedish and Norwegian Registry Databases and found an increased meningioma risk with increasing numbers of affected first degree relatives with persons having one or two first degree family members with meningioma. In the recent study of Claus et al. (2011) a first-degree family history of meningioma was associated with an increased meningioma risk (OR 4.4 95% CI 1.6-11.5). The risk was even higher if the meningioma diagnosis of the relative was done at a young age. There also seems to be a small non-significant risk also with second-degree family history of meningioma (OR 3.2, 95% CI 1.2-24.3).

2.1.2.5  Hormonal treatments

For the first time, the suspicion of an increased meningioma risk among women using MHT was raised by the Nurses’ Health Study (Jhawar et al., 2003). They found an elevated risk of meningioma in postmenopausal women who were current users of MHT (RR 1.86). After that, this finding was confirmed in two other studies (Blitshteyn et al., 2008; Wigertz et al., 2006) with odd ratios from 1.7 to 2.1. Three other studies have attempted to clarify the relationship between meningioma risk and MHT but the results
have been inconclusive or shown a non-significant protective effect of MHT on meningioma (Hatch et al., 2005; Custer et al., 2006; Lee et al., 2006). In a large European cohort study with 194 meningioma cases among 276,212 women, use of MHT, especially current use, increased meningioma risk (HR 1.79; 95% CI 1.19-2.71) (Michaud et al., 2010). The Million Women study was a prospective study of 1,147,894 postmenopausal women. During a mean follow-up time of 5.3 years, 311 meningiomas were observed. The relative risk of meningioma among current users was 1.34, (95% CI 1.03-1.75) and among past users it was 1.29, (95% CI 0.96-1.72). Among current users, the highest meningioma risk was associated with women using estrogen-only therapy (1.44, 95% CI 1.03-2.02). The risk increased with longer exposure time. Also, oral estrogen intake was associated with slightly but non-significantly higher meningioma risk than transdermal estrogen (RR 2.42, 95% CI 1.30-4.50 vs. 1.59, CI 0.98-2.58) (Benson et al., 2010). Opposite to these findings, no association between MHT or OC and meningioma risk was found in a large population-based cohort study in the UK (Cea-Soriano et al., 2011). They followed over 2 million patients for an average follow-up period of six and half years and during this time they identified 745 meningioma cases. The results of these previous studies on meningioma risk and MHT are shown in Table 1.

Results on oral contraceptives and meningioma risk are even more inconclusive. In a study on risk factors for spinal meningiomas, OCs had a protective effect and this effect was greater with longer exposure (Preston-Martin, 1995). The protective effect of OCs was also demonstrated in the study of Lee et al. (2006), but there was no association with duration of use and meningioma risk. Non-significantly elevated risk in past (OR = 1.5, CI 0.8-2.7) and current (OR = 2.5, CI 0.5-12.6) users was reported by Custer et al. (2006). In the European cohort study, an increased risk with current use of OC was found with HR of 3.61 (95% CI 1.75-7.46) and there was also a statistically significant dose-response relationship for the duration of OC use
(Michaud et al., 2010). Other studies have failed to demonstrate the relationship between OCs and meningioma (Wigertz et al., 2006; Lee et al., 2006; Hatch et al., 2005).

Only one study has evaluated other modes of contraception such as subdermal implants, injections and intrauterine devices and meningioma risk (Wigertz et al., 2006). In this study, the odds ratio of meningioma was 1.5 (CI 0.9-2.6). For use of 10 years or more the risk increased to 2.7 (CI 0.9-7.5).

The effects of parity, age at first birth, menarche and menopause on the risk of meningioma are unclear. Meningiomas appear to enlarge during pregnancy and the luteal phase of a menstrual cycle, which suggests that reproductive history may be related to the meningioma risk. Increased risk for meningioma with increasing age at menarche was found in a prospective study of Jhawar et al. (2003). Earlier, similar results with menarche were reported by Preston-Martin et al. (1995) in their study of spinal meningiomas.

A few study groups have observed increased risk for meningioma with ever having been pregnant (Jhawar et al., 2003; Michelsen and New, 1969) but opposite results have also been published (Roelvink et al., 1987; Schlehofer et al., 1992; Preston-Martin et al., 1995; Hatch et al., 2005). Custer et al. (2006) found an increased risk for meningiomas among women who were currently menopausal. In turn, post-menopausal status 10 years before the reference date had a non-significant protective effect. The authors suggested that the risk of developing meningioma may be associated with changing hormonal levels.
Table 1. Previous studies on use of menopausal hormone therapy (MHT), oral contraception (OC) and meningioma risk.

<table>
<thead>
<tr>
<th>Author</th>
<th>Study design</th>
<th>Number of cases</th>
<th>Comparison</th>
<th>MHT RR (CI 95%)</th>
<th>OC RR (CI 95%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Benson 2010</td>
<td>Cohort</td>
<td>311</td>
<td>Ever</td>
<td>1.3 (1.0-1.8)</td>
<td>-</td>
</tr>
<tr>
<td>Michaud 2010</td>
<td>Cohort</td>
<td>194</td>
<td>Current</td>
<td>1.8 (1.2-2.7)</td>
<td>3.6 (1.8-7.5)</td>
</tr>
<tr>
<td>Blitshteyn 2008</td>
<td>Cohort</td>
<td>1390</td>
<td>Ever</td>
<td>2.2 (1.9-2.6)</td>
<td>-</td>
</tr>
<tr>
<td>Wigertz 2006</td>
<td>Case-control</td>
<td>178</td>
<td>Ever</td>
<td>1.7 (1.0-2.8)</td>
<td>1.0 (0.6-1.6)</td>
</tr>
<tr>
<td>Custer 2006</td>
<td>Case-control</td>
<td>143</td>
<td>Current</td>
<td>1.0 (0.5-2.2)</td>
<td>2.5 (0.5-12.6)</td>
</tr>
<tr>
<td>Lee 2006</td>
<td>Case-control</td>
<td>219</td>
<td>Ever</td>
<td>0.7 (0.4-1.2)</td>
<td>0.5 (0.4-0.8)</td>
</tr>
<tr>
<td>Hatch 2004</td>
<td>Case-control</td>
<td>151</td>
<td>Ever</td>
<td>0.8 (0.5-1.4)</td>
<td>1.0 (0.6-1.7)</td>
</tr>
<tr>
<td>Jhawar 2003</td>
<td>Cohort</td>
<td>125</td>
<td>Current</td>
<td>1.9 (1.1-3.2)</td>
<td>1.3 (0.2-10.0)</td>
</tr>
</tbody>
</table>
2.2 Diagnostic and pathologic classification

2.2.1 Location

Meningiomas are extra-axial lesions that most often arise in superficial locations associated with meninges. The most common locations include the convexity and sphenoid ridge. Other locations are the parasellar region, the olfactory groove and the sylvian fissure. It’s rare for meningioma to involve the optic nerve sheath. Approximately 10% occur infratentorially. Meningiomas can also arise intraventricularly but this is more common in children. In children, intraventricular meningiomas account for 17% of meningiomas and 19% are located infratentorially. Anatomical location has been shown to be a risk factor for atypical and malignant meningiomas. Nonskull base meningiomas are twice as likely to be atypical or malignant (Kane et al., 2010).

The majority of spinal meningiomas are located in the thoracic region in women. In men, 50% of meningiomas are located in thoracic region and 40% in the cervical region. Spinal meningioma is seldom multiple. Multiplicity is usually associated with NF2 (Traul et al., 2007).

2.2.2 Classification

2.2.2.1 Benign meningiomas

Meningiomas are classified histologically according to World Health Organization (WHO) criteria (Louis, 2007). They arise from the meningothelial cells of the arachnoid membranes or their precursors or stem
cells. Meningiomas can be differentiated histologically into 15 different subtypes and the majority of meningiomas are benign (grade I). Benign meningiomas have a variety of histological subtypes, which do not differ in behavior or prognosis. The most common subtypes are mengiothelial, fibrous, and transitional meningiomas. Other subtypes include psammomatous, angiomatous, microcystic, secretory, lymphoplasmocyte rich and metaplastic meningioma.

2.2.2.2 Atypical meningiomas

Atypical meningiomas (grade II) constitute about 4-7% of meningiomas. Atypical meningiomas are characterized by increased mitotic activity (= 4 mitosis/10 high power fields). Tumors with less mitotic activity must have three or more of the following features: increased cellularity, small cells with high nucleus to cytoplasm ratio, prominent nucleoli, uninterrupted patternless or sheet-like growth, and necroses. In the new WHO classification (Louis, 2007), brain invasion is also an independent criterion for atypical meningiomas. Clear cell meningioma is a rare subgroup of atypical meningiomas with increased propensity to recur locally. Another variant of atypical meningioma is choroid meningioma, which resembles chordoma histologically and is considered grade II because of its higher recurrence rate.

2.2.2.3 Malignant meningiomas

There is not much data on the clinical behavior of malignant meningiomas. Malignant or anaplastic (grade III) meningiomas constitute 1-3% of all meningiomas. There is male predominance of malignant meningiomas and they tend to appear earlier in life. Malignant meningiomas have high mitotic
indices (= 20 mitoses/10 high power fields) or loss of meningotheelial differentiation resulting in sarcoma, carcinoma or melanoma-like appearance. Papillary and rhabdoid meningiomas are rare histological subgroups that are included in grade III meningiomas. They are associated with increased risk of recurrence and distant metastases. Papillary meningiomas occur mostly in children.

2.2.2.4 Proliferation index

Proliferation index is usually measured with the antibody MIB-1, which targets the antigen Ki-67. Ki-67 is expressed in proliferating cells throughout the cell cycle (Gerdes et al., 1984). Labeling index (LI) is the percentage of immunoreactive tumor cell nuclei. As tumors are histologically heterogenic with regional differences of cell proliferation, tumor sampling is a source of error in determining LIs. The area in the tumor that is histologically most malignant, is usually selected for the analysis of LI.

Increased MIB-1 is associated with higher histological grade and an increased risk of recurrence in meningiomas (Hsu et al., 1998). The average LI for benign meningioma is 3%, for atypical meningioma 8% and for malignant meningioma 17% (Abry et al., 2010). Most studies report a higher proliferation index for recurrent meningiomas than non-recurrent ones. Meningiomas with MIB-1 staining index of 4 % or more have a significantly higher tendency to recur. MIB-1 has also been reported to be higher in males than females and in meningiomas with edema in MRI. MIB-1 is lower in meningiomas with calcifications (Matsuno et al., 1996). NF2-associated meningiomas have higher LI than sporadic ones, reflecting the more aggressive behavior of these meningiomas (Antinheimo et al., 1997).
2.2.2.5 Molecular genetics

The NF2 gene, which encodes the merlin tumor suppressor, is located in chromosome 22. Merlin is located on the cell membrane at regions that regulate cell-cell contact motility. The absence of merlin stimulates cell proliferation in meningioma cells. Monosomy of chromosome 22 represents the most common gene alteration in meningiomas. About 60% of sporadic, solitary meningiomas occur due to inactivation of this gene. In 40% of sporadic meningiomas genetic background is unknown (Ruttledge 1994).

NF-2 associated meningiomas have deletions on chromosome arm 22q in almost 100% of cases (Lamszus et al., 2004). Different subtypes of benign meningiomas have different frequency of NF2 gene alterations. Fibroblastic and transitional subtypes have NF2 mutations in 70-80% of cases whereas meningothelial subtype is only seen in 25% of cases (Wellenreuther, 1995). The NF2 mutation is likely to represent an early event in tumorigenesis because the frequency of NF2 mutations does not vary according to different histologic grades (Lamszus et al., 2004).

In addition to the NF2 gene, several other chromosomal alterations in meningiomas have been detected. DAL-1 protein resembles merlin, as it is a transmembrane protein and harbors tumor suppressor properties. It is located on chromosome 18. DAL-1 expression is lost in 76% of sporadic meningiomas. Loss of expression is similar among different grades, suggesting that DAL-1 mutation also is an early event in tumorigenesis (Perry et al., 2000). Deletions on chromosome 1 have been associated with tumor progression. Expression of 1p deletions increases from 13% to 26% in benign meningiomas to 70-100% in anaplastic variants (Bello et al., 1994). The third most frequently found chromosomal alteration in meningiomas after those in chromosome 22 and 1 is deletion on chromosome arm 14q. This deletion is also associated with tumor progression as its frequency increases with tumor grade.
Meningioma tumors in women and men differ cytogenetically from each other. Female patients have lower frequency of monosomy 14 and higher frequency of numerical abnormalities of chromosomes 22 and X than male patients. Complex karyotypes are more often found in men. 14% of the female patients have a loss of chromosome X. A chromosome Y nulisomy is detected in one quarter of male patients. Based on 8 genes that are differentially expressed between sexes, meningiomas from female and male patients can be classified as different. The differentially expressed genes in males and females are coded in the sex chromosomes emphasized in the Y chromosome. Interestingly, these genes are not directly related to sex hormone receptor expression. This may indicate that non-steroid hormone associated genetic factors encoded in the sex chromosomes may be involved in the development of tumors (Tabernero et al., 2007).

2.2.3 Sex hormone receptors in meningiomas

2.2.3.1 Progesterone receptors

Normal adult meninges express only low levels of progesterone receptors (PRs) (Maxwell et al., 1993) whereas the majority of meningiomas express PRs. A decrease in PR expression from low grade to high grade has been reported (Roser et al., 2004; Ruiz et al., 2010) and PR negative meningiomas are more aggressive than PR positive ones (Whittle et al., 1984). Even a small number of tumor cells expressing PR is a favorable prognostic factor for meningiomas (Hsu et al., 1997). Meningiomas that have transformed from benign to atypical have lower expression of PR than de novo atypical meningiomas (Krayenbühl et al., 2007). Meningothelial subtype has a higher PR expression than the transitional and fibrous subtypes. However, few study groups have reported higher PR expression in
females than males (Hsu et al., 1997; Blankenstein et al., 2000). In a recent study, Claus et al. (2008) examined gene expression for meningioma and found evidence of overexpression of number of genes located on the long arm of chromosome 22 for PR positive cases compared to PR negative cases. This association between PR status and chromosome 22q regulation suggests a role for hormones in tumorigenesis of meningiomas.

2.2.3.2 *Estrogen receptors*

Normal meningeal tissues do not express estrogen receptors (ER) (Koehorst et al., 1993). The level of ER expression in meningiomas has been equivocal in different studies. In more recent studies approximately one third of meningiomas express ER (Konstantinidou et al., 2003, Guevara et al., 2009). There is no association between ER and PR expression, suggesting PRs in meningiomas are estrogen independent.

Expression of ER has been associated with more aggressive clinical behavior of meningiomas (Pravdenkova et al., 2006) but opposite results with lost or reduced ER expression in atypical meningiomas have been published (Konstantinidou et al., 2003). In the study of Krayenbühl et al. (2007) meningiomas that had transformed from benign to atypical, had ER expression of 30% compared to 0% expression in de novo atypical meningiomas.

2.2.3.3 *Androgen receptors*

Expression of androgen receptors (ARs) is less investigated in meningiomas than PRs or ERs. By immunohistochemistry, ARs are present
in two thirds of meningiomas (Carroll et al., 1993). AR expression is very low in normal meninges compared to meningiomas (Maxwell et al., 1993). Androgen receptors are more abundant in women compared to men (Black et al., 1996). Meningiomas with higher proliferation rate have been shown to express more ARs than meningiomas with lower proliferation rates (Konstantinidou et al., 2003). Androgens induce the synthesis of epidermal growth factor (EGFR) in human prostatic carcinoma cells (Mulder et al., 1989). Active EGFR has also been detected in meningiomas (Carroll et al., 1997). It is possible that AR promotes menigioma cell growth via autocrine or paracrine mechanisms. Androgens can stimulate meningioma growth in vitro and anti-androgens show anti-proliferative effects (Adams et al., 1990). Cyproterone acetate (CPA) is a progestin with anti-androgenic effects. It is used for conditions caused by increased androgen production. Patients using high dose CPA have an increased risk of meningioma. In a population-based case-control study with cases exposed to high dose CPA over one year, an incidence rate of 60 meningiomas per 100,000 person-years was detected (Gil et al., 2011).

2.2.3.4 Other molecular markers in meningiomas

Several receptors are gained in meningiomas by tumorigenesis. In addition to sex hormone receptors, somatostatin and glucocorticoid receptors are present in meningiomas. Meningiomas also express at least epidermal growth factor (EGFR), gonadotropin-releasing hormone (GnRH), platelet-derived growth factor (PDGF), cyclooxygenase-2 (COX-2), transforming growth factor alpha (TGF-alpha) and P-glycoprotein.

GnRH receptors are found in half of the meningiomas (Hirota et al.,
but its clinical significance is largely unknown. Most meningiomas express somatostatin receptors (Schultz et al., 2000). The activation of these receptors may be associated with an anti-proliferative effect. Activation of EGF and TGF-alpha receptors may promote tumor growth. EGF receptor inhibitors have been studied in recurrent meningiomas but no significant benefit was found (Norden et al., 2010). PDGF probably has a role in tumorigenesis of meningiomas. Higher expression of PDGF is found in atypical and malignant meningiomas compared to benign tumors (Nagashima et al., 2001). Increased COX-2 expression is found in recurrent meningiomas. Ragel et al. (2005) evaluated the effects of COX-2 inhibitor on meningioma using a mouse xenograft model. Tumor growth was inhibited by celecoxib. P-glycoprotein mediates resistance to a range of antitumoral drugs. Expression of P-glycoprotein is higher in transitional compared to meningothelial meningiomas (Andersson et al., 2004).

2.2.4 Carbonic anhydrases in meningiomas

2.2.4.1. Function of carbonic anhydrases

CAs belong to a family of metalloenzymes. They catalyze the reversible hydration of carbon dioxide.

\[ \text{CO}_2 + \text{H}_2\text{O} \leftrightarrow \text{HCO}_3^- + \text{H}^+ \]

In humans there are fifteen CA isoforms of which 12 are catalytically active. They participate in numerous biological processes including acid-base balance, carbon dioxide and ion transport, respiration, bone development and function and mucosal protection. Different isoforms vary in tissue
distribution and subcellular localization. Abnormal levels of CA II, IX and XII have been reported in several cancers and other diseases as glaucoma and neurological disorders (Supuran 2008). Small molecule inhibitors can modulate CA activity. There are already at least 25 different clinically used drugs that possess CA inhibitor properties, including drugs used in treatment of hypertension, glaucoma and hyperglycemia.

2.2.4.2 CA II

CA II is the most widely distributed of the carbonic anhydrases. It is found in almost all human tissues including the CNS. In the brain, CA II is expressed in the oligodendrocytes, choroid plexus epithelium, in subset of neurons with GABAergic phenotype and seldom in astrocytes. CA II positivity is transiently found during brain development in the endothelial cells of microvessels (Tashian, 1992; Kida et al., 2006). CA II deficiency causes the recessive inherited human syndrome of osteopetrosis with tubular acidosis and cerebral calcification (Sly & Hu, 1995).

Cytoplasmic CA II interacts with CA IX and an anion exchanger protein to produce acidosis in the extracellular space of tumors (Pastorekova et al., 2004). Hypoxic changes lead to necrosis of tumor tissue. Cytoplasmic CA II expression has been found in tumor cells as pancreatic and renal cancer cells (Parkkila et al., 1995; Parkkila et al., 2000). Recently, expression of CA II has been discovered in tumor vessel endothelia, which suggests a role in tumor angiogenesis (Yoshiura et al., 2005). Expression of CA II is common in the endothelial cells of high-grade oligodendrogliomas and infiltrating astrocytomas. CA II positivity also correlates with tumor grade and poor prognosis in astrocytomas (Haapasalo et al., 2007).

Activation of CA II constitutes a possible therapeutic approach for the enhancement of synaptic efficacy, which may be useful in the treatment of
Alzheimer’s disease. In addition to Alzheimer’s disease, diminished CA II activation has been detected in Parkinson’s disease (Korolainen et al., 2006).

2.2.4.3 CA IX

The distribution of CA IX expression is very limited in normal tissue. CA IX is found in the GI-tract, fetal lung and muscle and the male reproductive tract but it is not expressed in a normal human brain. Ectopic CA IX expression has been detected in several cancers including malignant brain tumors as astrocytic tumors and anaplastic meningiomas (Haapasalo et al., 2006; Ivanov et al., 2001; Yoo et al., 2007). CA IX is a transmembrane-associated enzyme and it is strongly induced by microenvironmental hypoxia. Its expression is usually limited to the perinecrotic areas. Conversion of glucose into lactic acid in tumor cells creates an acid load, which should cause intracellular acidosis. Upregulation of CA IX in tumor cells may buffer the acidic conditions. During the reversible hydration of CO2, bicarbonate is formed and H+ ions are transferred to the extracellular space, which leads to acidification of the extracellular space with activation of proteolytic enzymes and enhanced tumor invasiveness (Webb et al., 1999). In addition to this, CA IX influences other processes, such as cell adhesion and cellular proliferation. Expression of CA IX is often associated with poor responsiveness to radiotherapy and chemotherapy and thus with poor prognosis in several human tumors.

The most important class of CA IX inhibitors, is the sulfonamides. The sulfonamides bind specifically only to the hypoxic cells expressing CA IX, which leads to more physiologic pH extracellularly (Švastová et al., 2004). In hypoxic LS174Tr tumor cells, the inhibition of CA IX leads to a 40% reduction of xenograft tumor volume. When another carbonic hydrase
isoform CA XII is inhibited together with CA IX, 85% reduction of tumor growth is achieved (Chiche et al., 2009).

2.3 Treatment and prognosis

2.3.1 Diagnosis

Magnetic resonance imaging (MRI) is the method of choice when diagnosing meningiomas even though computed tomography (CT) is more available. CT is superior to MRI when demonstrating effects of meningioma on adjacent bone and calcifications seen in some meningiomas. Meningiomas appear as rounded or elongated extraaxial masses. Often, a dural attachment is seen. After administration of contrast material, meningiomas enhance most often homogenously but can sometimes show some heterogeneity (Saloner et al., 2010). 2% to 10% of meningiomas are cystic and they present a diagnostic challenge (Zee et al., 1995). Cysts can appear intratumourally or peritumourally. Cysts are more common in pediatric patients than in adults, the reported incidence being 24% (Ferrante et al., 1989). Meningioma can cause hyperostosis of adjacent skull, which is often visible in MRI and represents direct tumor invasion of the bone (Pieper et al., 1999).

There is no special feature in MRI or CT that differentiates benign meningiomas from malignant ones. Diffusion and perfusion MRI can be used to help to predict aggressive histological features. In diffusion MRI, reduced water diffusivity has been correlated with more aggressive tumor behavior and recurrence (Nagar et al., 2008). Perfusion MRI provides information on the vascular supply of meningiomas. Benign meningiomas get their blood supply from the external carotid artery via dural branches.
These vessels lack blood brain barrier and are permeable to gadolinium and thus provide a typical curve. Pial-cortical blood supply is usually associated with higher grade meningiomas. These branches contain a blood brain barrier which result in perfusion scans with intensities that return to baseline levels (Saloner et al., 2010).

2.3.2 Natural history of meningiomas

Widespread use of computer tomography and magnetic resonance imaging has led to identification of patients with incidental meningiomas. The majority of these patients stay asymptomatic during follow-up. Most incidental meningiomas show minimal growth and can be treated expectantly with serial imaging. In particular, meningiomas with calcifications are associated with lack of growth. The initial tumor size is not a predictive factor for tumor growth but meningiomas of patients less than 60 years old seem to have higher growth potential than those of older patients (Nakamura et al., 2003). Based on studies of the natural history of meningiomas, about one third of meningiomas progress (Yano et al., 2006). Unfortunately, in most of these studies only asymptomatic, elderly patients are selected for observation, and observation times are seldom more than 5 years, which is a relatively short time to study slow growing tumors.

Meningiomas can progress from benign to atypical or malignant. Up to 2% of benign meningiomas progress to higher grades. Almost one third of recurrent meningiomas transform to higher grade (Al-Mefty et al., 2004).
2.3.3 Treatment

2.3.3.1 Endovascular embolization

Embolization results in tumor necrosis and it can be used preoperatively to reduce morbidity and mortality associated with significant blood loss during operating large, hypervascular meningiomas. After embolization, tumors shrink and become softer and easier to resect. The most commonly used embolic agent is polyvinyl alcohol (PVA). In addition to PVA particles, glue embolizes, as Onyx can be used. The optimal timing for surgical procedure after embolization is within 7 to 9 days (Kai et al., 2002). If surgery is delayed after this, there is substantial potential for recanalization of embolized vessels. The overall risk of embolization is 5-6%. Serious complications include inadvertent delivery of embolizate into the intracranial circulation, intracranial or intratumoral hemorrhage, blindness and cranial nerve injury. Large PVA particles have lower risk of penetrating adjacent tissues and causing stroke or cranial nerve palsy. Smaller particles get deeply into the tumor but may also migrate into normal tissue (Carli et al., 2010).

2.3.3.2 Surgery

Surgery is the treatment of choice for most meningiomas and in most cases it is curative. Small, incidental, asymptomatic meningiomas especially in elderly patients may be managed expectantly with serial imaging. The most reliable predictive factor of meningioma recurrence is the extent of surgical resection (Simpson, 1957). Complete resection of meningioma is not always possible in difficult locations such as the olfactory groove, supracellar
region, foramen magnum, or lateral ventricle. The extent of surgical resection is graded by the Simpson five-level grading system (Simpson, 1957) in which grade I is synonymous with total resection. In grade II the tumor is macroscopically totally resected and dural attachments coagulated. Grade III equals total resection without resection or coagulation of dural attachments. Grade IV is partial resection and grade V biopsy. In the population-based study of Cahill et al. (2011), with over 12,000 patients with benign meningioma, the 1- and 3-year survival rates after resection were 95.4% and 92.4%. Recurrence rates after incomplete resection in benign meningiomas are 30%, 60%, and 90% at 5, 10, and 15 years (Marosi et al., 2008). In the study of Jääskeläinen (1986), after a seemingly complete resection of the tumor, an overall recurrence rate after 20 years was 19%. In the same study, strong risk factors for recurrence were coagulation of the dural insertion, invasion of bone, and soft consistency of tumor.

Histological grade of the tumor predicts the likelihood of recurrence. Benign meningiomas have a recurrence rate of 7-20% after gross total resection. Atypical and malignant meningiomas have a 40% and 50-80% risk of recurring (Lamszus, 2004). The mean overall survival for atypical meningiomas is 142.5 ± 6.0 months and 39.8 ± 7.8 months for malignant meningiomas (Yang et al., 2008). In the case series of Sughrue et al. (2010), 63 patients with malignant meningioma were followed for an average of 5 years. Patients with near total gross resection had improved overall survival compared to patients with gross total resection (median survival of 107 months compared with 50 months). They speculated that gross total resection can affect the patient’s functional status and thus influence decisions on further treatment. Simpson’s recommendation of total resection for treatment of meningioma has also been challenged in case of benign meningiomas. In the cohort of 373 grade I meningiomas, there was no significant difference in recurrence-free survival between patients going through Simpson grade I, II, III or IV resection (Sughrue et al., 2010).
The amount of cytogenetic abnormalities in meningioma also affects the recurrence rate. The more there are cytogenetic abnormalities, the higher the recurrence rate is after surgical resection even with the same histological grade. Convexity meningiomas have more chromosomal changes than skull base meningiomas. Recurrence rate of completely resected convexity meningioma is higher than skull base meningioma or meningioma in the spinal canal (Ketter et al., 2007, 2008).

In case of spinal meningioma, surgical resection is the preferred treatment. Complete resection is possible in most cases. Long-term outcome of spinal meningioma treated with complete resection is good and recurrence of grade I tumors is uncommon. Recurrence rates for spinal meningiomas are smaller than for intracranial ones (Baird and Gallagher, 1989).

2.3.3.3 Radiation therapy

All meningiomas cannot be resected safely or completely due to location, large size or proximity of critical structures. Meningiomas are relatively radio-resistant but still, radiotherapy is the most effective adjuvant therapy available. For benign meningiomas, total doses of 50 to 60 Gy are needed for prolonged tumor control (Mendenhall et al., 2003). Atypical or malignant meningiomas require even higher doses. Postoperative radiation therapy is associated with better PFS and local control rates compared to subtotal resection alone and is comparable to those in patients with complete resection (Taylor et al., 1988). The goal of the radiation therapy is to halt tumor progression. The size of the tumor may remain the same or decrease only slightly after radiotherapy. When tumor shows no progression in size, radiotherapy is considered to be curative.

Literature supports the use of immediate postoperative radiation for
patients with malignant meningioma. In the study of Dziuk et al. (1998), the 5-year PFS for patients with total resection increased from 28% to 57% with postoperative radiation. Increasing the dose of radiation is associated with improved local control.

In stereotactic radiosurgery, a high single dose of radiation is delivered to a discrete treatment volume of less than 3 to 4 cm. The radiation dose falls rapidly at the edge of the target volume. Hence, only a clinically insignificant radiation dose reaches the normal tissue. Most meningiomas are small and well circumscribed which makes them a good target for stereotactic radiosurgery (SRS). In addition to partly resected meningiomas, SRS has been used as primary therapy in surgically inaccessible tumors. In the study of Kondziolka et al. (2008), 536 patients with meningiomas without histological confirmation, had stereotactic radiosurgery with an overall control rate of 97% at a median of four years. Follow-up imaging after 8 years revealed a 94% control rate.

The most common side effects of SRS are cranial nerve defects and peritumoral edema. Rare but uncommon side effects include radiation necrosis, peritumoral cyst formation, carotid artery stenosis and hypothalamic dysfunction (Kondziolka et al., 2008).

Use of radiation therapy with spinal meningiomas is adversarial because of the indolent clinical course of most spinal meningiomas. Radiation therapy may be justified in cases of relapsing meningioma, inaccessible tumor or pre-existing comorbidities. There is preliminary data showing that frameless stereotactic radiosurgery, as a single fraction therapy, is safe and effective in treatment of benign spinal tumors (Gerszten et al., 2004).

2.3.3.4 Medical Therapy

Chemotherapy is seldom used in treatment of meningiomas. It is mainly
applied in inoperable cases with tumor progression after radiotherapy. Traditional cytotoxic drugs have not been particularly effective even though modest activity has been shown in some subgroups of patients. Furthermore, the slow growth rate of benign meningiomas makes it difficult to estimate the effect of chemotherapy, as meningioma can appear radiologically stable for a long period of time.

Hydroxyurea is currently used mainly in treatment of myeloproliferative disorders. Hydroxyurea inhibits ribonucleotide reductase and can cause apoptosis in meningioma cell cultures. It is the most promising chemotherapeutic agent in the treatment of meningiomas. It has shown modest clinical activity in several studies in treatment of inoperable or recurrent meningiomas (Schrell et al., 1997; Mason et al., 2002) but quite often causes hematological side effects. Temozolomide, which has proved to be effective treatment in glioblastoma, has not been shown to have an effect in treatment of recurrent meningiomas (Chamberlain et al., 2004).

Promising results in breast cancer treatment have supported approaches based on hormone manipulation in meningioma treatment. Treatment with antiestrogenic agents, such as Tamoxifen, has not been effective (Schrell et al., 1990). The antiprogesterone agent Mifepristone inhibits growth of meningioma cells in vitro (Matsuda et al., 1994) but has failed to demonstrate clinical treatment effect. A phase III double-blind randomized trial of Mifepristone showed no benefit (Grunberg et al., 2001). At this point, antiprogesterone and antiestrogen drugs have a limited role in the treatment of meningiomas. Published data on androgen receptor inhibitors in clinical trials does not exist. In a small unpublished trial consisting of six patients treated with antiandrogen flutamide, no response was seen (Wen et al., 2010).

In addition to sex hormone receptors, most meningiomas express somatostatin receptors (Arena et al., 2010). Somatostatin has shown to inhibit meningioma cell growth in vitro. In a preliminary study of 16
patients, one third of the patients had partial response and one third had stable disease. In a phase II study 11 patients with recurrent meningioma and one patient with hemangiopericytoma were treated with subcutaneous ocreotide (a somatostatin receptor agonist). Eleven patients experienced tumor progression with a median time to progression of 17 weeks. Median survival time was 2.7 years (Johnson et al., 2011).

Immunoglobulins are produced by the immune system in response to various disturbances including tumors. Interferon-α (IFN-α) inhibits growth of cultured meningioma cells in cell lines (Koper et al., 1991). Small reports on efficacy of IFN-α on treatment of recurrent meningiomas have been published earlier. The largest study had six patients (Kaba et al., 1997). Chamberlain et al. (2008) published a prospective phase 2 study of meningioma patients with grade I recurrent meningiomas that had recurred after one or more surgeries and had also failed radiotherapy and/or chemotherapy. 26 of 35 patients demonstrated a stable disease after three cycles of interferon. The progression-free survival rate was 54% at 6 months and 31% at 12 months.
3. AIMS OF THE STUDY

The present study aimed to clarify the possible role of sex hormones in tumorigenesis of meningiomas. The specific aims were defined the following:

1) To evaluate the expression of sex hormone receptors and carbonic anhydrases II and IX in meningiomas.

2) To analyze their association with sex, age, tumor grade, cell proliferation and recurrence.

3) To find out the association between hormonal and reproductive factors, in particular,

4) Menopausal hormone treatment on meningioma risk.
4. DATA AND STATISTICAL ANALYSIS

4.1 Study Population

4.1.1 Case-control study

The source population consisted entirely of women aged 20-69 years in Finland. Only Aland and the northernmost Lapland were excluded. The study period ranged from November, 2000, to October, 2002. The study was a part of INTERPHONE, an international case-control study of adult brain tumors (Cardis et al., 2007). Women aged 20-69 years old diagnosed with meningioma during the study period were eligible. Diagnosis was always verified histologically and confirmed by a single neuropathologist. Cases were identified continuously during the study period from neurosurgery departments at all five of the university hospitals in Finland. Patient lists were browsed weekly by research nurses to identify new cases. Of the 320 identified cases 292 patients were eligible for the study. Diagnosis date preceding the start of the study, language problems, mental retardation and so on were the reasons for exclusion. Of these 292 cases, 26 (9%) refused. Control subjects were randomly identified from the source population, and were stratified by age (in 5-year groups) and residential area to match the distribution among cases (frequency matching). Controls were selected from the nationwide Population Register Centre approximately every second month throughout the study period. A total of 506 women (43%) of the
1180 eligible controls consented and were successfully interviewed. The most common reasons for failure to recruit the controls were refusal (60%) and failure to contact (23%).

Research nurses were hired and trained to carry out all interviews and contacts with cases and controls. Information on use of hormonal treatments and other possible risk factors, such as parity, exposure to ionizing radiation, and family history of brain tumors, was collected through personal interviews. Exposure information related to exogenous female hormones was acquired by asking the subject if she has ever used any kind of contraceptives, or received hormonal treatment for gynecological problems, fertility problems or menopausal symptoms. A positive answer was followed by supplemental questions on the brand name of the preparate and duration of use. Postmenopausal status was defined by not having menstrual periods or by having a bilateral oophorectomy at least a year prior to the reference date (date of diagnosis for the cases and the date preceding the interview by a similar period, i.e. days 39 for controls).

4.1.2 Cohort study

For the cohort study, all women at the age of 50 years or higher who had bought systemic menopausal hormone therapy (MHT) during the study period (1994-2009) were identified. Regimens for MHT are available only by doctor’s prescription and they are partly reimbursed through the National Social Insurance Institution. Thus, the individual MHT use can be traced from the medical reimbursement register, which covers all systemic MHT use in Finland. Only women who had used estradiol-only (ET) or estradiol-progestin therapy (EPT) for at least six months were included in the final study population.
In Finland, estradiol is the only systematically administered estrogen available. As post-menopausal therapy, it can be administered either orally or transdermally. The route of administration refers to oral or transdermal use of EPT. Oral EPT is defined as regimen wherein both estradiol and progestin were taken orally, whereas the term “transdermal EPT regimen” is used when estradiol was added with transdermal progestin. The dosages of transdermal ET were 50 µg/d in fixed EPT preparations and those of oral ET were 1 or 2 mg/d. The progestin component can be administered sequentially or continuously. Some women used individual EPT regimens by combining oral (1.0-2.0 mg/d) or transdermal estradiol (25-100 µg/d from the patch or 0.5-1.5 mg from gel) with oral progestin courses of 10-14 days, with 1-3 month intervals. The sequential regimen included progestin courses of 10-14 days duration that were combined at a 1-3 month interval with estradiol. Use of progestin every day was defined as a continuous combined regimen. The EPT regimen was classified according to the first EPT. The cohort was followed for incident meningioma cases up to end of 2009 through the nationwide Finnish Cancer Registry.

4.1.3 Tumor samples

The study samples were from patients who underwent surgery for intracranial meningioma at the Tampere University Hospital during 1989-1999. Patients less than 16 years old were excluded. 443 primary and 67 recurrent tumor specimens were chosen for the study. Tumors were classified and graded using the World Health Organization scheme (grades I – III). The most common histological subtype was meningothelial (33%), followed by transitional (29%) and fibroblastic (21%) subtypes. 407 of the meningiomas (92%) were diagnosed as benign (grade I), 33 (7%) atypical (grade II), and 3 (1%) malignant (grade
III). Of the 67 recurrent meningiomas 48 (71.6%) were benign (grade I), 16 (23.9%) were atypical, and 3 (4.5%) were malignant. For statistical analysis, atypical and malignant tumors were grouped together.

4.1.4 Immunohistochemistry

4.1.4.1 Sex hormone receptors

In immunohistochemical studies, tissue microarray (TMA) was used. In TMA hundreds of tissue cores can be arranged on a single slide and analyzed by a single immunostaining reaction (Kononen et al., 1998). The tumor samples were fixed in 4% phosphate buffered formaldehyde and processed into paraffin blocks using standard methods. A neuropathologist selected histologically representative tumor regions of hematoxylin-eosin-stained slides and corresponding areas were sampled in tissue microarray blocks using a custom-built instrument (Beecher Instruments, Silver Spring, MD). The sample diameter of the tissue core in the microarray block was 600 μm.

For analysis of proliferation index, sections cut from TMA block were immunostained with MIB-1 (Ki-67) antibody (DakoCytomation, Glostrup, Denmark). Heat-induced epitope retrieval (Tris-EDTA buffer (pH 9.0, 2x7 min in a microwave oven) and an automated immunostaining protocol (TechMate immunostainer) was used. The tissue sections were counterstained with methyl green. The proliferation was reported as the percentage of immunopositive nuclei and evaluated by analysing all the tumor cells in the core tissue with an image analysis system (CAS-200 Software, Becton Dickinson & Co., USA) as described previously (Sallinen et al., 1994).

Monoclonal antibodies 6F11, PGR312, and 2F12 were used for
immunohistochemistry of PR, ER, and AR, respectively (Novocastra Laboratories, Newcastle, UK). Antigen retrieval was carried out as described above. All antibodies were diluted at 1 μg/ml, and detected with a peroxidase-polymer based detection kit (PowerVision+TM, ImmunoVision Technologies, Daly City, CA). Counterstaining was performed with hematoxylin.

4.1.4.2 Carbonic anhydrases II and IX

The automated immunostaining for CA II and IX was performed using Power Vision+TM Poly-HRP IHC Kit (ImmunoVision Technologies, CA), reagents and antibodies specific for CA II and CA IX. The following steps were included in the immunostaining method: a) rinsing in Tris-buffered saline (TBS) + 0.05% Tween-20 wash buffer (TBST); b) treatment in 3% H2O2 in ddH2O for 5 min and rinsing in TBST; c) blocking with cow colostrum for 20 min; d) rinsing in TBST; e) incubation with rabbit anti-human CA II serum (produced and characterized earlier by Parkkila et al., 1995), M75 antibody against human CA IX 48, or normal rabbit serum (NRS). Anti-CA II serum and NRS were diluted 1:2000 and M75 1:200, respectively, in Universal IHC diluent for 30 min; f) washing with TBST for 3x, 5 min; g) blocking with postblocking solution for 20 min (only in M75 staining); h) rinsing in TBST for 3x, 5 min; i) incubation in Poly-HRP-conjugated anti-rabbit/mouse IgG for 30 min; j) washing in TBST for 3x, 5 min; k) incubation in DAB (3,3',5-diaminobenzidine tetrahydrochloride) solution (one drop DAB solution A and one drop DAB solution B with 1 ml ddH2O) for 6 min; l) rinsing with ddH2O; m) CuSO4 treatment for 5 min to enhance the signal; and n) rinsing with TBST and counterstaining with hematoxylin. All procedures were carried out at room temperature. The sections were mounted in Entellan Neu (Merck; Darmsadt, Germany).
4.1.5 Statistics

In the analysis of the association of sex hormone expression and association with age, gender and histological grade, a chi-square test was used. The relationship between receptor status and proliferation index was assessed by Mann-Whitney U test.

The chi-square test was also used when analyzing associations between CA expression, sex hormone receptor status, tumor grade and patient age group. The relationship between CA expression and cell proliferation was assessed with the Mann-Whitney U-test.

In the case-control study, odds ratios with 95 percent confidence intervals were used as measures of the effect of hormonal and reproductive factors on risk of meningioma. Case-control analysis was conducted using unconditional logistic regression with adjustment for age and residential area as well as any family history of brain tumors. Duration of hormonal treatment was divided into approximate quartiles based on frequencies among controls, with full years as cut-points. No exposure was used as the referent category.

Analyses by hormone receptor status were carried out by comparing subgroups of cases with all of the controls.

In the cohort study, expected numbers of meningioma case were calculated by multiplying the number of person-years in each 5-year age group by the corresponding average meningioma incidence among all Finnish women during the same year of observation. To calculate standardized incidence ratios, the observed numbers of cases were divided by expected numbers. Exact 95% confidence intervals were defined under
the assumption that the observed numbers follow a Poisson distribution. This assumption was confirmed by a negative binomial regression analysis showing little evidence for overdispersion. All reported probability values were 2-sided and values of < 0.05 were considered statistically significant.

4.1.6 Ethics

The study protocol was approved by the Ethical Committee of Tampere University Hospital and the National Authority for Medicolegal Affairs of Finland. Written informed consent was obtained from all study participants in the case-control study. For the cohort study, permission to identify women from the medical reimbursement register was given by the Finnish National Institute for Health and Welfare.
5. RESULTS

5.1 Expression of sex hormone receptors

A total of 510 specimens from 447 patients were included in the study. 81% of patients were women. 443 of specimens were from primary meningiomas. The average age of patients was 59.5±12.7 years. Of the primary meningiomas, 92% were classified as benign, 7% were atypical and 1% were malignant (Table 2). Histologically, the meningothelial subtype was the most common, representing 36% of meningiomas (Figure 1). The second most common subtype was transitional (32%). To evaluate association between age and hormone receptor expression, patients were divided into three age groups: 26-49, 50-59 and 60-84 years.

The majority (88%) of meningiomas were PR positive. There was no difference in PR immunostaining between men and women of different age groups. Women were also analyzed separately and no difference was found. Expression of PR was slightly higher in benign meningiomas but the difference was not significant when compared to atypical and malignant meningiomas (p= 0.097).

159 (40%) of the primary meningiomas had a positive staining reaction for ER. ER status did not vary by gender or age group. Benign meningiomas were more frequently ER positive than atypical and malignant meningiomas (41% vs. 27%, p= 0.10). There was no difference between primary and recurred meningiomas. In the subgroup of recurred meningiomas, half of the benign meningiomas were ER positive, compared to 19% of atypical and malignant meningiomas (p= 0.04).
Table 2. Characteristics of the tumor specimens of primary meningiomas

<table>
<thead>
<tr>
<th>Tumor grade</th>
<th>Benign</th>
<th>Atypical/Malignant</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Characteristics</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No of cases</td>
<td>407</td>
<td>36</td>
<td>443</td>
</tr>
<tr>
<td>Male/Female</td>
<td>86/357</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hormone receptor status</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PR+ (%)</td>
<td>340 (88.8)</td>
<td>26 (78.8)</td>
<td>366 (88.0)</td>
</tr>
<tr>
<td>ER+</td>
<td>150 (41.6)</td>
<td>9 (26.5)</td>
<td>159 (40.3)</td>
</tr>
<tr>
<td>AR+</td>
<td>140 (40.3)</td>
<td>7 (22.6)</td>
<td>147 (38.9)</td>
</tr>
<tr>
<td>MIB-1</td>
<td>3.1 ± 2.8</td>
<td>6.8 ± 7.5</td>
<td>3.4 ± 3.6</td>
</tr>
</tbody>
</table>

PR = progesterone, ER = estrogen, AR = androgen receptor, MIB-1 = proliferation index

Figure 1. Histological subtype of meningiomas
Of the primary tumors, 39% stained positive for AR. Staining in the recurrent meningiomas was slightly higher (48%, \(p=0.25\)) (Table 3). Men and women and different age groups did not show clear differences in AR expression. AR expression was slightly more frequent in benign meningiomas compared to atypical and malignant ones but significance was not reached (40% vs. 23%, \(p=0.06\)). In recurrent meningiomas, benign tumors expressed significantly more often AR than atypical and malignant ones (57% vs. 24%, \(p=0.02\)).

Almost 40% of tumor specimens stained positive for both PR and ER. On the other hand, negativity for both PR and ER was rare (9%). Only 1% of the cases were positive for ER but negative for PR.

PI differed significantly between benign and higher grade meningiomas (3.2±2.9 and 7.3±7.4, \(p<0.001\)). No difference in PI was detected between primary and recurrent tumors when benign meningiomas were analyzed separately. ER positive meningiomas had higher PI than ER negative ones (median 3.1% vs. 2.6%, \(p=0.038\)). No difference between PIs was found for PR or AR.

Table 3. Hormone receptor status of recurring tumors

<table>
<thead>
<tr>
<th>Grade</th>
<th>I</th>
<th>II-III</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>PR+</td>
<td>43 (95.6)</td>
<td>14 (87.5)</td>
<td>57 (93.4)</td>
</tr>
<tr>
<td>ER+</td>
<td>20 (50.0)</td>
<td>3 (18.8)*</td>
<td>23 (41.1)</td>
</tr>
<tr>
<td>AR+</td>
<td>24 (57.1)</td>
<td>4 (23.5)**</td>
<td>28 (47.5)</td>
</tr>
</tbody>
</table>

PR = progesterone, ER = estrogen, AR = androgen receptor, + = positive
* \(p=0.039\), ** \(p=0.024\)
5.2 Expression of carbonic anhydrases II and IX

5.2.1 CA II

Tumor samples were divided into categories according to staining reactivity. 85% of cases were negative (no or weak reaction) and the rest were CA positive (moderate or strong reaction). Only 4% of the fibrous histological subtype was CA-positive compared to 16% and 15% of meningothelial and transitional subtypes. CA II expression did not differ in tumor samples acquired from primary meningiomas, meningiomas with second operation or recurrent meningiomas. Only 13% of benign meningiomas were CA II-positive compared to atypical and malignant meningiomas of which 30% were positive (Table 4.). The difference was statistically significant (p=0.002). Also PI was higher in CA II-positive meningiomas compared to CA II-negative tumors (4.8±4.0 vs. 3.5±3.8, p= 0.002). Progesterone or estrogen receptor status did not have an effect on CA II expression but AR-negative meningiomas differed statistically from AR-positive meningiomas. Moderate or strong CA II immunostaining was found in 19% of AR-negative specimens and in 8% of AR-positive specimens (p=0.001).

5.2.2 CA IX

12% of meningiomas were CA IX-positive and 88% were negative. There was no difference in CA IX expression between different histological
subtypes. Primary operation, second operation or recurrent tumors did not differ in CA IX immunopositivity. In addition, no association between tumor grades, proliferation index or sex hormone receptor expression was found. There was a trend toward more frequent CA IX positivity with increasing age but significance was not reached. Endothelial CA II expression did not correlate with cytoplasmic CA IX expression in tumor cells.

Table 4. Association of CA II and IX expression with grade, proliferation and recurrence

<table>
<thead>
<tr>
<th></th>
<th>N (%)</th>
<th>Primary</th>
<th>Recurrent</th>
<th>MIB-1</th>
</tr>
</thead>
<tbody>
<tr>
<td>CA II positive</td>
<td>72 (14.8)</td>
<td>65 (15.3)</td>
<td>7 (11.5)</td>
<td>4.82 ± 3.96**</td>
</tr>
<tr>
<td>Grade I</td>
<td>57 (13.0)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Grade II-III</td>
<td>15 (30.6)*</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CA IX positive</td>
<td>55 (11.6)</td>
<td>48 (11.6)</td>
<td>7 (11.7)</td>
<td>3.87 ± 4.06</td>
</tr>
<tr>
<td>Grade I</td>
<td>47 (11.1)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Grade II-III</td>
<td>8 (16.0)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* p= 0.002, ** p= 0.002, compared to MIB-1 of CA II negative tumor cells

CA = carbonic anhydrase, MIB-1 = proliferation index

5.3 Case-control study

A total of 264 meningioma cases and 505 controls were included in the study (Table 5.). The mean ages for meningioma patients and for controls were 54 ± 10 (±SD) and 51 ± 12 years, respectively. Of the tumor specimens, 86% (170) were positive for progesterone receptor and 50% (98) for estrogen receptor.
The odds ratio of meningioma when for oral contraceptives had been used was 1.3 (95% confidence interval (CI): 0.9, 1.9). Other long-term hormonal contraception such as subdermal implants, injections and hormonal intrauterine devices carried a slightly increased risk of borderline significance for meningioma (OR 1.4, CI: 1.0, 2.1). The use of menopausal hormone therapy was not associated with an elevated risk of meningioma (OR 0.9, CI: 0.6, 1.3). Past use of fertility treatments did not carry an elevated risk of meningioma (OR 0.7, CI: 0.4, 1.4). Hormonal treatment for gynecological problems (e.g., menorrhagia or irregular menstruation) did not show an association with elevated meningioma risk (OR 1.1, CI: 0.8, 1.7). Age at menarche and postmenopausal status were not associated with the risk of meningioma. The number of children was positively, but non-significantly related to the risk of meningioma. Non-significant positive association was found between tumors expressing PR and use of oral contraceptives (Table 6). Some evidence of an increased risk of estrogen receptor-positive meningiomas in relation to hormonal treatment for infertility was also obtained. Expression of sex hormone receptors was not associated with post-menopausal status.

Table 5. Descriptive statistics of meningioma cases and controls

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Meningioma cases</th>
<th>Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total</td>
<td>264</td>
<td>505</td>
</tr>
<tr>
<td>Mean age (years)</td>
<td>54 ±10</td>
<td>51 ±12</td>
</tr>
<tr>
<td>Number of children</td>
<td>2.0 ± 1.3</td>
<td>1.8 ± 1.3</td>
</tr>
<tr>
<td>Mean age at menarche</td>
<td>13.2 ± 1.6</td>
<td>13.2 ± 1.5</td>
</tr>
<tr>
<td>Menopausal status</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Post-menopausal</td>
<td>179 (68 %)</td>
<td>285 (57 %)</td>
</tr>
</tbody>
</table>
Table 6. Comparison of risk of estrogen receptor (ER)- and progesterone receptor (PR)- positive meningiomas and exposure to exogenous and endogenous sex hormones

<table>
<thead>
<tr>
<th>Hormone use</th>
<th>ER+ Meningiomas</th>
<th>p value</th>
<th>PR+ Meningiomas</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>OR (95% CI)</td>
<td></td>
<td>OR (95% CI)</td>
<td></td>
</tr>
<tr>
<td>Oral contraceptives</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Never</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Ever</td>
<td>1.3 (0.8-2.2)</td>
<td>0.3</td>
<td>1.4 (0.9-2.1)</td>
<td>0.1</td>
</tr>
<tr>
<td>Menopausal hormone therapy</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Never</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Ever</td>
<td>0.8 (0.5–1.3)</td>
<td>0.8</td>
<td>1.0 (0.7–1.5)</td>
<td>0.9</td>
</tr>
<tr>
<td>Other hormonal contraception</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Yes</td>
<td>1.4 (0.8–2.4)</td>
<td>0.3</td>
<td>1.5 (1.0–2.4)</td>
<td>0.1</td>
</tr>
<tr>
<td>Hormonal treatment for infertility</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Yes</td>
<td>1.3 (0.6–3.0)</td>
<td>0.5</td>
<td>0.8 (0.4–1.7)</td>
<td>0.6</td>
</tr>
<tr>
<td>Hormonal treatment for gynecological problems</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Yes</td>
<td>1.1 (0.6–2.0)</td>
<td>0.7</td>
<td>0.8 (0.5–1.3)</td>
<td>0.4</td>
</tr>
</tbody>
</table>
5.4 Cohort study

A total of 131,480 women using post-menopausal estradiol-only therapy (ET) and 131,248 women using estradiol-progestin combination therapy (EPT) were followed during 1994-2009. A total of 289 estradiol users and 194 estradiol-progestin users were diagnosed with meningioma (Table 7).

Table 7. Number of women and number of person-years using estradiol-only or estradiol-progestin therapy at least for six months.

<table>
<thead>
<tr>
<th>Therapy</th>
<th>N</th>
<th>Woman-years</th>
<th>Meningioma cases</th>
</tr>
</thead>
<tbody>
<tr>
<td>Estradiol-only</td>
<td>131,480</td>
<td>1,189,236</td>
<td>289</td>
</tr>
<tr>
<td>Estradiol-progestin</td>
<td>131,248</td>
<td>1,020,110</td>
<td>194</td>
</tr>
<tr>
<td>Estradiol-MPA</td>
<td>35,859</td>
<td>318,537</td>
<td>68</td>
</tr>
<tr>
<td>Estradiol-NETA</td>
<td>62,632</td>
<td>483,108</td>
<td>86</td>
</tr>
<tr>
<td>Estradiol-dydrogesterone</td>
<td>17,876</td>
<td>105,382</td>
<td>16</td>
</tr>
</tbody>
</table>

Number of women at the beginning of the follow-up, woman-years by age at the follow-up, meningioma cases up to 31.12.2009.
MPA=medroxyprogesterone acetate, NETA= norethisterone acetate

The use of ET at least for six months was associated with a significantly increased meningioma risk with SIR 1.3, 95% CI 1.2-1.4, p< 0.001. SIR
increased with longer exposure time and after at least 36 months of use was 1.40 (CI 1.2-1.6, p< 0.001). Among EPT users the SIR after 6 months of use was 0.9 (CI 0.8-1.1) and after at least 5 years of use 1.1 (CI 0.9-1.2) (Table 8). In the subgroup of women using NETA, the SIR was 0.9 (CI 0.8-1.1) after 5 years of use. There was no difference in SIR whether NETA was used orally or transdermally. When comparison between oral and transdermal route was made with all EPT users, non-significantly increased risk estimates were obtained for long-term transdermal administration. There was no difference between continuous and sequential administration of EPT with short-term or long-term use.

Table 8. Meningioma SIR among women using estradiol-only or estradiol-progestin therapy in 1994-2009 in Finland.

<table>
<thead>
<tr>
<th>Hormone type</th>
<th>n</th>
<th>Obs</th>
<th>Exp</th>
<th>SIR</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>use at least 6 months</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ET</td>
<td>131,480</td>
<td>289</td>
<td>223</td>
<td>1.3</td>
<td>1.2-1.4*</td>
</tr>
<tr>
<td>EPT</td>
<td>131,248</td>
<td>194</td>
<td>210</td>
<td>0.9</td>
<td>0.8-1.1</td>
</tr>
<tr>
<td>use at least 36 months</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ET</td>
<td>53,552</td>
<td>139</td>
<td>99</td>
<td>1.4</td>
<td>1.2-1.6</td>
</tr>
<tr>
<td>EPT</td>
<td>74,280</td>
<td>92</td>
<td>103</td>
<td>0.9</td>
<td>0.7-1.1</td>
</tr>
<tr>
<td>use at least 60 months</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ET</td>
<td>45,015</td>
<td>131</td>
<td>98</td>
<td>1.3</td>
<td>1.1-1.6**</td>
</tr>
<tr>
<td>EPT</td>
<td>111,143</td>
<td>204</td>
<td>192</td>
<td>1.1</td>
<td>0.9-1.2</td>
</tr>
</tbody>
</table>

ET= estradiol-only therapy, EPT= Estradiol-progestin therapy, SIR = standardized incidence ratio, Obs= observed meningioma cases, Exp= expected meningioma cases, CI= confidence interval, *p<0.001, p<0.01
6. DISCUSSION

6.1 Molecular markers in meningiomas

6.1.1 Sex hormone receptors

Most meningiomas express PR and less than half express ER and AR. Age and sex have no effect on sex hormone receptor status. Expression of these hormone receptors seems to be higher in benign meningiomas compared to atypical and malignant tumors even though no significant difference was reached. In recurrent meningiomas, ER and AR expression was significantly higher in benign tumors.

PR expression in our study is consistent with other studies where expression level varies between 60-100%. The significance of PR expression in meningiomas is still unknown. Preponderance of female patients, acceleration of growth during pregnancy and association with breast cancer all suggest that sex hormones have an effect on meningioma growth. PRs are functional in meningiomas but their regulation remains unclear. Previous studies have shown low numbers of ER expression, which suggests that PRs in meningiomas seem to be estrogen independent, which in turn differs, for example, from breast cancer. According to this study, 40% of PR positive meningiomas are also ER positive. PR expression in women did not differ from men which is in agreement in other studies (Wolfsberger et al., 2004, Konstantinidou et al., 2003) However, recently, PR expression was shown to be significantly higher in women than in men.
PR positivity correlates inversely to tumor grade (Bouillot et al., 1994; Hsu et al., 1997; Nagashima et al., 1995). Also, PR negativity is a strong predictor of poor prognosis (Hsu et al., 1997). We could not confirm this finding with significance, which probably is caused by a low number of atypical and malignant cases. If meningiomas lose their PR expression during transformation to higher grades, this makes it more difficult to use antiprogesterones as treatment for atypical and malignant meningiomas.

Promising results have been detected by antiprogesterones in meningioma cell cultures (Matsuda et al., 1994). Unfortunately, the results of the clinical studies have been somewhat disappointing. In a series of 28 patients treated with Mifepristone, a minor response was noted in eight (Grunberg et al., 2006). Overall, antiprogestosterone treatment is well tolerated.

Estrogen and androgen receptors are less investigated in meningiomas than progesterone receptors. The significance of ER expression in meningiomas is still under debate. Some investigators believe that meningiomas lack ERs and others report expression levels varying from 10% to 68% (Takei et al., 2008; Guevara et al., 2010; Bouillot et al., 1994). Normal meningeal tissue does not express ER. Opposite to PRs, the presence of ERs in meningiomas correlates with increasing potential of aggressive behavior. Also, ER expression correlates with karyotype abnormalities (Pravdenkova et al., 2006). These findings are opposite to our results where ER expression was lower in atypical and malignant cases. Yet, ER expression was higher in recurrent meningiomas and, again, a low number of atypical and malignant cases may have contributed to these results. Results of in vitro studies have been controversial. Tamoxifen, an antiestrogen, can cause stimulation, inhibition or show no result (Olson et al., 1986).

Expression of AR is covered in only a few studies. Reported expression
rates are 30-67% and a significant gender difference has also been reported women expressing ARs twice as often as men. As AR expression may equal PR expression, it has been suggested that ARs can modulate PR activity (Konstantinidou et al., 2003; Carroll et al., 1995; Maxwell et al., 1993). Immunohistochemical examination has revealed that AR immunostaining is mostly located in the nucleus. In one study, AR expression was significantly higher in malignant meningiomas compared to benign and atypical tumors (Chen and Chen 2001). Our AR expression numbers are in agreement with previous studies but no gender difference was found. Instead, in our material, atypical and malignant meningiomas seemed to lose their AR expression even though significance was quite not reached. Antiandrogens have antiproliferative effects on meningioma cells in culture but there have not been any clinical studies yet.

As ERs, ARs are also missing in normal arachnoid tissue. Aromatase is an enzyme that converts androgens to estrogens. This enzyme is common in several hormone-dependent cancers as ovarian, breast and endometrial cancer. Normal arachnoid tissue expresses aromatase but interestingly it is not found in meningioma cells. In the study of Leães et al. (2010), aromatase expression was constant in normal meninges (all positive) and in meningiomas (all negative). They hypothesized that progesterone may inhibit aromatase activity in meningiomas. A similar phenomenon is also seen in breast cancer (Hardy et al., 2008).

6.1.2 Carbonic anhydrases II and IX

According to our results, expression of carbonic anhydrases is uncommon in benign meningiomas. One third of atypical and malignant meningiomas
express CA II but tumor grade has no effect on CA IX expression.

It has been shown earlier that expression of CA IX is associated with poor prognosis in several human cancers. Hypoxia is one of the main features of malignant tumors. It is probably caused by inadequate blood supply to highly cellular tumor tissue by poorly organized neovessels. CA IX is suggested to be a hypoxia biomarker. In the study of Yoo et al. (2007), almost half of the meningioma cases were CAIX-positive. Of the grade III meningiomas, 85% stained positive. The low rate of CA IX expression (11.6%) in our material may be due to small number of atypical and malignant cases compared to theirs (41 atypical or malignant cases out of 510 meningiomas in our material vs. 37/62 in their study). Recently, expression of CA IX was evaluated in clear cell meningiomas of which 39% were CA IX positive. In most cases staining was local, involving less than 5% of neoplastic cells (Prayson et al., 2010).

Studies on CA II expression in meningiomas are rare. Parkkila et al. (1995) tested several brain tumors for expression of CA II but few meningiomas tested remained negative. Subsequently, it was confirmed that CA II is commonly found in malignant brain tumors (Haapasalo et al., 2007). We showed that CA II expression is significantly higher in atypical/malignant meningiomas compared to benign tumors. CA II expression also correlates with cell proliferation. As in other higher-grade brain tumors, CA II staining in malignant meningiomas is located in tumor vessel endothelia which suggests that CA II may have an important role in tumor cell metabolism.

Progesterone and estrogen receptor expression had no association with CA II staining but CA II expression was more abundant in AR-negative tumor samples. Regulation of CA II expression by androgens has been studied in rat epididymis where acidification of epididymal fluid by CA II and CA IV is under control of androgen effects (Kaunisto et al., 1999). However, response of CA II to androgen varies. In the lateral prostate
decrease of testosterone level decreases CA II expression but opposite happens in the dorsal prostate (Härkönen et al., 1991). Also, there is some evidence that androgens have many beneficial effects in the central nervous system and they regulate neurone survival by actions that are not yet fully understood. Recently, androgen protection was suggested to be specific to cell death that involves apoptosis (Nguyen et al., 2010).

The vasculature in malignant solid tumors is often poor and cannot provide enough oxygen for rapidly growing tumor cells. This leads to hypoxia, which in turn induces activation of the hypoxia-inducible transcription factor (HIF) (Brahimi-Horn and Pouyssegur, 2009). One of the genes HIF induces, is CA IX. To maintain physiological intracellular pH, tumor cells require a high bicarbonate flux. Carbonic anhydrase IX promotes this flux and hence its inhibition should result in the growth inhibition of tumor cells. In a transfected colorectal cancer xenograft, silencing of CA IX gene resulted in 40% tumor volume reduction. There are numerous CA IX inhibitors that are evaluated and showed efficacy in vitro. Still, many more studies are needed in order to understand the behavior of these compounds in vivo. CA II inhibition also leads to inhibition of tumor cell growth but, in general, as CA II expression is ubiquitous, its inhibition may be detrimental.

6.2 Hormonal exposure and meningioma risk

6.2.1 Menopausal hormone therapy

Mixed results have been obtained from previous studies concerning MHT and meningioma risk. Conclusion in several case-control studies was that
MHT and meningioma risk have no association. Earlier studies have often been too small to be able to indicate correlation between rare CNS tumors and MHT. Recently, two large cohort studies have found a significant association between MHT and meningioma. In the Million Women study (Benson et al., 2010) ever use of MHT carried a small but significant risk for meningioma (RR 1.32, CI 95% 1.05-1.66, p= 0.02). Current users of MHT showed a similar risk. When current users were divided into two groups according to type of MHT, estrogen-only therapy was associated with higher risk than estrogen-progestogen use. This is in agreement with our results. Benson et al. (2010) also evaluated meningioma risk in relation to route and mode of MHT administration. There was no difference between continuous and sequential administration. Oral estrogen was associated with higher risk than transdermal estrogen (RR 2.42, CI 1.30-4.50 vs. 1.59 CI 0.98-2.58) but the number of meningioma cases was small in both groups (11 and 19). In this study, long-term use of continuous EPT was associated with similar risk estimates than sequential EPT. These findings differ from those concerning breast cancer, which is regarded as a strongly hormone-dependent cancer. With breast cancer, a higher risk is carried with EPT than ET use and continuous EPT is associated with a higher cancer risk than sequential therapy (Bakken et al., 2011).

Concomitantly with the Million Women Study, results of the EPIC study were published (Michaud et al., 2010). A total of 194 meningioma cases occurred among 276,451 women. Current MHT use carried an increased meningioma risk with RR 1.79, 95% CI 1.19-2.71. They also reported an increased meningioma risk associated with current OC use. Former OC use did not increase the risk. Also, a dose-response among premenopausal women was found. Higher risk among current users suggests that meningioma growth rate is accelerated during a hormonal exposure period, and when the exposure stops, the risk diminishes with time. In the case-control study analysis by hormone receptor status, there was some
indication of an increased risk of progesterone receptor-positive meningiomas associated with oral contraceptive use (OR 1.39, 95% CI 0.92-2.10) and other hormonal contraception (OR 1.50, 95% CI 0.95-2.36). There is one earlier study evaluating meningioma risk and exogenous sex hormone exposure by sex hormone receptor status (Custer et al., 2006). According to their results, only 2% of cases expressed ER which differed substantially from ours. PR expression was similar to ours with 68% of tumors having >25% of cells expressing PR. They found a strong association between OC use and risk of a tumor with low PR expression (< 25%). In our data, the cutoff value for PR positivity was >10% cells expressing PR, so these results cannot be directly compared.

Similarly, meningioma risk and use of other than oral contraception (hormonal intrauterine devices, subdermal implants and injections) has been evaluated earlier in only one study. RR of meningioma was estimated to be 1.5 (CI 0.9-2.6) (Wigertz et al., 2006). As in our material, the number of patients using these products was small. Likewise, the number of patients using hormonal treatments for gynecological problems was too low in their and our material to draw conclusions.

In the population-based case-control study, the subjects were asked to report the exact name of the compound they used for hormonal treatment. Less than half of the cases and controls could remember the brand names of menopausal hormonal treatments or oral contraceptives and analyses by type of hormones could not be made. Further, we did not have information on time period since hormone use and were therefore unable to distinguish between current and past users. The participation proportion of cases was high, but only moderate among controls (mainly due to failure to contact the controls), so selection bias could not be ruled out. No information on, for example, the socioeconomic factors among nonparticipants was able to be obtained, but an earlier report suggests a higher level of education among participating than non-participating cases and controls (Lahkola et al.,
2005). Also, when self-reported data is used, misclassification can occur. The validity of age at menarche reported in middle age compared with that recorded in adolescence is only moderate (Cooper et al., 2006).

In the cohort study, a large, nationwide study material was identified. Use of MHT was traced from the medical reimbursement register which minimizes misclassification of MHT exposure. Meningioma cases were identified from the Finnish Cancer Registry. This registry receives notifications on menigioma with, for example, histologic type and grade information from all hospitals and pathologic laboratories in Finland, and its coverage is about two thirds of all meningioma cases (Larjavaara et al., 2008). The limitations of the cohort study are that no information on socioeconomic status, pregnancies, smoking or age at menarche was available. In addition, body mass index also has an effect on meningioma risk. A significantly increased meningioma risk is associated with an increased weight. Hazard ratio for BMI >30 was 1.48, 95% CI 0.98-2.23 compared to meningioma patients with BMI of 20-24.9 (Michaud et al., 2011).

### 6.2.2 Reproductive factors

There is little evidence regarding the effect of reproductive factors on meningioma risk. In a large population-based study, < 50 years old women had an increased risk of meningioma associated with an increasing number of pregnancies that led to live births (Wigertz et al., 2008). This finding suggests that hormones affect the growth of the tumor rather than its initiation. Earlier studies could not show any association between number of pregnancies and meningioma risk (Hatch et al., 2005; Custer et al., 2006; Lambe et al., 1997) except for Jhawar et al. (2003), who reported increased meningioma risk among parous women compared to nulliparous women.
An opposite result was reported by Lee et al. (2006), who found that there was a protective effect of pregnancy. For three or more pregnancies compared to no pregnancy the RR was 0.3 (CI 0.2-0.6). We found a trend of an increasing risk of meningioma being related to increasing number of children, but this finding did not reach statistical significance.

In the Nurses’ Health Study, late menarche was associated with an increased meningioma risk. Other studies are in agreement with our results, which show no effect of age at menarche on meningioma risk. Results concerning menopausal status and meningioma risk are equivocal. Both decreased (Schlehofer et al., 1992) and increased (Hatch et al., 2005; Custer et al., 2006) meningioma risk has been reported. We did not find evidence for an association between menopausal status and meningioma risk.

6.3 Methodological considerations and future prospects

6.3.1 Tumor samples

Of the tumor samples, only 8% were atypical or malignant. This makes it more difficult to show a difference between benign and higher-grade tumors. It has been shown earlier that higher-grade meningiomas lose their PR-positivity (Bouillot et al., 1994). In our material, PR-, ER- and AR-positivity were more common in benign than grade II and III meningiomas but did not reach statistical significance probably due to the small number of malignant cases. Similarly, low rate of CA IX expression may be explained with the small number of grade II and III meningiomas.

Progesterone positive and progesterone negative meningiomas differ in
their gene expressing profiles but ER+ and ER- meningiomas do not (Claus et al., 2008). In the future, microarray tests may be used for increased diagnostic accuracy and perhaps more individualized treatments can be offered. Suppression of angiogenesis has shown promising results inhibiting malignant glioma cell growth in in vitro and in vivo rodent models (Im et al., 1999). In addition to gene therapy, CA inhibition can be used to prevent angiogenesis in subsets of meningiomas expressing CA II. There are no clinical trials reported on meningioma and gene therapy or CA II inhibition.

The wide expression of PRs in meningiomas makes it an attractive candidate for antitumoral treatment but the role of endogenous sex hormone inhibition is still limited in meningiomas. As long as the pathways of sex hormone function on meningioma’s growth are unclear, it is unlikely that progesterone antagonists can be effectively used in antitumoral therapy. Clinical hormonotherapy trials performed on well-defined subgroups of meningiomas with different sex hormone receptor status could be beneficial.

6.3.2 Cohort and case-control study

In the case-control study of women aged 20-69 years old, 320 meningiomas were identified from 5 university hospitals during November 2000 and October 2002. We were not able to identify all meningioma cases, as in 2001 the Finnish Cancer Register received notifications for 230 meningioma cases and, in 2002, for 181 meningioma cases among women in this age group. Also, the coverage of the Finnish Cancer register is not complete for benign tumors, as half of the meningiomas are treated without surgery and remain without histological confirmation. We have probably missed meningioma cases both in the case-control and cohort studies but it is not likely that gaining these cases would weaken our finding on the positive
association between MHT and meningioma risk.

We did not investigate possible confounding from socioeconomic factors or body mass index. In the cohort study, we did not have information on previous irradiation therapy and there can also be confounding from factors we don’t know, e.g. genetic factors. It has been recently shown that meningioma risk is significantly higher if BMI is over 30 (Michaud et al., 2011). Non-participation associated with socioeconomic factors may cause bias and they are assumed to influence more controls than cases. This is not a problem if participation is similar among cases and controls but in our study 60% of the controls refused to participate compared to 9% of the cases. Results of studies published on socioeconomic factors and meningioma risk are conflicting. Positive association has been found for income level and meningioma (Inskip et al., 2003). Instead, in a Swedish register study, socioeconomic factors were not associated with meningioma risk (Wigertz et al., 2010).

Misclassification of exposure history may occur with self-reported information. According to Klungel et al. (2000), only 70% of drugs currently in use could be recalled through self-administered questionnaires. In the study of accuracy of self-reported use of noncontraceptive estrogens dispensed during the previous 12 years, 78% recalled the name and 26% recalled the name and dose (West et al., 1995). These studies show that underascertainment of self-reported prescription drug exposure is possible but it is not likely that exposures are overreported. In addition, cases may recall exposure differently than controls as a result of them having a tumor and thereby causing a biased result. The strength of the cohort study compared to the case-control study is that exposure data is based on a register. It diminishes the misclassification of exposure history but still does not tell us anything about drug compliance.
The slow growth rate of meningioma is a challenge for epidemiological studies. In the cohort study, we had an average follow-up time of nine years. With an even longer follow-up time, we might have found a stronger association between estrogen use and meningioma risk. Addition of sex hormone receptor status and histological grade to a large population-based register study would give us valuable information of the risks of different meningioma subtypes and MHT.
7. CONCLUSIONS

The present study demonstrates:

Expression of sex hormone receptors is common in meningiomas. Presence of ER is associated with a higher proliferation index compared to ER negative meningiomas. ER may have more significance in pathogenesis of menigiomas than it is earlier thought.

Minority of meningiomas express CA II and CA IX. CA II is located in the tumor vessel endothelia of meningiomas. Expression of CA II is more common in atypical and malignant meningiomas than benign tumors. CA II may be a target molecule for antitumor therapy.

Endogenous sex hormone exposure seems not to be a risk factor for meningiomas. Non-significant positive association is found between tumors expressing PR and use of oral contraceptives and needs to be confirmed in future studies.

Estradiol-only post-menopausal therapy slightly increases meningioma risk. Continuous and sequential estradiol-progestin therapy have comparable effects. The risk for spinal or intracranial meningioma does not differ among estradiol-only or EPT users. The effect of long-term transdermal administration does not materially differ from oral administration.

Overall, hormonal and reproductive factors have some involvement in etiology of meningioma, but their effect is of modest size and unlikely to explain the differences between men and women in menigioma incidence.
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Sex hormone receptors in meningiomas

Female predominance in meningiomas can not be explained by differences in progesterone, estrogen, or androgen receptor expression

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Summary

The female predominance in meningioma incidence and association between meningioma and breast cancer suggest that growth of meningiomas is hormone-dependent. There are several discrepancies in literature about the proliferative effect of sex hormones on meningiomas. This study aims to evaluate the hormone receptor status of meningiomas and assess its relation to age, sex, histological grade, recurrence, and proliferation activity. The material was based on consecutive patients operated for meningioma at Tampere University Hospital in 1989-1999. The occurrence of progesterone, estrogen and androgen receptor in patients with primary and recurrent meningiomas was studied immunohistochemically by using specific monoclonal antibodies. Hormonal status was determined in 510 tumor samples. 443 samples were from primary meningiomas and 67 from recurrent tumors. Of the samples, 455 were benign (WHO grade I), 49 atypical (grade II), and 6 malignant (grade III). Of the primary tumor samples, 88% were progesterone receptor positive, 40% were positive for estrogen and 39% for androgen receptors. Grade I meningiomas had significantly higher incidence for estrogen and androgen receptors than higher grade meningiomas. Estrogen positive tumor samples had significantly higher proliferation index than estrogen negative samples. No difference in expression of sex hormone receptors was observed by sexes or age group. Estrogen and androgen receptors may have more influence on the pathogenesis of meningiomas than earlier thought. The higher incidence of meningiomas in women can not be explained by differences of sex hormone receptor expression.

Key words: androgen receptor, estrogen receptor, meningioma, MIB-1, progesterone receptor
Introduction

Meningiomas are common tumors and account for up to 20% of all intracranial neoplasms. Most of them are benign and their incidence increases with age. The incidence in women is more than twice as high as in men [16, 40]. The sex hormone dependency of meningiomas has been under investigation for more than two decades. The higher incidence in women, as well as increased growth rate during pregnancy and luteal phase of menstrual circle [4, 46] suggest that sex hormones may have a role in growth of meningiomas. Several authors have reported an association between meningioma and breast cancer [17, 37, 50].

Previous studies have shown the presence of progesterone (PR), estrogen (ER), and androgen receptors (AR) in meningiomas [6,11,12,27,36,48,51]. Most studies have focused on progesterone receptors as most of meningiomas contain PRs. PRs are located in the nucleus and have been shown to be functional [12]. The finding that growth of meningioma can be manipulated in vitro by progesterone and antiprogesterone [42] and the higher concentration of PRs in recurrent meningiomas than in primary tumors [48] indicate a role in the development of meningiomas. In vitro studies led to attempts to treat patients with meningiomas with antiprogesterones [20,31]. Modest results achieved in these studies suggest more complicated relation between sex hormones and meningioma growth.

ARs have not been investigated as extensively as ER and PR and their role remains unclear. Androgen antagonists have been shown to inhibit meningioma cell growth in vitro [2] As in case of PRs, the expression of ARs is low in normal meninges compared to meningiomas [36]. Several studies suggest that ER has no biological relevance in the development and growth of meningiomas. Majority of meningiomas express ERs only weakly or lack them completely [5,8,9,22,27,36]. In
every respect, the role of sex hormones in the development and growth of meningioma is still controversial.

The aim of this study was to determine the progesterone, estrogen and androgen receptor status in primary and recurrent meningiomas by using specific monoclonal antibodies and to evaluate the effect of gender, age and tumor grade on hormone receptor status. We also used MIB-I (Ki-67), the proliferation marker, to determine the association between proliferation activity and the hormone receptor status of meningiomas.

**Material and Methods**

*Tumor specimens*

Material consists of patients who underwent surgery for intracranial meningioma at the Tampere University Hospital during 1989-1999. Two patients were excluded because of their young age (4 and 15 years). A total of 443 primary and 67 recurrent tumor specimen were chosen for the study. The study protocol was approved by the Ethical Committee of Tampere University Hospital.

Tumors were classified and graded using the World Health Organization scheme (grades I – III) [28]. Atypical meningiomas (WHO II) were defined by high mitotic index (>4/10 HPF or 2.5/mm²), brain invasion, or the presence of at least three of following variables: sheeting architecture, macronucleoli, hypercellularity or small cells. Anaplastic meningiomas (WHO III) were characterized by excessive proliferation ( >20 mitoses/10 HPF or 12.5 mitoses/mm²) or loss of meningothelial differentiation.

*Immunohistochemistry*
In immunohistochemical studies, tissue microarray (TMA) technique was used [29]. The tumor samples were fixed in 4% phosphate buffered formaldehyde and processed into paraffin blocks with standard methods. Histologically representative tumor regions of hematoxylin-eosin-stained slides were selected by a neuropathologist (HH) and corresponding areas were sampled in tissue microarray blocks using a custom-built instrument (Beecher Instruments, Silver Spring, MD). The sample diameter of the tissue core in the microarray block was 600 μm.

For proliferation analysis sections cut from TMA block were immunostained with MIB-1 (Ki-67) antibody (DakoCytomation, Glostrup, Denmark). Heat-induced epitope retrieval (Tris-EDTA buffer (pH 9.0, 2x7 min in an microwave oven) and an automated immunostaining protocol (TechMate immunostainer) were used. The tissue sections were counterstained with methyl green. The proliferation was reported as the percentage of immunopositive nuclei and evaluated by analysing all the tumor cells in the core tissue with an image analysis system (CAS-200 Software, Becton Dickinson & Co., USA) as described previously [49].

For immunohistochemistry of hormone receptors, monoclonal antibodies 6F11, PGR312, and 2F12 were used for PR, ER, and AR, respectively (Novocastra Laboratories, Newcastle, UK). Antigen retrieval was carried out described above. All antibodies were diluted at 1 μg/ml, and detected with a peroxidase-polymer based detection kit (PowerVision+™, Immunovision Technologies, Daly City, CA) according to manufacturer’s instructions.

In 29 cases immunostaining for progesterone failed or samples were lost during the section preparation and immunostaining steps. The numbers for estrogen and androgen were 55 and 72, respectively. Specimens with failed immunostaining were excluded from the analysis.
Statistical methods

Associations between hormone receptor expression and grade, gender, or age group were evaluated based on the chi-square test. Relationship between receptor status and cell proliferation by MIB-1 was tested with Mann-Whitney test. All the reported p-values are 2-sided and a value of p<0.05 was considered statistically significant.

Results

A total of 510 biopsy specimens from 447 patients were included in the study. Of the patients operated for primary meningioma 357 (80.6%) were women and 86 (19.4%) were men. The average age of patients was 59.5 (SD 12.7) years (for women 59.6±12.4 and for men 59.0±13.7). Of the 443 primary meningiomas, 407 (92%) cases were classified as grade I, 33 (7%) grade II and 3 (1%) grade III. Sixty-seven tumor samples were obtained at re-operation for recurrency (Table 1). Three initially benign meningiomas progressed into atypical or malignant. One meningioma changed from grade II to grade I meningioma.

Receptor immunoreactivity in meningiomas

Progesterone receptor (PR) status

Of the primary tumour specimens, 366 (88%) had positive reaction for PR (Table 2). The PR positivity was equally common among men and women (91.5% and 87.1%). No differences in proportion of PR positive tumor specimens were observed between three age groups: 87.3% in age
group 26-49 years, 90.7% in patients aged 50-59 years, and 86.9% in ages 60-84 years. No difference was found between age groups when women were evaluated separately.

PRs were detected slightly more frequently in benign than grade II-III meningiomas (88.8% versus 78.8%, p=0.097). PR-positive staining was more common in recurrent than primary tumors (93.4% vs. 88.0%, respectively) but the difference did not reach statistical significance (p=0.28).

**Estrogen receptor (ER) status**

Overall, 159 (40.3%) of the primary meningiomas were positive for ER (Table 2). No substantial difference was seen between men and women (32.9% vs. 42.1%, p=0.16). ER status did not vary by age. Yet, grade I tumors were more frequently ER positive than grade II-III meningiomas (41.3% vs. 26.5%, p=0.10). Of the recurrences 41.1% stained positive for ER. In the recurred tumors the difference between benign and of grade II-III tumors was statistically significant (50% vs. 18.8% ER positive, p=0.04).

**Androgen receptor (AR) status**

Recurrent tumors were AR positive slightly more commonly than primary tumors (47.5 vs. 38.9%, respectively, p=0.25) (Table 2). There were no clear differences between men and women (43.4% and 37.7%) or between age groups (44.2% in ages 26-49 years, 38.7% in 50-59 years, and 36.7% in 50-84 years). Benign tumors expressed AR more frequently than grade II-III meningiomas (40.3% vs. 22.6%) the difference being of borderline significance (p=0.06). The difference was statistically significant in the recurrences (57.1% vs. 23.5%, p=0.02).

**Progesterone, estrogen and androgen hormone receptor combinations**

Of the tissue specimens, 173 (39.7%) stained positive for both PR and ER. Only 41 (9.4%) were negative for both PR and ER (Table 3).
**MIB-I and hormonal status**

There was a significant difference in MIB-1 proliferation index (PI) between benign and grade II and III meningiomas. PI of benign tumor samples was 3.2 ± 2.9 and 7.3±7.4 in more aggressive meningiomas (p<0.01). When benign meningiomas were considered separately, there was no significant difference between primary and recurrent tumors (PI=3.1±2.8 vs. 3.9±3.4, p=0.195). Men and women did not differ in PIs (p=0.33). ER positive meningiomas had a significantly higher MIB-1 PI than ER negative tumors (median 3.12 vs. 2.62 %, p=0.038, Mann-Whitney test) but no such association was found for PR (median 2.80 vs. 2.38, p = 0.43) or AR (median 2.82 vs 2.91, p = 0.24).

**Discussion**

Several studies confirm the female predilection in occurrence of meningiomas, with incidence rates 1.5 to 2-fold compared to men [16, 40]. The contribution of sex hormones to the development of meningiomas has been suggested but the hypothesis still remains to be proven. The results concerning the frequency of these hormone receptors, especially of estrogen receptors, are also controversial. We were able to use a large, representative meningioma material collected from a university hospital providing neurosurgery service for a catchment population of approximately one million. Due to the comprehensive public health care system in Finland, the population-based material provides a representative case mix, unselected by disease or patient characteristics, which is not achievable in other settings. Our material was also larger than in previous studies of sex hormone receptors, providing better precision and allowing more detailed analyses.
Almost all investigators have found abundant progesterone receptors in most meningiomas [7, 8, 10, 30, 36, 48]. Our work confirms the high incidence of PRs (Figure 1). Similar numbers has been reported by other investigators [22, 30, 48]. On the other hand we did not observe any difference in incidence of PRs between male and female patients as reported in some earlier studies [5, 10, 22]. Even though the prevalence of meningioma increases with age [40], patients’ age did not seem to have an effect on the PR status.

Of primary meningiomas, 40% expressed ER in our study (Figure 1). Using immunohistochemistry, some groups have failed to detect ER in meningiomas [7, 8, 9, 27,36, 44, 51] and others have reported a presence of ERs in a small fraction of tumors. In the study of Hsu et al. [22] less than 10% of meningiomas had positive nuclear staining for ER. In a recent work of Konstantinidou et al 35.4% stained positive [30], while Carroll et al [13] demonstrated the presence of mRNA for ER-alpha3 and ER-beta in 68 and 44 % of meningiomas, which is in concordance with our immunohistochemical findings. The variability in results received by immunohistochemistry is probably due to varying sensitivity of different methods to detect hormone receptors.

Few studies have focused on the role of AR in the pathogenesis of meningiomas. The AR binding sites have been found in approximately 2/3 of meningiomas. Carrol et al [11] reported mRNA for androgen receptor in 67% of meningiomas, and Maxwell et al in 66% [36]. More abundant androgen receptors have been reported in women than in men [5,11]. In our study 39 % of meningiomas expressed AR (Figure 1) and there was no difference in AR or ER expression between the sexes or age groups.

Histology, proliferation and hormone receptors
Even though meningiomas are most often benign, slowly growing tumors they have a high tendency to recur [25, 32]. The recurrence rate of meningiomas increases with histological grade. According to Jääskeläinen et al. [25] after complete operation the recurrence rate at five years is 3% for benign (grade I) meningiomas but 38% and 78% for atypical and anaplastic meningiomas. Meningiomas of young patients may also have a higher growth potential than of older patients [39]. Other factors to affect recurrence are patient’s sex, tumor location and extent of surgical removal.

The potential of sex hormones in the development, growth, and recurrence of meningiomas has been investigated in several studies. The PR status has been inversely related to tumor grade: PRs are more frequent in grade I than in atypical/malignant meningiomas [9, 22, 47]. Although PR positivity was very common in both benign and grade II-III meningiomas in our study PR expression slightly lower in higher grade tumors. Similar non-significant decrease was found with ER and AR expression. The rarity of grade II and III tumors (<10%) in the group of primary meningiomas limits the power to detect differences. Rubinstein et al. [48] found a significantly higher concentration of PRs in recurrent meningiomas compared to primary tumors while Fewings et al. [18] found that PR positive meningiomas are less likely to recur than PR negative. In the present study we found no difference between tumor recurrences by PR status. However, expression of estrogen and androgen receptors was significantly less frequent in grade II and III tumors than in benign grade I meningiomas. Similar finding with ER was made by Konstantinidou et al. [30] who found diminished ER levels in atypical meningiomas.

MIB-1 is a monoclonal Ki-67 antibody used commonly to evaluate cell proliferation in neoplasms. Several studies show that high MIB-1 labelling index is associated with tumor aggressiveness and recurrence in meningiomas [23,35]. Our results are in accordance with previous studies showing that proliferation activity increases with histological grade [1,3,26,41]. High MIB-1 PIs
have been found in young male patients suggesting they have high risk for recurrence [35]. We could not confirm the difference of PIs between sexes or different age categories in our material.

Hsu and colleagues [22] showed that mitotic index inversely correlates with PR status and patients with any PR positive tumor cells have significantly better progression-free survival rates. Similar results showing lower MIB-1 indices in PR positive meningiomas have been published [38]. In our study, AR positive and negative tumors did not differ significantly in proliferation by MIB-1. However, ER-positive meningiomas had significantly increased proliferation compared with ER-negative tumors. To our knowledge this is a novel finding and could implicate that ERs have a role in the pathogenesis of meningiomas.

Clinicopathological implications

Antiprogesterones seem to have some antitumor effect in meningiomas but this occurs independently of tumor hormonal status [34]. In their study of meningioma spheroid cultures Tonn et al. [52] found an inverse correlation between proliferation index and PR expression suggesting that PRs are down-regulated in proliferating tumor cells. This could explain why growth of meningioma can not be influenced by progesterone or antiprogestosterone treatment. On the other hand, Jhawar et al. [24] found an increased risk for meningiomas among women currently exposed to endogenous or exogenous sex hormones. Sex hormones may influence growth of meningioma by different pathways than hormone receptors. It has been suggested that steroid hormones regulate target tissues via locally produced growth factors in an autocrine/paracrine fashion [45]. Epidermal growth factor, insulin like growth factor-1, and TGF-alpha have all been considered as possible mediators [21, 36]. Also an association between PR and two steroid receptor cofactors SRC-1 and
TIF2 has been found [14]. The differential expression of these steroid receptor coactivators may explain the differential response of meningiomas to hormonal therapy.

Few studies have been reported using antiestrogens, mainly tamoxifen, in the treatment of patients with meningiomas [19,33]. Although some studies showed no significant therapeutic efficacy, regression of tumors e.g. with antiestrogen mepitiostane has been reported [43]. Our results indicate that with a sensitive immunohistochemical method, ERs and ARs occur in a quarter of atypical and malignant cases. In addition, the present study shows that ER positive meningiomas have higher proliferation indices than ER-negative meningiomas. Thus, there could be a subset of high-grade ER+ meningiomas, that may benefit from antiestrogen therapy

Conclusions

Using sensitive immunohistochemical methods and a large, representative material, we found ERs in 40 % of all meningiomas. The frequency of ARs is comparable with ERs. Nine out of ten meningiomas express PR. No difference in sex hormone receptor expression between sex and age categories was found indicating that, the higher incidence of meningiomas in women can not be explained by differences of sex hormone receptors. Atypical and malignant meningiomas seem to have a lower frequency of ERs and ARs. ER-positive meningiomas proliferated significantly more rapidly than ER-negative meningiomas. Further studies are needed to investigate the possibility to use antiestrogen treatment for the small subgroup of ER positive patients with unfavourable prognostic factors.

Acknowledgements
We thank Sari Toivola, Salla Kolmihaara and Anssi Lagerstedt for their excellent technical assistance.

References


Figure 1. Immunohistochemical staining of sex hormone receptors. From top to bottom: Progesterone receptor staining (x400); nearly all tumour cell nuclei are positive. Estrogen receptor staining (x400); nearly half of the nuclei are positive (arrows). Androgen receptor staining (x600); some of the nuclei are positive (arrows).
Table 1. Histological subtypes of meningiomas and the rate of recurrences

<table>
<thead>
<tr>
<th>Histological type of meningiomas</th>
<th>Primary</th>
<th>1st Recurrence</th>
<th>Subsequent recurrences</th>
</tr>
</thead>
<tbody>
<tr>
<td>Benign, WHO I (%)</td>
<td>407 (91.9)</td>
<td>37 (82.2)</td>
<td>11 (50.50)</td>
</tr>
<tr>
<td>fibroblastic</td>
<td>93 (22.9)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>meningothelial</td>
<td>145 (35.6)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>transitional</td>
<td>129 (31.7)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>other</td>
<td>40 (9.8)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Atypical, WHO II</td>
<td>33 (7.4)</td>
<td>6 (13.3)</td>
<td>10 (45.5)</td>
</tr>
<tr>
<td>Malignant, WHO III</td>
<td>3 (0.7)</td>
<td>2 (4.4)</td>
<td>1 (4.5)</td>
</tr>
<tr>
<td>Total</td>
<td>443</td>
<td>45</td>
<td>22</td>
</tr>
</tbody>
</table>
TABLE 2
Characteristics of tumor specimens

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Benign</th>
<th>Atypical/Malignant</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>No of cases</td>
<td>407</td>
<td>36</td>
<td>443</td>
</tr>
<tr>
<td>Male/female ratio</td>
<td>1:4.4</td>
<td>1:2.6</td>
<td>1:4.2</td>
</tr>
<tr>
<td>Age (yrs)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>mean+SD</td>
<td>59.4+12.6</td>
<td>60.3+13.9</td>
<td>59.5+12.7</td>
</tr>
<tr>
<td>range</td>
<td>27-84</td>
<td>26-84</td>
<td>26-84</td>
</tr>
<tr>
<td>Recurrence(^1)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No of cases (%)</td>
<td>37 (9.1)</td>
<td>8 (22.2)</td>
<td>45 (10.2)</td>
</tr>
<tr>
<td>Hormone receptor status of primary tumors</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PR+ (%)</td>
<td>340 (88.8)</td>
<td>26 (78.8)</td>
<td>366 (88.0)</td>
</tr>
<tr>
<td>ER+</td>
<td>150 (41.6)</td>
<td>9 (26.5)</td>
<td>159 (40.3)</td>
</tr>
<tr>
<td>AR+</td>
<td>140 (40.3)</td>
<td>7 (22.6)</td>
<td>147 (38.9)</td>
</tr>
<tr>
<td>MIB-1(^3) (mean+SD)</td>
<td>3.1+2.8</td>
<td>6.8+7.5(^#)</td>
<td>3.4+3.6</td>
</tr>
<tr>
<td>Hormone receptor status of recurring tumors(^2)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PR+ (%)</td>
<td>43 (95.6)</td>
<td>14 (87.5)</td>
<td>57 (93.4)</td>
</tr>
<tr>
<td>ER+</td>
<td>20 (50.0)</td>
<td>3 (18.8)(^*)</td>
<td>23 (41.1)</td>
</tr>
<tr>
<td>AR+</td>
<td>24 (57.1)</td>
<td>4 (23.5)(^**)</td>
<td>28 (47.5)</td>
</tr>
<tr>
<td>MIB-1</td>
<td>3.9+3.4</td>
<td>8.2+7.2(^##)</td>
<td>5.1+5.0</td>
</tr>
</tbody>
</table>

PR = progesterone receptor, ER = estrogen receptor, AR = androgen receptor, + = positive
\(^1\) first recurrence after primary operation
\(^2\) numbers include first and subsequent recurrencies
\(^3\) proliferation index percentage
\(^*\) significance of difference between benign and atypical/malignant groups (chi-square test)
\(^\#\) significance of difference between benign and atypical/malignant groups (Mann-Whitney test) p<0.01
\(^\##\) p=0.026
<table>
<thead>
<tr>
<th>Hormone receptor status combinations</th>
<th>PR-</th>
<th>PR+</th>
<th>AR-</th>
<th>AR+</th>
</tr>
</thead>
<tbody>
<tr>
<td>ER- (%)</td>
<td>41 (9.4)</td>
<td>218 (50.0)</td>
<td>159 (39.3)</td>
<td>80 (19.8)</td>
</tr>
<tr>
<td>ER+</td>
<td>4 (0.9)</td>
<td>173 (39.7)</td>
<td>79 (19.5)</td>
<td>87 (21.6)</td>
</tr>
<tr>
<td>AR- (%)</td>
<td>28 (6.7)</td>
<td>221 (53.0)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>AR+</td>
<td>14 (3.4)</td>
<td>154 (36.9)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Hormone receptor status was determined by immunohistochemical analysis (PR = progesterone receptor, ER = estrogen receptor, AR = androgen receptor, + = positive, - = negative)
Carbonic anhydrases in meningiomas: association of endothelial carbonic anhydrase II with aggressive tumor features

Laboratory investigation

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Object. Carbonic anhydrase (CA) II and IX are enzymes involved in pH homeostasis and have been shown to be upregulated in several types of cancer. In this study, the authors evaluate the expression of CA II and IX in meningiomas and assess their relationship to patient age, tumor type and grade, tumor sex hormone receptor status, tumor cell proliferation, and tumor recurrence.

Methods. This study was conducted in consecutive patients who underwent meningioma surgeries at Tampere University Hospital between 1989 and 1999. The expression of CA II and IX was studied immunohistochemically using a tissue microarray technique and specific antibodies.

Results. Immunohistological staining with CA II and IX was assessed in 443 primary and 67 recurrent tumor specimens. Of these samples, 455 were benign (WHO Grade I), 49 atypical (Grade II), and 6 malignant (Grade III). Endothelial cells in 14.8% of the tumors stained positively for CA II. Tumor cells were positive for CA IX in 11.6% of the cases. Endothelial CA II expression correlated with increasing histological grade (p = 0.002), and tumor proliferation rates were higher in CA II+ versus CA II− cases (p = 0.002). Androgen receptor–negative tumors were found to be CA II+ significantly more often than androgen receptor–positive tumors (p = 0.001). No associations were found with the CA IX enzyme.

Conclusions. Carbonic anhydrase II positivity in the endothelium was associated with cell proliferation and malignancy grade. These results suggest that CA II expression is associated with malignant progression of meningiomas and could thus be a target molecule for anticancer therapy. (DOI: 10.3171/2008.10.17672)

Key Words: • cancer • carbonic anhydrase • immunohistochemistry • meningioma • tissue microarray

Carbonic anhydrases belong to a family of metalloenzymes that catalyze the reversible hydration of carbon dioxide. Carbonic anhydrases participate in numerous biological processes such as acid-base balance, carbon dioxide and ion transport, respiration, and mucosal protection. Distribution patterns of different CA isoforms differ within normal human tissues as well as in tumors. Particular interest has been focused on isoforms CA IX and XII, and to a lesser degree, CA II. The authors of several studies have demonstrated their expression in a wide variety of malignant neoplasms.1,2,6,15,19,21,31

Carbonic anhydrase II is widely expressed in normal organs and is considered a very important enzyme in a number of physiological reactions. In addition, it is also found in malignant brain tumors,25 leukemia,19 lung cancer,2 pancreatic cancer,27 and colorectal cancer.2 Carbonic anhydrase II was recently shown to be expressed in tumor vessel endothelia,39 and is the most widely expressed CA isoenzyme in the CNS. Parkkila et al.25 found CA II in myelin, oligodendrocytes, astrocytes, and the choroid plexus. They also demonstrated expression of CA II in several brain tumors, but the few meningiomas tested did not express this enzyme.

Hypoxia in tumors is associated with aggressive growth and poor response to cancer treatments.4,11 Carbonic anhydrase IX has been proposed as a new hypoxia biomarker because it is strongly induced by hypoxia and is found in hypoxic areas of many cancers, such as those originating from the kidneys,3,23 lungs,6,7 breasts,5 cervix,21,24 brain,9,31 bladder,12 and ovaries.13 Hypoxia-in-
ducible expression of CA IX in tumors suggests that the enzyme has an important role in tumor cell survival in hypoxic environments.\textsuperscript{29,32} It has been proposed that CA IX helps maintain a neutral intracellular pH\textsuperscript{19} and contributes to the acidification of extracellular space around tumor cells, thereby facilitating tumor growth and invasion. The feasibility of CA IX as a potential hypoxia biomarker has been realized by its very limited expression in the alimentary tract in normal human tissue.\textsuperscript{15} Expression of CA IX is very low in the normal brain. It is confined to the cells lining the ventricles and the choroid plexus.\textsuperscript{15} Induced expression has been found in high-grade gliomas.\textsuperscript{9,15} Ivanov et al.\textsuperscript{15} studied a small number of meningiomas and found enhanced CA IX expression in hypoxic/necrotic areas. A correlation between CA IX expression and poor prognosis has been shown in several cancers.\textsuperscript{5,7,20,21,23}

In the present study, we assessed the expression of CA II and IX in a large series of primary and recurrent meningiomas to evaluate the relationship between their expression and clinicopathological features such as tumor grade, patient age, sex hormone expression, and tumor cell proliferation rate.

**Methods**

**Tumor Specimens**

All tumor samples were obtained in patients who underwent surgery for intracranial or intraspinal meningiomas at the Tampere University Hospital between 1989 and 1999. Two patients were excluded because of their young age (4 and 15 years). A total of 443 primary and 67 tumors of second operations were chosen for the study. The study protocol was approved by the Ethical Committee of Tampere University Hospital.

Tumors were classified and graded using the WHO scheme (Grades I–III).\textsuperscript{16} Of the 443 primary tumors, 145 (32.7\%) were meningothelial, 129 (29.1\%) were transitional, 93 (21.0\%) were fibroblastic, 44 (10.5\%) were other benign tumors, 29 (6.5\%) were atypical, and 3 (0.7\%) were malignant. Four atypical and malignant meningiomas were irradiated after recurrence. Recurrence was defined as new tumor growth after Simpson Grade I or II removal. Of the 29 recurrent meningiomas, 22 (75.9\%) were benign, 5 (17.2\%) were atypical, and 2 (6.9\%) were malignant. The second operation group consisted of all tumors that required a repeated operation despite the extent of the primary operation. Of these 67 meningiomas, 48 (71.6\%) were benign, 16 (23.9\%) were atypical, and 3 (4.5\%) were malignant. Seven meningiomas in the second operative group were embolized. For statistical analysis, atypical and malignant tumors were grouped together.

**Immunohistochemical Analysis**

Tissue microarray techniques were used for immunohistochemical analysis.\textsuperscript{17} The tumor samples were fixed in 4\% phosphate-buffered formaldehyde and processed into paraffin blocks using standard methods. Histologically representative tumor regions (highest grade and highest proliferation) of H & E–stained slides were selected by a neuropathologist (H.H.) and the corresponding areas were sampled in tissue microarray blocks using a custom-built instrument (Beecher Instruments). The sample diameter of the tissue core in the microarray block was 600 \(\mu\)m.

For proliferation analysis, sections cut from the tissue microarray blocks were stained with MIB-1 (Ki 67) antibody (DakoCytomation). Heat-induced epitope retrieval, in Tris-ethylenediamine tetraacetic acid buffer (pH 9.0; samples were microwaved twice for 7 minutes each time) and an automated immunostaining protocol (TechMate immunostainer) were used. The tissue sections were counterstained with methyl green. The proliferation was evaluated by analyzing all tumor cells in the tumor core of the multitissue block with an image analysis system (CAS-200 Software, Becton Dickinson & Co.) as previously described.\textsuperscript{33} The MIB-1 PI was reported as the percentage of immunopositive nuclei.

The hormone receptor analyses (AR, estrogen receptor, and progesterone receptor) were performed as previously described.\textsuperscript{18} Automated immunostaining for CA II and IX was performed using Power Vision+ Poly-HRP IHC Kit (Immunovision Technologies, Co.) reagents and CA II– and CA IX–specific antibodies. The immunostaining method included the following steps: 1) rinsing in Tris-buffered saline and 0.05\% TBST; 2) treatment in 3\% H\(_2\)O\(_2\), in double-distilled H\(_2\)O for 5 minutes and rinsing in TBST; 3) blocking with cow colostrum for 20 minutes; 4) rinsing in TBST; 5) incubation with rabbit anti–human CA II serum (produced and characterized by Parkkila et al.\textsuperscript{20}), M75 antibody against human CA IX,\textsuperscript{30} or normal rabbit serum. Anti–CA II serum and normal rabbit serum were diluted 1:2000 and M75 1:200, respectively, in Universal IHC diluent (Immunovision Technologies, Co.) for 30 minutes; 6) washing with TBST 3 times for 5 minutes each time; 7) blocking with postblocking solution for 20 minutes (only in M75 staining); 8) rinsing in TBST 3 times for 5 minutes; 9) incubation in poly-horseradish peroxidase–conjugated anti–rabbit/mouse IgG for 30 minutes; 10) washing again in TBST 3 times for 5 minutes; 11) incubation in DAB solution (1 drop DAB solution A and 1 drop DAB solution B with 1-ml double-distilled H\(_2\)O) for 6 minutes; 12) rinsing with double-distilled H\(_2\)O; 13) CuSO\(_4\) treatment for 5 minutes to enhance the signal; and 14) rinsing with TBST and counterstaining with hematoxylin. All procedures were performed at room temperature. The sections were mounted in Entellan Neu (Merck) and examined and photographed with a Zeiss Axioskop 40 microscope (Carl Zeiss).

The reactivity of staining for endothelial CA II and cytoplasmic CA IX was scored from the multitissue blocks on a scale of 0–3. In terms of staining reactivity, the scores were evaluated as follows: 0, no reaction; +, weak reaction, < 10\% of cells stained; ++, moderate reaction, 10–50\% of cells stained; and ++++, strong reaction, > 50\% of cells stained. The scoring was performed by 4 observers (H.H., K.K., R.V., and S.P.) who were blinded to tumor histological characteristics. In the statistical analyses, the specimens were grouped into 2 categories based on the staining reactivity. The positive group (CA II+ or CA IX+) included tumors with moderate or strong reactions, and the negative group (CA II– or CA IX–) included tumors with weak or no reaction.
Out of 510 cases, immunostaining for CA II was successful in 486 cases and for CA IX in 475 cases. Specimens with inadequate samples were excluded from the analysis. The hormone receptor status of meningioma specimens has been described earlier.\textsuperscript{18}

\textbf{Statistical Analysis}

Associations between hormone receptor expression, tumor grade, patient age group, and CA expression were evaluated with the chi-square test. The relationship between CA staining and cell proliferation assessed by MIB-1 staining was tested with the Mann-Whitney U-test. All reported probability values were 2-sided, and values < 0.05 were considered statistically significant. Mean values are presented ± SDs.

\textbf{Results}

\textbf{Carbonic Anhydrase II Expression}

Endothelial CA II immunostaining in blood vessels was strong in 8, moderate in 64, and weak in 175 cases, while in 239 cases there was no reaction. Tumor cells were negative for cytoplasmic CA II. When tumor specimens were grouped into categories based on the endothelial staining reactivity, 414 (85.2\%) cases were CA II\textsuperscript{−} (no or weak reaction) and 72 (14.8\%) CA II\textsuperscript{+} (moderate or strong reaction). Carbonic anhydrase positivity was found in 15.9 and 15.2\% of meningothelial and transitional meningiomas, respectively, compared to only 4.3\% of the fibrous subtype. No significant difference in CA immunopositivity was found between those from primary operations, second operations, and recurrent meningiomas. Carbonic anhydrase II staining reactivity correlated with tumor grade: 13.0\% of benign tumor specimens were CA II\textsuperscript{+} compared with 30.6\% of Grade II–III tumor specimens (p = 0.002). Carbonic anhydrase II positivity was more frequent in AR-negative tumors than in AR-positive tumors. Moderate or strong CA II reactivity was found in 19.1\% of AR-negative specimens and in 7.7\% of AR-positive specimens (p = 0.001). No such association was found for progesterone or estrogen receptor status (Table 1). Results of CA II immunostaining did not show significant correlation with age or sex. The mean MIB-1 PI for CA II+ tumors was 4.82 ± 3.96 and for CA II− tumors was 3.48 ± 3.79. The difference between these groups was statistically significant (p = 0.002).

\textbf{Carbonic Anhydrase IX Expression}

Carbonic anhydrase IX immunostaining was strong in 24 (5.1\%), moderate in 31 (6.5\%), and weak in 63 (13.3\%) specimens. Staining of the plasma membrane remained completely negative in 357 specimens (75.2\%). Thus, 420 (88.4\%) of 475 specimens were CA IX\textsuperscript{−} (no or weak staining), and 55 (11.6\%) were CA IX\textsuperscript{+} (moderate or strong staining) when the specimens were grouped into 2 categories. The frequencies of CA IX\textsuperscript{+} tumors in meningothelial, transitional, and fibrous meningioma specimens were 7.1, 9.9, and 9.2\%, respectively. Primary operation, second operation, and recurrent tumors did not differ in CA IX immunopositivity. Carbonic anhydrase IX immunostaining did not correlate with the tumor grade—11.1\% of Grade I meningiomas and 16.0\% of Grade II–III tumors were CA IX\textsuperscript{+} (p > 0.05). There was no association between CA IX immunopositivity and the sex hormone status of the tumors. Comparison between age groups was not significant, but there was a trend toward more frequent CA IX positivity with increasing age (p = 0.092). Sex differences had no effect on CA IX expression, nor did PI correlate with CA IX reactivity. The mean PI values for CA IX\textsuperscript{+} tumor samples were 3.87 ± 4.06 and 3.66 ± 3.78 for CA IX\textsuperscript{−} samples (p > 0.05). There was no endothelial immunopositivity for CA IX in the tumor vessels. Endothelial CA II expression did not correlate with cytoplasmic CA IX positivity in tumor cells.

\textbf{Discussion}

Meningiomas are the most common benign tumors of the CNS. Despite their mostly benign nature, their rate of recurrence is high.\textsuperscript{22} Total resection is often the goal for treatment although it can be laborious because these tumors are often found in places where surgery is most challenging. Carbonic anhydrase IX and II are possible target molecules for new therapeutic interventions in several types of tumors. To our knowledge, this is the first study to evaluate the expression of both CA II and IX in a large series of meningiomas.

Cytoplasmic CA II is a very efficient enzyme. It has been proposed to interact with CA IX and an anion exchanger protein to produce acidosis in the extracellular space of tumors.\textsuperscript{29} Cytoplasmic CA II expression has
Carbonic anhydrase II and IX in meningiomas

been found in some tumor cells such as pancreatic and renal cancer cells. In addition, expression of CA II has been recently discovered in tumor vessel endothelia, suggesting a role for this enzyme in tumor angiogenesis (Fig. 1). Yoshiura and colleagues found that the expression of endothelial CA II increased in hypoxic conditions. Hypoxic changes lead to necrosis of tumor tissue, a common feature of higher grade meningiomas. In line with this, one-third of atypical and malignant meningiomas in our study stained positively for CA II, compared to 13% in low-grade tumors. Based on our results, CA II expression also correlated with cell proliferation, while CA II and AR showed reciprocal staining reactivities. Although the reason for more abundant expression of CA II in AR-negative tumors is unclear, it is known that androgens do regulate CA II expression at least in the reproductive system. The regulatory mechanisms are complex, however, because androgens induce CA II expression in some organs and inhibit it in others. Expression of CA II has recently been found to be common in the endothelial cells of high-grade oligodendrogliomas and diffuse infiltrating astrocytomas. Carbonic anhydrase II positivity also correlated with tumor grade and poor prognosis; CA II reactivity was highest in high-grade astrocytomas, which are vascularized tumors containing necrotic areas.

Our findings suggest that CA II may be important in the development of atypical/malignant meningiomas, which are often highly vascular. If the role of CA II in tumor angiogenesis proves to be important, CA inhibitors may present new possibilities for tumor treatment in combination with other therapies such as surgery. Carbonic anhydrase inhibitors have been shown to inhibit growth and invasion of cancers. Teicher et al. showed that the CA inhibitor acetazolamide was a beneficial adjunct to chemotherapy because it extended tumor growth delay with anticancer drugs in vivo. It is also notable that some CA inhibitors are widely used drugs for neurological and ophthalmological diseases such as brain edema and glaucoma. Because CA II expression seems to be quite common in the worst cases of meningiomas, it could be beneficial to add a CA inhibitor to the treatment regimen, especially in cases with incomplete tumor resection.

The normal human brain shows only slight or no expression of CA IX, but ectopic expression of CA IX in malignant brain tumors has recently been confirmed. The transmembrane protein CA IX is strongly induced by hypoxia, and expression of CA IX is predominantly found in poorly perfused, perinecrotic areas of tumors. It has been suggested that the transmembrane location of CA IX allows it to participate in converting carbon dioxide (which diffuses in from the intracellular space) to bicarbonate and hydrogen ions. Because bicarbonate is exchanged for intracellular chloride, extracellular acidosis is maintained. Extracellular acidosis and intracellular alkalosis facilitate tumor growth; this is also in line with several previous observations that high expression of CA IX often correlates with a higher malignancy grade and...
poor prognosis.7,9 According to our findings, CA IX is expressed in only a minority of meningiomas (11.6%). This low level of expression in the present study is probably due to the mostly benign nature of meningiomas. Malignant and necrotic tumors only represented 1–2% of meningiomas. Grade II and III meningiomas showed only slightly higher expression of CA IX (16 vs 11%) than the benign specimens. Atypical meningiomas constituted the majority of meningioma specimens in the group of high-grade meningiomas. According to our results, CA IX is expressed in only a minority of meningiomas (11.6%). This low level of expression in the present study is probably due to the mostly benign nature of meningiomas.

Conclusions

Our findings indicate that CA II and IX are expressed in a minority of meningiomas. Endothelial CA II positivity was slightly more common than CA IX positivity in these tumor cells. One-third of atypical and malignant meningiomas expressed CA II, which was significantly more than in benign tumors. Carbonic anhydrase II positivity was also associated with a higher cell proliferation rate, which suggests that CA II expression is associated with the malignant progression of meningiomas. Thus, CA II could be a potential target molecule for antitumor therapy, especially of recurrent meningiomas. Our results also suggest that CA IX is not likely to be a suitable target in the search for alternative treatments of meningiomas because its expression did not differ between malignancy categories or correlate with cell proliferation.

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References

Carbonic anhydrase II and IX in meningiomas


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Sex hormones and meningioma risk

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International Union Against Cancer
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Abstract

**Background:** Previous studies on association of exogenous female sex hormones and risk for meningioma have yielded conflicting results. The aim of this study was to evaluate the potential relation between prior use of menopausal hormone therapy or oral contraception and risk of meningioma.

**Methods:** This population-based case-control study was conducted during years 2000-2002 in Finland. All women aged 20-69 years with meningioma diagnosis were identified from five university hospitals and frequency-matched controls were randomly chosen from population register. A total of 264 cases and 505 controls were interviewed on their use of menopausal hormone therapy, oral and other contraception, fertility treatment, treatment for gynecological problems, age at menarche and number of children. We also analyzed separately tumors expressing progesterone or estrogen receptors. Of the successfully stained tumor specimens, 86.3% were positive for progesterone receptor and 50% for estrogen receptor.

**Results:** Postmenopausal hormonal treatment, use of contraceptives or fertility treatment did not influence the risk of meningioma. In further analysis by hormone receptor status, there was some indication for an increased risk of progesterone-receptor positive meningiomas associated with oral contraceptive use (OR 1.39, 95% confidence interval 0.92-2.10) and other hormonal contraception (OR 1.50, 95% CI 0.95-2.36).

**Conclusions:** Overall, we found little indication that reproductive factors or use of exogenous sex hormones affect meningioma risk.

Key words: case-control, meningioma, sex hormones, estrogen receptor, progesterone receptor
Introduction

Meningioma is at least twice as common in women than in men with an increasing trend in the female: male ratio [1,2]. The only well-established risk factors besides age [3] are ionizing radiation [4, 5], and some rare hereditary conditions such as neurofibromatosis. Many other risk factors as head trauma [6], medical conditions [7-9], occupational exposures [10,11], and recently cell phone use [12] have been suggested as risk factors but no consistent associations have been reported. The fact that most meningiomas express sex hormone receptors suggests a possible role for sex hormones in the etiology of meningiomas. Progesterone receptors are found in 90% of meningiomas, estrogen, and androgen receptors in about 40% of meningiomas [13-15]. Further, increased tumor growth rate during pregnancy [16, 17] and an association between meningioma and breast cancer [18,19] have been reported. Hormonal agents have been evaluated in treatment of meningiomas, but antiprogesterone and antiestrogen agents have yielded only minor responses in small trials [20, 21].

Studies on the association of meningioma risk and parity, menopausal status or age at menarche have yielded mixed results [22-24] A few studies have recently evaluated association of oral contraceptives and menopausal hormone therapy (MHT) with risk of meningioma [22,24-30]. Jhawar et al. [27] found an increased risk of meningioma associated with current use of MHT (RR 1.86, CI 1.07-3.24). Wigertz and colleagues [28] showed similar results; among postmenopausal women, use of MHT resulted in elevated meningioma risk with RR of 1.7 (CI 1.0-2.8). Opposite findings have also been reported [22, 24, 26]. The results regarding oral contraceptives are inconclusive. In two papers, OC use was associated with a reduced risk of meningioma [24, 29], while a non-significantly increased risk was suggested in one study [26] and no association was found in others [22, 27, 28]. The association between hormonal treatment for gynecological
problems and menigioma risk has been evaluated in only one study [28]. However, the number of women using such treatments was very low and no strong conclusions could be drawn.

The use of oral contraceptives and menopausal treatments is widespread, even though the number of MHT users has decreased since the publication of the Women's Health Initiative randomized controlled trial in 2002 [31,32]. The study showed that current use of menopausal treatment is associated with increased risks of breast cancer, stroke, coronary heart disease and venous thrombosis.

In this population based case-control study we evaluated the association of oral and other hormonal contraceptives, menopausal hormone therapy, fertility treatments, and hormonal treatments on gynecological problems with development of meningiomas. We also analyzed separately tumors expressing progesterone or estrogen receptors to evaluate possible effect modification.

**Subjects and methods**

**Study population**

The source population consisted of all women aged 20-69 years in Finland excluding Åland and northernmost Lapland (with 1.5% of the entire population excluded). The study period ranged from November, 2000, to October, 2002. The study was part of INTERPHONE, an international case-control study of adult brain tumors [33]. The primary aim of the INTERPHONE was to evaluate the association of brain tumors with use of mobile phones. During the planning of the study, additional questions were included in the interview to allow analyses of other risk factors, such as menopausal hormone therapy, for brain tumors.

All women diagnosed with intracranial meningioma (ICD -10 C70.0 and D32.0) during the study
period were eligible. Diagnosis was independently confirmed histopathologically by a single neuropathologist (Hannu Haapasalo). Cases were identified continuously during the study period from neurosurgery departments at all five university hospitals in Finland. Trained research nurses at the clinics browsed patients lists weekly to identify new cases. Of the 320 identified cases 292 patients were eligible for the study. The reasons for exclusion were diagnosis date preceding the start of the study, language problems, mental retardation, refusal by patient or relative or death prior to interview. Of these 292 cases, 26 (8.9%) refused and two (0.7%) were not interviewed despite initial consent.

Control subjects were randomly identified from the source population, stratified by age (in 5-year groups) and residential area to match the distribution among cases (frequency matching). Controls were selected from the nationwide Population Register Centre approximately every second month throughout the study period. Of the 1180 eligible women, 505 (43%) consented and were successfully interviewed. The most common reasons for failure to recruit the controls were refusal (60%) and failure to contact (23%).

All interviews and contacts with cases and controls were by study nurses hired for this purpose. Information on hormone usage and other possible risk factors, such as parity, exposure to ionizing radiation, family history of brain tumors, was collected through personal interviews. Exposure information related to exogenous female hormones was acquired by asking the subject if she has ever used oral or other hormonal contraceptives, received hormonal treatment for gynecological problems, fertility problems or menopausal symptoms. Also, information of age at menarche, number of children and menopausal status were obtained. An affirmative answer was followed by additional questions on the brand name of the preparate and duration of use. Vaginally administered estrogen formulations were excluded as they do not increase serum concentrations similarly to other preparations and are not associated with a similarly increased risk of breast cancer [34]. Definition for postmenopausal status was having no menstrual periods or bilateral oophorectomy at least a
year prior to the reference date (date of diagnosis for the cases and for controls the date preceding the interview by a similar period than the lag between diagnosis and interview for cases i.e. days 39). The study protocol was approved by the Ethical Committee of Tampere University Hospital. A written informed consent was obtained from all study participants.

Immunohistochemistry

In immunohistochemical studies, tissue microarray (TMA) technique was used [35]. The tumor samples were fixed in 4% phosphate buffered formaldehyde and processed into paraffin blocks with standard methods. Histologically representative tumor regions of hematoxylin-eosin-stained slides were selected by a neuropathologist (HH) and corresponding areas were sampled in tissue microarray blocks using a custom-built instrument (Beecher Instruments, Silver Spring, MD). The sample diameter of the tissue core in the microarray block was 600 μm. For immunohistochemistry of hormone receptors, monoclonal antibodies 6F11 and PGR312 were used for ER and PR, respectively (Novocastra Laboratories, Newcastle, UK). All antibodies were diluted at 1 μg/ml, and detected with a peroxidase-polymer based detection kit (PowerVision+TM, Immunovision Technologies, Daly City,CA) according to the manufacturer´s instructions.

Histological analysis for progesterone receptor was successful in 197 cases (74%) and for estrogen receptor in 196 of 264 cases (74%). The failure in immunostaining was due mainly to the fact that some of the TMA tissue cores were detached from slides during the immunohistochemical stainig process. A tumor was considered receptor-positive if >10% of cells expressed progesterone or estrogen receptors. The 10% cutoff is commonly used in hormone receptor analysis of breast cancer [36].
Statistical analyses

Odds ratios with 95 percent confidence intervals were used as effect measures for exogenous sex hormone exposure and of meningioma. Case-control analysis was conducted using unconditional logistic regression with adjustment for age and residential area (matching factors) as well as family history with brain tumours (potential confounder). Duration of hormonal treatment was divided into approximate quartiles based on frequencies among controls, with full years as cut-points. No exposure was used as the referent category. Subjects reporting neurofibromatosis (three cases, no controls) or previous brain tumor diagnosis with radiation therapy for head or neck region (two cases and one control) were excluded from the analyses.

Results

A total of 264 meningioma cases (90.4% of eligible) and 505 controls (43% of the eligible subjects, 65% of those contacted) were included in the study (Table 1). The mean ages (±SD) for women with meningioma and for controls were 54 ± 10 years and 51 ± 12 years, respectively. The mean number of children for meningioma patients was 2.0 ± 1.3 compared to 1.8 ± 1.3 in the control group. The mean age at menarche was similar among cases and controls (13.2 ± 1.6 vs. 13.2 ± 1.5 years). A total of 179 (68.1%) of the cases and 285 (56.6%) of the controls were post-menopausal. Of the tumor specimens 86.3% (170) were positive for progesterone receptor and 50% (98) for estrogen receptor which is in accordance with thirteen meningioma patients had a family history of brain tumors as did 16 women in the control group.

For ever use of oral contraceptives, the odds ratio of meningioma was 1.33 (95 percent confidence interval (CI): 0.94, 1.89 (Table 2). There was no monotonous association with duration of use, but the highest risk was among women who had used oral contraceptives for 1-4 years (OR 2.05, CI:
The risk was slightly higher for tumors expressing estrogen receptors than for progesterone receptors (OR 2.67, CI: 1.46, 4.88 vs. OR 2.20, CI: 1.31, 3.70). Other long-term hormonal contraception (subdermal implants, injections, hormonal intra-uterine device) was also associated with an increased risk for meningioma (OR 1.42, CI: 0.95, 2.11) but number of users was low. The use of menopausal hormone therapy was not associated with an elevated risk of meningioma (OR 0.90, CI: 0.63, 1.27) and there was no trend with duration of use. Analyses of MHT were also carried out restricted to postmenopausal women, but the results were similar (for ever vs. never use OR = 0.97, 95% CI 0.67-1.42). A total of 45 women had received fertility treatments. Six cases and 13 controls had received only one course of treatment while nine women had multiple treatments (ranging 2-12) and 17 women could not recall the number of treatments. The odds ratio for past use of fertility treatments did not indicate an elevated risk of meningioma (OR 0.73, CI: 0.37, 1.43). Hormonal treatment for gynecological problems (e.g. menorrhagia or irregular menstruation) did not show an association with elevated meningioma risk (OR 1.13, CI: 0.76, 1.67). Neither age at menarche nor postmenopausal status was associated with increased risk of meningioma. An increasing number of children was associated with a slightly increased risk of meningioma, but this finding did not reach significance.

A consistent though non-significant positive association was found between tumors expressing PR and use of oral contraceptives (Table 3). Some evidence for increased risk of estrogen-receptor positive meningiomas and hormonal treatment for infertility was also obtained. Menopausal hormone therapy was not associated with hormone receptor status.

**Discussion**

We conducted a population-based case-control study to explore the association between meningioma risk and use of exogenous female sex hormones. Menopausal hormonal treatment or
use of contraceptives was not strongly associated with the risk of meningioma. Moreover, we assessed the meningioma risk by hormone receptor status and found some indication for an increased risk in relation to duration of oral contraceptive use for progesterone-receptor positive cases.

Few studies have assessed the association between meningioma risk and menopausal treatment. Their results are inconsistent varying from a protective association to an increased risk for meningioma. The first study to report an increased risk of meningioma associated with use of menopausal treatment was the Nurses’ Health Study [27] which showed an increased risk among postmenopausal women who were current hormone users (OR 1.86, CI 1.07-3.24). After that Wigertz et al. [28] reported a population-based case-control study with similar results among postmenopausal women who had ever used menopausal hormone treatment. In a recent study positive association was found between meningioma diagnosis and current or past menopausal treatment also by Blitsheyn et al. [30]. The association appeared strongest in younger women (aged 26-55) with an OR of 4.1 (CI 2.7, 6.4). Recently, results regarding MHT use and central nervous system tumor risk in the Million Women Study cohort were published [25]. Ever users of MHT had a significantly increased risk of meningioma compared to never users (RR 1.32, CI: 1.05-1.66, p=0.02). For current users, the risk was higher when using estrogen-only therapy compared to estrogen-progestagen combination therapy (RR 1.44 CI: 1.03-2.02 vs. RR 1.10, CI: 0.77-1.56). Our results do not support a link between menopausal treatment and meningioma risk and the confidence interval excludes even a modest risk (OR 1.3 or greater).

The evidence for an association of oral contraceptive (OC) use with meningioma risk is even weaker than for menopausal treatment. A non-significantly increased risk has been found in one study [26] among past and current users with higher risk (OR 2.5, CI 0.5-12.6) in current users, but with very wide confidence intervals. There was no association between meningioma risk and duration of use. A protective association between OC use and meningioma risk has been reported in
two studies. The clearest finding was reported by Lee et al. [24] among current OC users but some indication was also among past users. In another study of intraspinal meningioma the protective association increased with longer duration of use [29]. Three other studies have found no significant association between OC use and meningioma risk [22, 27, 28]. In the Million Women cohort study [37], only height and BMI were associated with an increased risk of meningioma. In our study, the OR for patients who had used oral contraceptives 1-4 years was 2.05 (CI 1.7, 3.0) but the lack of association after 4 years of use suggested that this finding may due to chance. We did not find any association with long-term use of other hormonal contraception (subdermal implants, injections or intrauterine devices) and the risk of meningioma. Few studies have evaluated the association between hormonal treatments for subfertility or gynecological bleeding disorders and the risk of meningioma. Our results are in agreement with Wigertz et al. [28], who found no association between hormone use for gynecological problems and meningioma. However, the strength of conclusions in both studies is limited by the small number of women with such hormonal treatments.

Indicators of endogenous hormonal factors such as parity, age at menarche or menopausal status have not been consistently associated with meningioma risk. Different studies have found parity being either protective factor or associated with an increased risk of meningioma. Two studies showed slight non-significant risks for ever-gravid and ever-parous women [22, 27]. An opposite finding was reported by Lee and colleagues [24] who found a protective influence of pregnancy directly proportional to the numbers of pregnancies. We found a trend with increasing risk of meningioma with increasing number of children, but this finding did not reach significance.

In the Nurses’ Health Study [27], menarche at age of 14 or later was associated with an increased risk (OR 1.97, CI 1.06-3.66), but this finding has not been confirmed by other studies [22, 24], or ours. Slightly increased risk for meningioma and postmenopausal women has been reported [22, 26] and a decreased risk of meningioma related to postmenopause has been reported in one
Few studies have assessed both sex hormone receptor expression and exposure to exogenous sex hormones. We found some indication of increased risk for progesterone receptor positive tumors in relation to duration of oral contraceptive use. In the study of Custer et al. [26] with sub-division of cases by hormone receptor expression, a strong association (OR 3.2, CI 1.3-8.0) was reported between oral contraceptive use and tumors with less than 25% of cells expressing progesterone receptors. An association between the number of live births and tumors expressing less than 25% of cells was found in the same study. Their results are difficult to compare with ours, because the sensitivity of immunohistochemical techniques are likely to differ. In their study only 2% of the tumors expressed estrogen receptors compared to our 50%. Also sub-division of cases by hormone receptor expression was made differently, cut-off value being 25% in their study and 10% in ours. A quarter of the histological specimens could not be analyzed for hormone receptors in our study. The failure is likely to be unrelated to hormone receptor status and the loss of information would slightly decrease the power of the study (precision of the results).

This study has some limitations. Use of exogenous female hormones was self-reported. Less than half of cases and controls could report the exact name of the compound they used and therefore sub-divisions of menopausal hormonal treatments or oral contraceptives by type of hormones could not be made. Further, we did not have information on time since hormone use and were therefore unable to distinguish between current and past users. The participation proportion of cases was high, but only moderate among controls (mainly due to failure to contact the controls) so we cannot rule out selection bias. We had no information on e.g. sociodemographic factors among non-participants, but an earlier report suggests higher level of education among participating than non-participating cases and controls [39]. However, in our study 33.7% of controls were current or past users of menopausal hormone therapy, which is in accordance with the large nation-wide Health 2000 Survey [40], in which 34.3% of Finnish women were current or past users.
The strengths of our study include histopathological diagnosis of all cases by a single neuropathologist. All contacts were made by the same trained nurses which ensures the standardized interview of all cases and controls and hence comparability of information. We were also able to control for the potential confounding due to the established risk factors i.e. hereditary syndromes and prior radiotherapy. Even if recruitment of cases was performed through hospitals, we were able to attain a population-based series due to the coverage of all university hospitals responsible for providing neurosurgery services for their source population. In the Finnish comprehensive public health care system, they are practically the only treatment centers in the field of neurosurgery. A comparison of a regional case series with the nation-wide cancer registry indicated that all cases notified to the registry were also identified from the neurosurgery department [41]. Overall, our results are reassuring as they do not indicate an increased risk of meningioma in conjunction with hormonal contraception or menopausal treatment. We could exclude risks of magnitude relevant for public health e.g. OR>1.5 in most analyses. Further confirmation for the relation of oral contraceptive use and progesterone-positive meningiomas is needed.

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European Commission (Quality of Life and Management of Living Resources), as well as Medical Research Fund of Tampere University Hospital and International Union Against Cancer, which administered the support from the Mobile Manufacturers Forum and GSM Association.

References


tumour development. Results from the international adult brain tumour study. Int J Cancer 82(2): 155-60.


Table 1. Descriptive characteristics of meningioma patients and controls

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Meningioma cases (%)</th>
<th>Controls (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total</td>
<td>264</td>
<td>505</td>
</tr>
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</table>

Age (years)

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<th>Age Range</th>
<th>Meningioma cases (%)</th>
<th>Controls (%)</th>
</tr>
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<tbody>
<tr>
<td>20-29</td>
<td>3 (1.1)</td>
<td>40 (7.9)</td>
</tr>
<tr>
<td>30-39</td>
<td>24 (9.1)</td>
<td>58 (11.5)</td>
</tr>
<tr>
<td>40-49</td>
<td>51 (19.3)</td>
<td>118 (23.3)</td>
</tr>
<tr>
<td>50-59</td>
<td>106 (40.2)</td>
<td>154 (30.5)</td>
</tr>
<tr>
<td>60-69</td>
<td>80 (30.3)</td>
<td>135 (26.7)</td>
</tr>
</tbody>
</table>

Number of children

<table>
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<tr>
<th>Number of Children</th>
<th>Meningioma cases (%)</th>
<th>Controls (%)</th>
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</thead>
<tbody>
<tr>
<td>0</td>
<td>33 (12.5)</td>
<td>101 (20.0)</td>
</tr>
<tr>
<td>1-2</td>
<td>151 (57.4)</td>
<td>269 (53.3)</td>
</tr>
<tr>
<td>≥3</td>
<td>79 (30.0)</td>
<td>135 (26.7)</td>
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Menarche

<table>
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<th>Menarche</th>
<th>Meningioma cases (%)</th>
<th>Controls (%)</th>
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</thead>
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<td>≤12</td>
<td>91 (35.0)</td>
<td>158 (31.5)</td>
</tr>
<tr>
<td>13</td>
<td>65 (25.0)</td>
<td>158 (31.5)</td>
</tr>
<tr>
<td>≥14</td>
<td>104 (40.0)</td>
<td>185 (37.0)</td>
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</table>

Menstrual status

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<th>Meningioma cases (%)</th>
<th>Controls (%)</th>
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<tr>
<td>menstruating</td>
<td>78 (30.4)</td>
<td>199 (41.1)</td>
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<tr>
<td>postmenopausal</td>
<td>179 (69.6)</td>
<td>285 (58.9)</td>
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Family history of brain tumors

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<tr>
<th>Family history of brain tumors</th>
<th>Meningioma cases (%)</th>
<th>Controls (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>13 (4.9)</td>
<td>16 (3.2)</td>
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Table 2. Risk of meningioma and exposure to exogenous and endogenous sex hormones.

<table>
<thead>
<tr>
<th>Hormone use</th>
<th>Meningioma</th>
<th>Control</th>
<th>OR (95% CI)</th>
<th>p value</th>
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<tr>
<td><strong>Oral contraceptives:</strong></td>
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<td></td>
<td></td>
<td></td>
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<tr>
<td>Never</td>
<td>81</td>
<td>162</td>
<td>1</td>
<td>0.11</td>
</tr>
<tr>
<td>Ever</td>
<td>182</td>
<td>342</td>
<td>1.33 (0.94-1.89)</td>
<td></td>
</tr>
<tr>
<td><strong>Duration of use (months):</strong></td>
<td></td>
<td></td>
<td></td>
<td>0.51</td>
</tr>
<tr>
<td>0-12</td>
<td>40</td>
<td>83</td>
<td>1.13 (0.70-1.82)</td>
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<tr>
<td>13-48</td>
<td>63</td>
<td>76</td>
<td>2.05 (1.30-3.22)</td>
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<tr>
<td>49-96</td>
<td>39</td>
<td>84</td>
<td>1.18 (0.72-1.95)</td>
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<tr>
<td>&gt;96</td>
<td>35</td>
<td>88</td>
<td>1.02 (0.61-1.69)</td>
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<tr>
<td><strong>Other hormonal contraception:</strong></td>
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<td></td>
<td></td>
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<tr>
<td>No</td>
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<tr>
<td>Yes</td>
<td>54</td>
<td>80</td>
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<td><strong>Menopausal hormone therapy:</strong></td>
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<tr>
<td><strong>Duration of use:</strong></td>
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<td></td>
</tr>
<tr>
<td>Never</td>
<td>161</td>
<td>334</td>
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<tr>
<td>0-24</td>
<td>32</td>
<td>52</td>
<td>0.98 (0.56-1.63)</td>
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<tr>
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<td>33</td>
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<tr>
<td>61-120</td>
<td>30</td>
<td>48</td>
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<tr>
<td>&gt;120</td>
<td>17</td>
<td>35</td>
<td>0.61 (0.32-1.18)</td>
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<tr>
<td><strong>Fertility treatment:</strong></td>
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<tr>
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<td>32</td>
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<tr>
<td>Yes</td>
<td>50</td>
<td>90</td>
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<td><strong>Number of children:</strong></td>
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</tr>
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<td>33</td>
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<td>104</td>
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<td>0.86 (0.59-1.26)</td>
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</table>
Table 3. Comparison of risk of estrogen receptor (ER) and progesterone receptor (PR) positive meningiomas and exposure to exogenous and endogenous sex hormones.

<table>
<thead>
<tr>
<th>Hormone use</th>
<th>Meningioma cases</th>
<th>p value</th>
<th>Meningioma cases</th>
<th>p value</th>
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<td>Expressing ER</td>
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<td>OR (95% CI)</td>
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<td>Oral contraceptives:</td>
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<td>Ever</td>
<td>1.31 (0.79-2.17)</td>
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<tr>
<td>Duration of use (months)</td>
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<td>0.53</td>
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<td>0.51</td>
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<tr>
<td>Never</td>
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<tr>
<td>1-12</td>
<td>0.79 (0.36-1.73)</td>
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<td>0.99 (0.55-1.79)</td>
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<tr>
<td>13-48</td>
<td>2.67 (1.46-4.88)</td>
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<td>2.20 (1.31-3.70)</td>
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<td>49-96</td>
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<td>&gt;96</td>
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<td>Other hormonal contraception</td>
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<td>1.37 (0.78-2.39)</td>
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<td>Menopausal hormone therapy</td>
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<td>1-24</td>
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<td>0.97 (0.65-1.46)</td>
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<td>25-60</td>
<td>0.74 (0.29-1.91)</td>
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<td>0.90 (0.44-1.86)</td>
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<td>61-120</td>
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<td>0.99 (0.54-1.81)</td>
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<td>&gt;120</td>
<td>0.42 (0.15-1.18)</td>
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<td>Hormonal treatment for infertility</td>
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<td>1.32 (0.57-3.01)</td>
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<td>0.80 (0.37-1.73)</td>
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<td>Hormonal treatment for gynecological problems</td>
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<td>Yes</td>
<td>1.13 (0.63-2.00)</td>
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<td>0.81 (0.51-1.27)</td>
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<tr>
<td>≥14</td>
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<td>0.81 (0.53-1.24)</td>
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<td>1.60 (0.92-2.76)</td>
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<td>≥3</td>
<td>1.70 (0.80-3.58)</td>
<td>1.49 (0.81-2.74)</td>
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