Molecular, genetic, patient and surgical factors involved in the development and outcome of central nervous system tumours

A thesis submitted to The University of Manchester for the degree of

MD

In the Faculty of Medical and Human Sciences

2010

Ian D. Kamaly-Asl, MB ChB, FRCS(SN)

School of Medicine

List of Contents

Total word count: 63,915

List of Figures

List of Tables

Abstract

The University of Manchester

Ian Daryoush Kamaly-Asl

MD

Molecular, genetic, patient and surgical factors involved in the development and outcome of central nervous system tumours

Prognostic factors come in a variety of forms and may be patient, tumour or environmental related.

This thesis examines the interaction of prognostic factors for a variety of tumour types. It particularly focuses on single nucleotide polymorphisms (SNPs) of the vascular endothelial growth factor (*VEGF*) gene.

The first section on meningiomas describes the frequency of sex steroid receptors in meningiomas. In this study, absence of progesterone receptors is associated with high tumour grade and male gender. Tumours that are progesterone receptor negative have an odds ratio for recurrence of 5.

Choroid plexus carcinomas are aggressive malignant tumours generally occurring in young children. Gross total surgical resection has been shown to be a highly significant factor in tumour recurrence and survival. This study describes a treatment paradigm of neoadjuvant ICE chemotherapy in these children which decreases the vascularity and increase the chance of a complete removal. The operative blood loss with this regimen is reduced to 0.22 blood volumes from 1.11 blood volumes without neoadjuvant chemotherapy.

The *VEGF* gene is highly polymorphic and SNPs of the region have previously been shown to influence VEGF protein expression. This study looks at cohorts of both adult gliomas and a variety of paediatric brain tumours; comparing them to controls. There are several associations described between the development of certain tumours and specific SNP genotypes. In addition to this, certain genotypes and haplotypes have an influence on survival of adult grade 2 astrocytomas and paediatric medulloblastomas and ependymomas. There are consistent themes to the prognostic genotypes throughout both the adult and the paediatric tumours.

Prognostic factors come in a variety forms as described in this thesis. It is vital to understand the complex interaction between factors to best utilise them for the benefit of patients.

DECLARATION

I declare that no portion of the work referred to in the thesis has been submitted in support of an application for another degree or qualification of this or any other university or other institute of learning.

COPYRIGHT STATEMENT

Personal contribution to the work

I have personally undertaken the bulk of the practical work and data analysis for this thesis. For the meningioma study I was one of the reviewers of the receptor imunnohistochemistry. I performed the volumetric analysis of scan data for the choroid plexus study and the calculations of blood loss. I undertook all the quantification of DNA and VEGF genotyping assays for both the adult and paediatric tumour patients and paediatric controls. I obtained the clinical information and undertook all the statistical analysis for each of the studies.

There have, however, been others who have contributed to the practical work and where this is the case I acknowledge them below.

Acknowledgements

There are many people who have been helpful to me in the undertaking of the work for this thesis.

I would first like to thank my overall supervisor for the thesis, Professor Paul Brenchley (Professor of Renal Immunology, The University of Manchester) who was the inspiration and driving force for this work.

For the meningioma project I have to thank Mr Charles Davis (Consultant Neurosurgeon, Royal Preston Hospital) for the initial impetus for the work. I also have to thank Angela Clayton (Laboratory Technician, Royal Preston Hospital) and the other technicians who undertook the immunostaining of the meningiomas over the years of the project. I am also very grateful to Dr Tim Dawson and Dr Dominic O'Donovan (Consultant neuropathologists, Royal Preston Hospital) who independently reviewed the histology and steroid receptor status with me.

The choroid plexus carcinoma study was undertaken with the guidance of Dr Michael Taylor (Paediatric Neurosurgeon, The Hospital for Sick Children, Toronto, Canada). I also have to thank Maria Lamberti-Pasculli (Research Nurse, The Hospital for Sick Children) for help in accessing the clinical research database.

The adult VEGF glioma study was supported by Dr Peter McLaren Black (Chief of Neurosurgery, The Brigham and Women's Hospital, Boston, USA). I am very grateful for him allowing me to access the hospital's tumour bank for the study. I am indebted to Dr Rona Carroll (Principle Scientist in the

Black Lab., Harvard Medical School, Boston) for her assistance in the laboratory.

The paediatric VEGF brain tumour study was undertaken with the help and guidance of Dr James Rutka, (Neurosurgery Program Director, The Hospital for Sick Children, Toronto, Canada) Dr David Malkin, (Senior Staff Oncologist, The Hospital for Sick Children) and Dr John McLaughlin (Vice President, Population Studies & Surveillance, Cancer Care Ontario). I am very grateful to Jodi Lees (Senior Research Scientist, The Hospital for Sick Children) for her help in the laboratory in Toronto.

This work was funded by grants from the Black Laboratory Research Fund, Boston and Brainchild Trust, Toronto.

I am grateful to all the other members of the teams at Boston Children's Hospital and Toronto Hospital for Sick Children who were and are an inspiration to me both for this work and my career as a paediatric and neuro-oncological neurosurgeon.

Lastly, I wish to thank my family, who have always been my greatest inspiration, for helping me through this as with many other mammoth tasks with their love and support.

Dedicated to

My Mother Ann and Father Yadoullah; My Children, Anna, Sara and Laurence and most of all my Wife Helen

Chapter 1 – Introduction

Prognosticate: To forecast or predict (something future) from present indications or signs; prophesy.

From Old Italian prognosticare, from Latin prognosticum (-con), from Ancient Greek προγνωστικόν (prognostikon), neutral of προγνωστικός (prognostikos) "foreknowing, prescient, prognostic", from prefix πρό- (pro-) + γνωστικός (gnostikos) "of or for knowing, good at knowing", from γιγνώσκω (gignosko) "to learn to know, to perceive, to mark, to learn".

This thesis is an examination of various factors and their influence the development and outcome in central nervous system (CNS) tumours.

The introduction will discuss the nature of prognostic factors in oncology, followed by an overview of the various CNS tumours involved in the thesis and then a more detailed review of angiogenesis and particularly vascular endothelial growth factor (VEGF) biology and its association with CNS tumours.

1.1 Prognostic factors in oncology

In the field of medicine and oncology in particular, the requirement for clinicians to provide an accurate prognosis for a condition is vital. Along with diagnosis and treatment, prognosis is one of the three core elements of the art of medicine,[2] and Hippocrates included prognosis as a principle concept of medicine.[3] The aspiration is to be able to predict if and when a tumour

will recur and what the life expectancy of a patient with cancer will be with 100% accuracy. Obviously given the complex nature of the development and progression of tumours this is impossible, but on a daily basis there is a need for individual patients to have this information in its most accurate form in order for decisions on their management to be made.

I will describe the various types of prognostic factors as well as the application of such factors. I will then discuss validation of factors and the guidelines for the development of prognostic factors in medicine.

1.1.1 Types of prognostic factors

There are three commonly used groups of prognostic factors in oncology; tumour, patient and environment (Figure 1.1).[2] There will be a complex interplay between factors in these different groups that will determine the outcome of the disease. It is unusual in modern medicine to be truly able to assess the "natural history" of a disease and the factors that may have influence on that in the absence of any intervention whatsoever. Therefore all the various factors may have different weighting of influence depending upon the treatments that the patient may receive.[2] In the literature there is an attempt to differentiate these factors by referring to prognostic factors as those that have an influence on patient outcome and predictive factors that will determine response to treatment.[4] Prognostic factors have some interplay with aetiological factors and there are many similarities in the

design and analysis of both types of studies but the prediction of outcomes is not synonymous with the explanation of the cause of a particular tumour.[5]

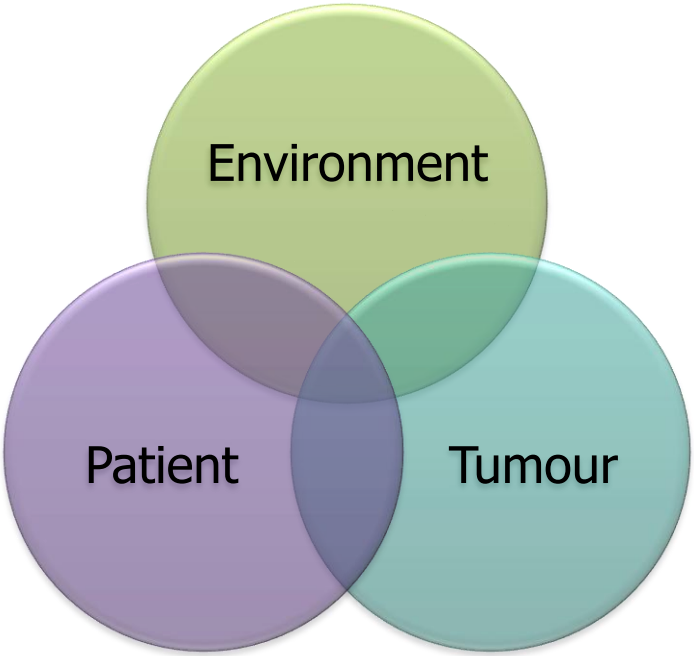


Figure 1.1 – Interaction of prognostic factor types – Tumour, Patient and Environment.

1.1.1.a Tumour related factors

The most common and longest used of factors relate to the nature and extent of the tumour itself. One of the primary and most useful factors is the phenotypic histological appearance of the tumour. For non-central nervous system tumours the International Union against Cancer (UICC) TNM (tumour, nodes and metastasis) classifications relating to the extent of local and distant invasion of the tumour are widely used.[6] Individual tumour biology has been recognised as important for many years as a marker of prognosis and response to treatment e.g. secretion of markers such as α-fetoprotein or β-human chorionic gonadotrophin. There has, though, been an explosion of studies describing biological and genetic factors in tumours

as the techniques required for their identification have become more widespread and easily performed.[1, 2, 4] Opening up this field has exponentially increased the complexity of interaction between prognostic factors for each tumour.

1.1.1.b Patient related factors

These can be split into those factors that may be related to the presence of the tumour e.g. age, gender and ethnicity and those that are independent of the tumour but may still have a significant impact on the disease course e.g. co-morbidities, performance status and immune status. For central nervous system tumours it has been recognised for many years that age and performance status have an influence on outcome.[7] Ethnicity has also been shown to have an influence via genetic differences in malignant glioma[8] and gender may have an influence in survival for patients with medulloblastoma.[9]

Somatic genetic changes within tumours are recognised to have an impact on outcome. Alterations in chromosomes 1p and 19q in Oligodendrogliomas are associated with a significant improvement in the response to chemotherapy.[10] Constitutional genetic makeup can also have an influence on the development and outcome of central nervous system tumours. A good example of this is neurofibromatosis type 1 (NF1) patients who are at increased risk of developing optic pathway gliomas but their clinical course is more indolent than those tumours occurring outside the setting of NF1.[11] Furthermore genetic traits have the potential to act as disease modifiers and angiogenesis is good model for this with known differences in individual's genetic expression of angiogenic factors at both constitutional and inducible

levels having the potential to affect the progression of tumours.[12, 13] This will be discussed in more detail later in this chapter.

1.1.1.c Environmental related factors

Environmental factors are clearly related to the development of certain cancers e.g. smoking and lung cancer, ultraviolet light exposure and skin cancer. In addition there are significant environmental factors that influence outcome of cancers. These relate to organisational and social issues such as availability of treatments, specialist care and health care policy.[14-16] Social deprivation has also been shown in several studies to have an influence on cancer outcomes.[17-19]

1.1.2 Application of prognostic factors

There are a variety of ways that prognostic factors are used and the applications are constantly increasing as research and knowledge of individual tumour types deepens.[2]

By identifying prognostic factors we can further our knowledge of the biology of certain tumours and also the factor related outcomes in both treated and untreated patients.

There are several ways that prognostic factors can be applied to patient care. Patients and families cannot make informed decisions regarding potential benefits of treatments and interventions if they are not accurately informed of the outcomes if no treatments are given. There may be the opportunity to either intensify or avoid treatments depending upon

prognostic factors. The most appropriate follow up care and monitoring will be determined by factors influencing the chance of tumour recurrence. Lastly there is a vital role for educating and informing patients and care givers, particularly in the palliative phase of cancer care.

There is a necessity for information on the spectrum of prognostic factors for an individual tumour to be more utilised in the conducting of research studies. For the effect of an intervention to be validated there is a requirement in study design, conduct and analysis to account for other known prognostic factors. Knowing that a prognosis is poor and response to other treatments limited can allow for appropriate recruitment into experimental/phase 1 and 2 studies.

In a wider setting, prognostic factors can help plan healthcare resources and monitor for the effect of screening programmes. They can be effectively used in the design and evaluation of clinical practice guidelines. Patient health education can be informed and enhanced with appropriate information from prognostic factors. Lastly as a greater understanding of the factors influencing prognosis for a particular tumour are known there will be better understanding of the apparent variation in clinical outcome.

1.1.3 Guidelines for the development of prognostic factors

There has been concern in the literature that the explosion of prognostic factors in oncology has led to studies that are badly designed, poorly analysed, poorly reported and subject to various types of bias.[1, 4, 20-28]

This problem has led to a variety of articles trying to standardise studies into prognostic factors.[1, 4, 27, 29]. One of the issues with previous studies have been the difficulties with multiple testing.[23] This is particularly problematic with the multiple variables that are generated with detailed genetic analysis. It is recognised that there is the potential need for adjusting the significance level for such multiple testing. The most commonly used method being the Bonferonni correction where the standard level of cut off for significance is divided by the number of variables with the idea of reducing the chance of a type 1 error (where the null hypothesis is falsely rejected and a significant factor is found where it does not exist). However, it is also recognised undertaking this correction, particularly in the context of a biologically or clinically relevant factor may lead to a type 2 error (where the null hypothesis is falsely accepted and a genuine prognostic factor is not shown to be significant).[30]

Sample size to determine significance is difficult to ascertain in prognostic multivariable studies.[5] A general rule of thumb is that for each candidate predictor studied there should be at least 10 outcome events, although one study showed that this may not be necessary in certain circumstances.[31].

For continuous variables there is a concern that cut off points which may not be appropriate are selected to optimise the significance of the variable.[20]

In order to correct for low sample size there have been methods available for some time to address this with systematic reviews and meta-analysis of both published and individual patient data undertaken.[32, 33] These methods

do have their own difficulties. A study on microvessel density in non-small cell lung cancer found that when individual patient data was entered into a meta-analysis the significance of microvessel density as a prognostic factor was lost contradicting the findings of a previous meta-analysis on published data only.[34]

Certain authors have outlined the different phases of studies in multivariable prognostic research.[4, 5, 21]

Phase 1 is developmental studies, identifying potential prognostic factors and determining the weight of their effect. Internal validation should be performed at this stage.

Phase 2 involves further validation studies. There are various forms of validation (essentially assessing whether the factor is predicting what it is designed to do) and various ways of testing it e.g. temporal validity where an already identified factor is retested on a later cohort of patients from the same institution. The best form of validity for a factor to be universally useful is external validity where the factor is tested on a cohort of patients external to the initial study.

Phase 3 involves impact studies looking at the utilisation of a factor in practice to see if it maintains its usefulness and relevance.

In an attempt to standardise studies, The National Cancer Institute and European Organisation for Research and Treatment of cancer have published

reporting recommendations for tumour marker prognostic studies (REMARK)(Table 1.1).[1]

INTRODUCTION
1. State the marker examined, the study objectives, and any prespecified hypotheses.

MATERIALS AND METHODS
Patients
2. Describe the characteristics (e.g., disease stage or co-morbidities) of the study patients, including their source and inclusion and exclusion criteria.
3. Describe treatments received and how chosen (e.g., randomized or rule-based).
Specimen characteristics
4. Describe type of biological material used (including control samples) and methods of preservation and storage.
Assay methods
5. Specify the assay method used and provide (or reference) a detailed protocol, including specific reagents or kits used, quality control procedures, reproducibility assessments, quantitation methods, and scoring and reporting protocols. Specify whether and how assays were performed blinded to the study endpoint.
Study design
6. State the method of case selection, including whether prospective or retrospective and whether stratification or matching (e.g., by stage of disease or age) was used. Specify the time period from which cases were taken, the end of the follow-up period, and the median follow-up time.
7. Precisely define all clinical endpoints examined.
8. List all candidate variables initially examined or considered for inclusion in models.
9. Give rationale for sample size; if the study was designed to detect a specified effect size, give the target power and effect size.
Statistical analysis methods
10. Specify all statistical methods, including details of any variable selection procedures and other model-building issues, how model assumptions were verified, and how missing data were handled.
11. Clarify how marker values were handled in the analyses; if relevant, describe methods used for cut point determination.

RESULTS
Data
12. Describe the flow of patients through the study, including the number of patients included in each stage of the analysis (a diagram may be helpful) and reasons for dropout. Specifically, both overall and for each subgroup extensively examined report the numbers of patients and the number of events.
13. Report distributions of basic demographic characteristics (at least age and sex), standard (disease-specific) prognostic variables, and tumour marker, including numbers of missing values.
Analysis and presentation
14. Show the relation of the marker to standard prognostic variables.
15. Present univariate analyses showing the relation between the marker and outcome, with the estimated effect (e.g., hazard ratio and survival probability). Preferably provide similar analyses for all other variables being analyzed. For the effect of a tumour marker on a time-to-event outcome, a Kaplan – Meier plot is recommended.
16. For key multivariable analyses, report estimated effects (e.g., hazard ratio) with confidence intervals for the marker and, at least for the final model, all other variables in the model.
17. Among reported results, provide estimated effects with confidence intervals from an analysis in which the marker and standard prognostic variables are included, regardless of their statistical significance.
18. If done, report results of further investigations, such as checking assumptions, sensitivity analyses, and internal validation.

Table 1.1 – Summary of reporting recommendations for tumour marker prognostic studies (REMARK).[1]

1.2 Central nervous system tumours

1.2.1 Meningiomas

1.2.1.a Definition

Meningiomas are tumours of meningothelial (arachnoidal) cell origin. Generally they are benign, slow growing tumours but there are a minority that are defined as atypical or anaplastic that are more aggressive lesions.[35]

1.2.1.b Epidemiology

After gliomas, meningiomas are the second most common primary central nervous system tumour. They generally occur at an older age (peak 50-70 years – median 65 years) and are more common in females (1.5 to 3 times). In autopsy series there is a prevalence of 2.7% of males and 6.2% of females over 80 years of age.[35] Annual incidence is approximately 5.35 (95% CI 5.28-5.42) per 100,000.[36]

1.2.1.c Aetiology

Most meningiomas are spontaneous but there is an association with ionising radiation. This was first determined in the group of immigrants into Palestine in the 1950s where tinea capitis was regularly treated with ionising radiation. There was a much higher incidence of meningiomas in this treated population with a delay in development of about 35 years.[37]

In addition the incidence of meningiomas in the population of Hiroshima and Nagasaki was abnormally high with a similar delay in presentation.[38] There is also an increased incidence of meningiomas post high dose therapeutic

cranial irradiation but with these doses the delay in presentation is only about five years.[39]

1.2.1.c.i Progesterone and oestrogen receptors

Given the increased incidence in post menopausal women and the observation of rapid growth of meningiomas in pregnancy (first documented by Harvey Cushing in 1929) there has been a longstanding link with sex steroids and meningiomas.[40] Growth has also been shown to accelerate during the luteal phase of the menstrual cycle.[41] Female preponderance is described in most series[36] and a significant association between meningiomas and breast cancer has been reported.[42]

It has been recognised for many years that some meningiomas do express sex steroid receptors.[43-59] The overall findings of these studies are that generally a proportion of meningiomas express progesterone receptors but they rarely express oestrogen receptors. Whilst this has been well documented in many series, the prognostic significance of the receptors has been less well studied.

Several papers have shown association with grade 2 and 3 meningiomas and progesterone receptor negativity.[57, 60]

A paper in 1995 was the first to show a correlation between steroid receptors and proliferative index using the MIB-1 antibody to Ki-67. The tumours that were progesterone receptor negative had a significantly higher proliferative index (6.53% +/- 4.3%) compared to progesterone positive tumours (2.35% +/-2.12%).[61] A further paper in 1997 confirmed this

finding of higher proliferative index in progesterone negative tumour spheroids and monolayer cultures.[62]

Another paper in 1997 from 70 selected patients with a variety of grade 1 to 3 meningiomas showed that absence of progesterone receptors, high mitotic index and higher tumour grade were significant factors for shorter disease free interval. The significance of these factors was maintained in a multivariable Cox proportional hazards model but the relative contribution of each to the overall model was not indicated. In this study they found, like others previously,[47, 56] that males were more likely to be progesterone receptor negative, but gender in itself was not a significant prognostic factor for tumour recurrence.

Papers from 2000 and 2007 showed that for grade 1 meningiomas, lack of expression of progesterone receptors was a significant prognostic indicator for higher tumour recurrence.[63, 64] In the earlier paper there was a low incidence of atypical tumours with 60 being grade 1 out of an unselected series of 62 patients.[63] In the second paper the cohort of 100 patients was very highly selected to be grade 1 tumours that had been completely excised. In their series they had a very high recurrence rate for completely excised grade 1 tumours of 50%.[64] In these grade 1 patients, they did find a significant correlation with mitoses per ten high powered field and both recurrence and progesterone receptor status.

In 2006 a study of 239 patients with meningiomas found that progesterone receptor negative tumours were associated with an accumulation of

qualitative and quantitative karyotype abnormalities and an increasing potential for aggressive clinical behaviour, progression, and recurrence of these lesions.[65]

Most recently a study on 31 meningioma patients found an association with progesterone receptor negativity and variations in gene expression using array analysis.[66] This was particularly true for genes on the long are of chromosome 22 near the *NF2* gene locus.

1.2.1.d Molecular pathology

Meningiomas are generally spontaneous but there is a strong association with neurofibromatosis type 2 (NF2). The majority of NF2 patients will develop meningiomas and approximately 60% of spontaneous tumours will have mutations of the *NF2* gene on chromosome 22q.[67] The *NF2* gene product, the protein merlin (schwannomin) belongs to the 4.1 family of proteins that links the cytoskeleton to proteins of the cytoplasmic membrane. Some meningiomas have been shown to be associated with loss of expression of other 4.1 family proteins e.g. 4.1B (DAL-1) and 4.1R.[68]

1.2.1.e Histopathology

The histological appearance of meningiomas is very variable. Broadly they fall into the World Health Organisation (WHO) grades 1, 2 and 3 representing benign, atypical and anaplastic lesions respectively.[69]

85-90% of lesions are WHO grade 1 benign lesion and are split into 9 variants: meningothelial (Figure 1.2), fibrous/fibroblastic, transitional

(mixed), psammomatous, angiomatous, microcystic, secretory lymphoplasmacyte-rich and metaplastic.

There are three variants of grade 2 lesions. The most common are atypical which are defined as tumours having 4 or more mitoses per high powered field or three or more of the following features: increased cellularity, presence of small cells with high nuclear/cytoplasmic ratios, prominent nucleoli, pattern-less growth or foci of necrosis. Meningiomas that show brain invasion but otherwise appear benign are known to act in a more aggressive manner and are therefore classified as grade 2 lesions.

The other grade 2 lesions are the less common clear cell and chordoid variants.

WHO grade 3 or anaplastic lesions show a more aggressive and invasive phenotype. There are again 3 variants: the most common are anaplastic meningiomas which show an obvious malignant morphology with 20 or more mitoses per high powered field and often significant brain invasion. The other rarer grade 3 variants are rhabdoid and papillary.

Immunohistochemically meningiomas typically are positive for vimentin and epithelial membrane antigen although the latter can sometimes be somewhat patchy. The Ki-67 expression is generally low in grade 1 meningiomas (<5%) and is higher in grade 2 and 3 lesions but these levels are variable and have not been made part of diagnostic criteria. Secretory meningiomas do show expression of cytokeratins and carcinoembryonic antigen (CEA). Immunoreactivity for S-100 or CD-34 has been shown in

some cases but is less prevalent than it is in schwannomas and other fibrous tumours.

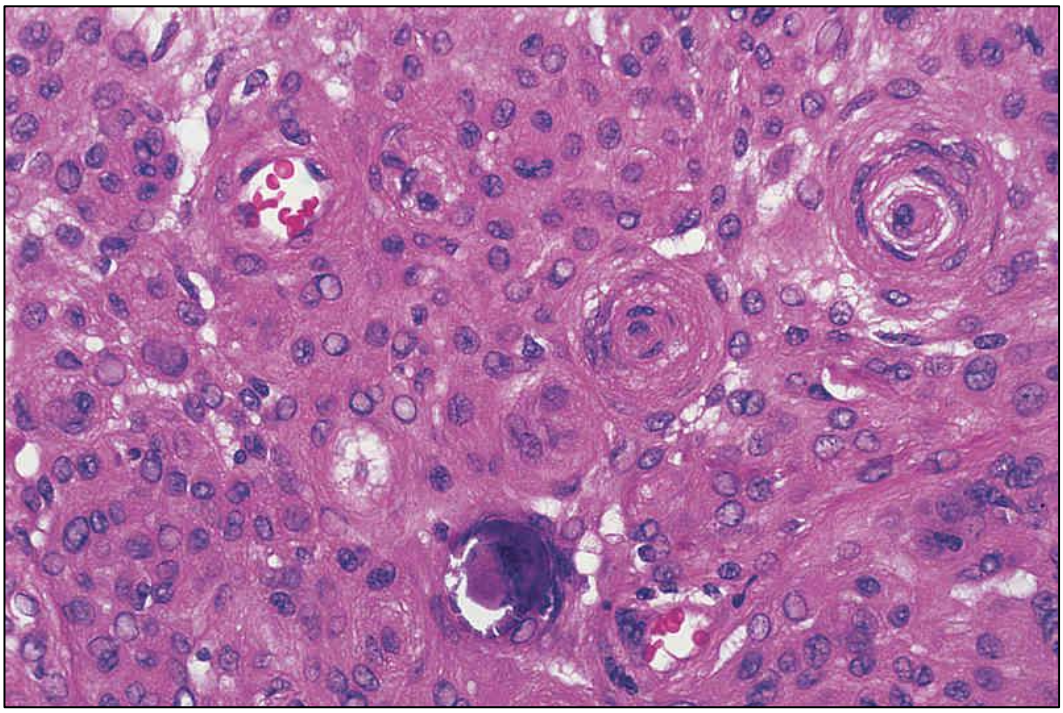


Figure 1.2 – Histology of a Meningioma. Indistinct cytoplasmic boundaries, nuclear clearing ('pseudoinclusions'), cellular whorls, and a psammoma body are all apparent in this view of a meningotheliomatous (syncytial) meningioma. (Image courtesy of PathConsult - http://www.pathconsultddx.com)[70]

1.2.1.f Macroscopic appearance and localisation

The majority of meningiomas are intracranial with some spinal and intraorbital. They are generally durally based and are typically found in a variety of locations such as convexity, parafalxine, sphenoid wing, olfactory groove, petrous ridge, tentorial, posterior fossa, parasellar and optic nerve sheath. Intraventricular meningiomas do occur as there are meningothelial cells present in the choroid plexus and tela choroidea.[35]

On magnetic resonance imaging they generally appear as a uniformly enhancing, durally based lesion (Figure 1.3). There would generally be an

attachment to the dura with a tail sign at the edge of the lesion. Meningiomas can invade into overlying bone and it is common to see a reactive hyperostosis in those circumstances.

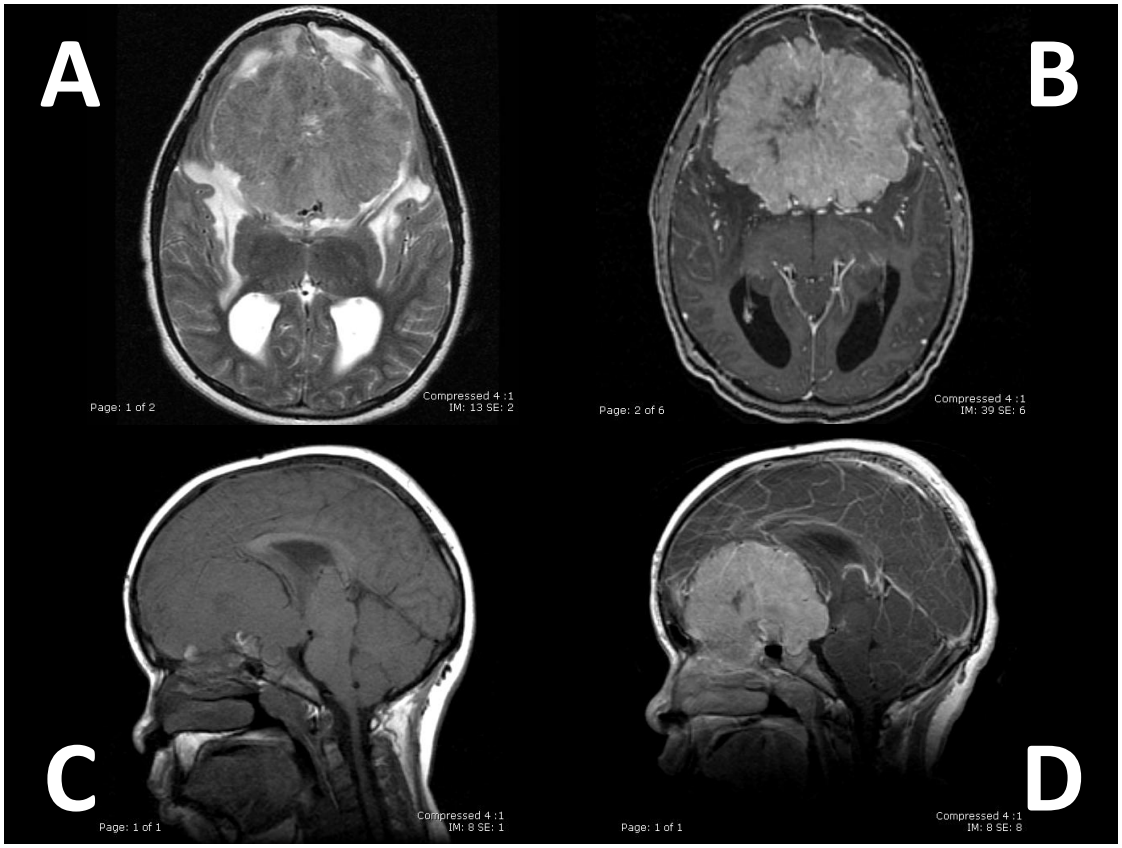


Figure 1.3 – MR Scan of patient with a large olfactory groove meningioma. A – axial T2, B – post contrast axial T1, C – sagittal T1 and D – sagittal T1 post contrast MR scans showing a large, brightly enhancing, extra-axial lesion arising from the floor of the anterior fossa.

1.2.1.g Treatment

Given the relatively high prevalence of meningiomas in the aging population, treatment would generally be given only for symptomatic or growing lesions.

1.2.1.g.i Surgery

For the majority of patients surgical resection would be the treatment of choice. The location of the lesion is critical to determine the surgical approach and pre-operative surgical goal.

It has been recognised for many years that the more complete the resection, the less likely of recurrence of a meningioma. This was first formally assessed by Simpson in 1957 who described a grading system with a score of 1 to 5 depending upon the extent of resection (Table 1.2).[71]

Simpson Grade	Description	Cases	Recurrences
I	Complete removal of tumour and dural attachment	90	8 (8.9%)
II	Complete removal of tumour with coagulation to dural attachment	114	14 (15.8%)
III	Complete removal of tumour without removal or coagulation of dural attachment	24	7 (29.2%)
IV	Partial removal leaving intradural tumour in situ	51	20 (39.2%)
V	Simple decompression with or without biopsy	9	8 (88.9%)

Table 1.2 – The original Simpson grade descriptions and rates of recurrence.[71]

1.2.1.g.ii Radiotherapy/radiosurgery

It would be usual to consider conventional radiotherapy post surgery for any tumours that were anaplastic or if there had been a partial resection of an atypical tumour. Furthermore radiotherapy is a treatment option as a primary treatment in patients where surgery would be high risk. This would usually halt the progression of a growing tumour with reports of 95% tumour control at 5 years.[72] Radiosurgery is a treatment option for smaller, surgically inaccessible or high risk tumours with control rates of 85%-97% at 5 years.[72]

1.2.1.g.iii Endovascular embolisation

There is an option to attempt pre-operative embolisation to reduce haemorrhage during a resection. Meningiomas tend to have a very rich blood

supply and there is the potential for significant, uncontrollable haemorrhage. Therefore the practice of pre-operative embolisation has become more established in recent years. Generally, however, the bulk of the blood supply to the lesion is taken away during the approach to the lesion and embolisation should be restricted to larger or less accessible lesions as it is not without complication as a procedure.[73, 74]

1.2.1.g.iv Chemotherapy

Generally there is no role for chemotherapy for meningiomas, even for anaplastic variants.[75, 76]

There has been a hope for some time that because of the presence of progesterone and also dopamine receptors in meningiomas that there may be the possibility of hormonally manipulating the tumours but as yet there is no proven agent that has achieved this.[77] Hydroxyurea has been shown to have a therapeutic effect in some patients but there is no good phase III trial confirming this observation.[78, 79]

1.2.1.h Prognosis

As mentioned above tumour grade, location and extent of surgical resection have all been shown to have an influence on recurrence. It is not clear from the literature, which of these factors has the most influence on an individual's chance of their tumour recurring.[35]

1.2.2 Choroid plexus carcinomas

1.2.2.a Definition

Choroid plexus carcinomas are rare tumours of neuroectodermal origin. They have a papillary pattern that resembles normal choroid plexus.

1.2.2.b Epidemiology

Together with choroid plexus papillomas they represent approximately 0.5% of all brain tumours with an annual incidence of 0.3 cases per million.[80, 81] However, choroid plexus carcinomas are mainly seen in childhood and account for approximately 3% of paediatric brain tumours overall[81, 82] and up to 20% of brain tumours presenting in the first year of life.[83] Choroid plexus carcinomas represent 37% of choroid plexus tumours.[81] The overall median age at diagnosis is 3.5 years.[81]

1.2.2.c Aetiology

There is no known environmental cause for choroid plexus carcinomas.[35] The majority of tumours are spontaneous but there are recognised genetic associations with the development of choroid plexus tumours.

1.2.2.d Molecular pathology

There are both germline and somatic abnormalities at several genetic loci associated with the development of these tumours.

1.2.2.d.i *TP53* Gene

The *TP53* gene is located on chromosome 17p13.1 and expresses the protein product p53 which has an influence on tumour suppression via a variety of mechanisms including DNA repair, apoptosis, cellular differentiation and angiogenesis.[84] Mutation of the gene causes loss of

function of p53 together with prolongation of the half life of the protein. This means that increased immunohistochemical staining for p53 protein can be used as a surrogate marker of gene mutation.[85]

Choroid plexus carcinomas are one of the tumours found in Li Fraumeni families with *TP53* germline mutations.[86-89] In addition spontaneous germline and somatic p53 mutations are both described in patients with choroid plexus carcinoma.[90] Positive nuclear staining for p53 protein is evident in the majority of choroid plexus carcinomas (10 of 11) whilst it is only rarely seen in choroid plexus papillomas (1 of 12).[91] *TP53* mutations have not been extensively studied in choroid plexus papillomas however germline mutations have been reported.[92, 93]

1.2.2.d.ii *hSNF5/INI1* Gene

The *hSNF5/INI1* gene is located on chromosome 22q11.2 and encodes a member of the SWI/SNF ATP-dependant chromatin-remodelling complex.[94] Germline mutations of the gene have been described as rhabdoid predisposition syndrome and families with the mutation develop both renal and extra-renal malignant rhabdoid tumours, choroid plexus carcinomas, atypical teratoid rhabdoid tumours (AT/RT) and medulloblastomas.[94, 95] Somatic mutations of the *hSNF5/INI1* gene have also been reported in choroid plexus carcinoma.[90] There are several studies describing the genotypic and phenotypic overlap between choroid plexus carcinomas and AT/RT.[96, 97] However, it has been reported that the majority of choroid plexus carcinomas have preserved hSNF5/INI1 protein expression on immunohistochemistry.[98, 99] It is therefore suggested that tumours described

as being choroid plexus carcinoma with *hSNF5/INI1* mutations may actually be AT/RTs.[98]

There is no evidence of *hSNF5/INI1* point mutations in choroid plexus papilloma.[92]

1.2.2.e Histopathology

Generally choroid plexus tumours show a cellular architecture that resembles normal choroid plexus.[35] There is a single layer of well-differentiated cuboidal epithelium around a fibro-vascular stalk. There are finger like projections that gives the typical papillary appearance. There is some controversy in the histological classification of choroid plexus tumours. For benign WHO grade 1 tumours (Figure 1.4) there should be a lack of malignant features and less than 1 mitosis per ten high powered fields. The average MIB-1 labelling index is 3.7%.[100]

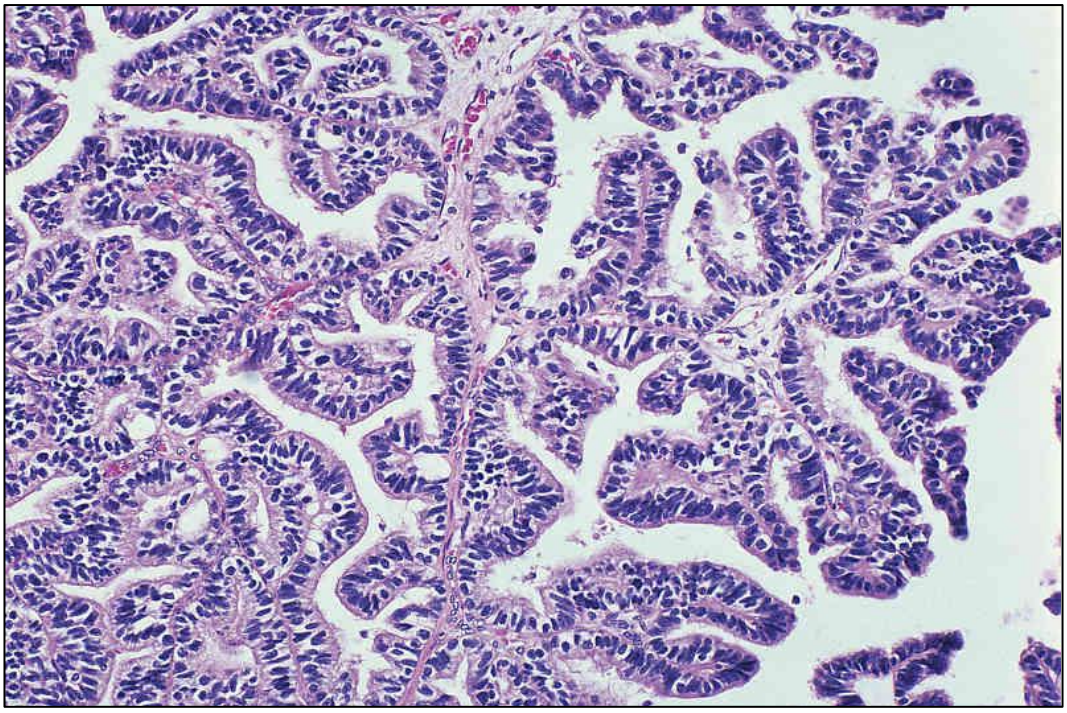


Figure 1.4 – Histology of a Choroid Plexus Papilloma. This example's delicate fibrovascular fronds are covered by an orderly, low columnar epithelium. (Image courtesy of PathConsult - http://www.pathconsultddx.com)[70]

The World Health Organisation recently recognised atypical choroid plexus tumours (WHO grade 2) as lesions showing increased cellularity, nuclear pleomorphism and particularly increased mitotic activity with two or more mitoses per ten high powered fields.[101]

Choroid plexus carcinomas are classified as grade 3 tumours by the World Health Organisation. Typically the features seen in a choroid plexus carcinoma would be cellular anaplasia, loss of papillary architecture, nuclear pleomorphism, necrosis and giant cell formation but often these features do not correlate with biological behaviour of an individual tumour (Figure 1.5).[102] One particular area of controversy is the classification of a lesion as a choroid plexus carcinoma based purely upon evidence of local brain invasion.[103] It was felt for many years that this was a prime differentiating

feature of carcinomas but there have now been lesions identified with invasion and otherwise benign histological appearance that do act in a biologically benign way.[104] The mean MIB-1 index for choroid plexus carcinomas is 14%.[100]

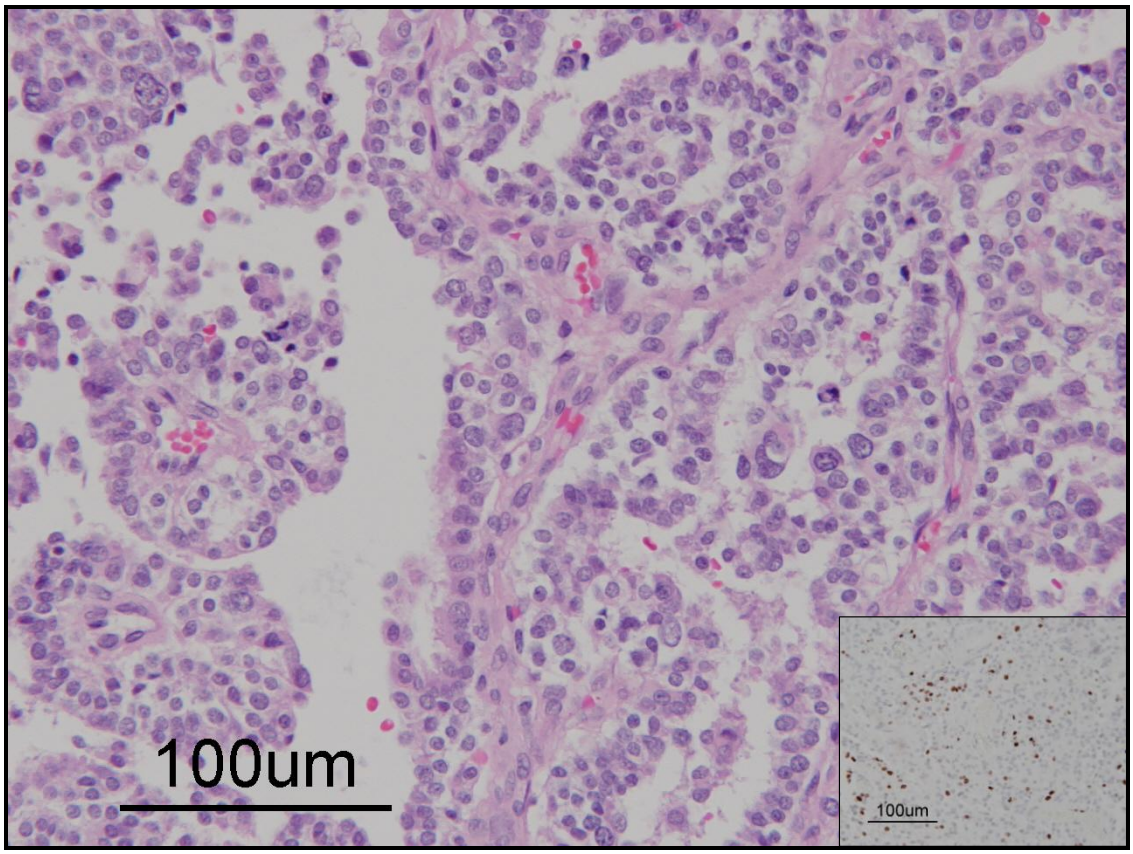


Figure 1.5 – Histology of a choroid plexus carcinoma. This example, compared to the choroid plexus papilloma, shows cellular anaplasia, loss of papillary architecture and nuclear pleomorphism. MIB-1 immunostaining (insert) shows a high proliferative index. (Image courtesy of Dr William Halliday, Hospital for Sick Children, Toronto)

It is a recognised phenomenon that choroid plexus papillomas can progress histologically to have a carcinoma phenotype.[105]

1.2.2.f Macroscopic appearance and localisation

There does not appear to be any difference in localisation of choroid plexus papillomas and carcinomas. In children approximately 75% of all choroid plexus tumours are located in the lateral ventricles (Figure 1.6). As the location becomes more caudal, the median age at presentation increases being 1.5 years in the lateral ventricles, 1.5 years in the third ventricle, 22.5 years in the fourth ventricle and 35.5 years in the cerebello-pontine angle.[81] The presence of metastases at presentation is recognized in 12% of patients being more common in choroid plexus carcinomas.[81]

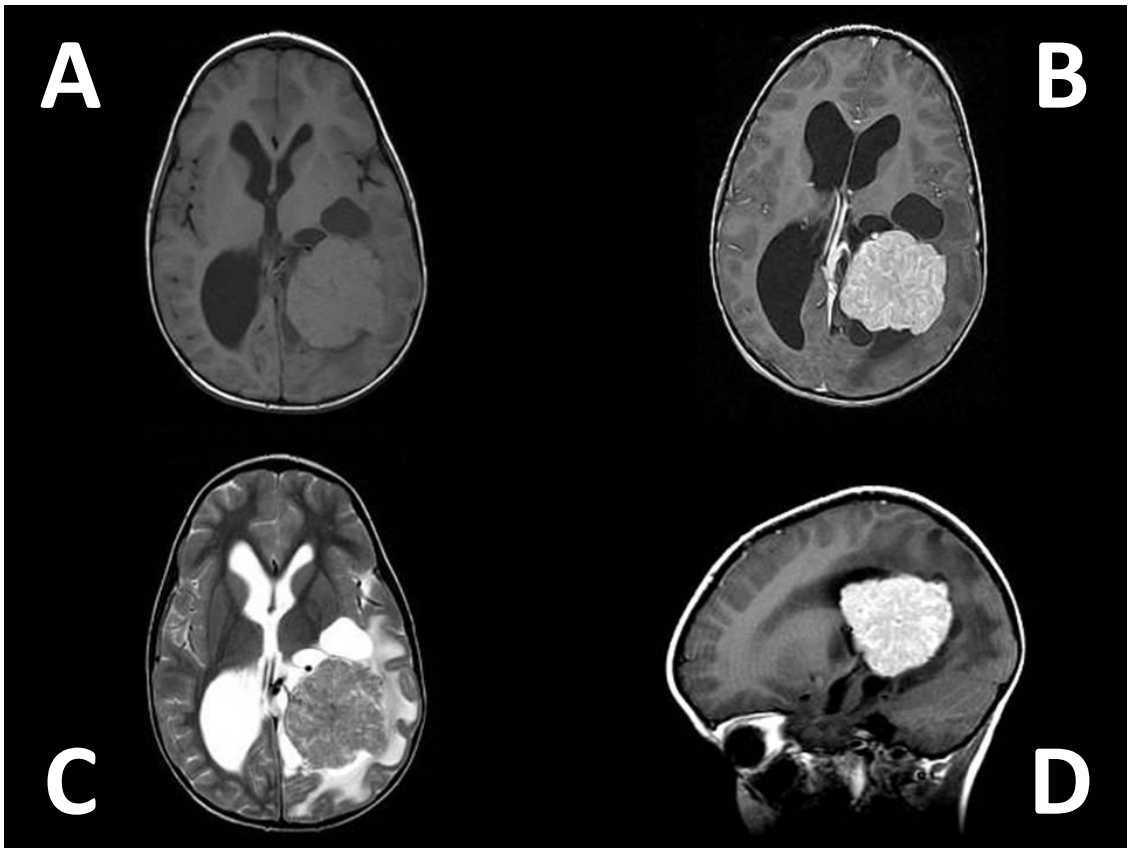


Figure 1.6 – MR scan of a patient with a choroid plexus carcinoma. A – axial T1, B – axial T1 post contrast, C – axial T2 and D – sagittal T1 post contrast images showing a brightly enhancing lesion in the left lateral ventricle with evidence of brain invasion.

1.2.2.g Treatment

1.2.2.g.i Surgery

In the available meta-analyses of choroid plexus carcinoma the primary prognostic factor is the extent of surgical resection with 2 year survival rates of 72% (+/- 10%) for gross total resection vs. 34% (+/- 10%) with incomplete resection.[81] However, given the increased vascularity, large size of these tumours and median age of presentation gross total resection can be difficult and is achieved in only 33% - 64%[81, 106-108] of cases in the literature. For incomplete resections second surgery should be considered as it has been shown to improve survival (2-year overall survival 69% versus 30% with no second surgery).[106]

1.2.2.g.ii Radiotherapy

Results of adjuvant therapy for residual disease are mixed. Radiotherapy is a limited option in choroid plexus carcinomas because the neurocognitive and endocrine sequelae would be marked given the young age at presentation of the majority of patients. A survival advantage has been shown following radiotherapy for patients with both gross total resection and residual disease.[81, 109, 110] However, the groups in these studies were not matched for age which is a potential independent prognostic factor.[81]

1.2.2.g.iii Chemotherapy

With only small case series and a limited number of systematic studies in the literature, determining the benefit of chemotherapy as adjuvant therapy has been restricted. Studies utilising a variety of protocols have demonstrated residual tumour control in a proportion of patients.[81, 111-113] A recent meta-

analysis has however shown a survival advantage in choroid plexus carcinoma patients.[110] This advantage was maintained irrespective of whether radiotherapy was given or if a gross total resection was achieved.[110]

Given the definite advantage of gross total resection, chemotherapy has been used by a variety of institutions to either reduce the size or more importantly the vascularity of choroid plexus carcinomas in order to facilitate second look surgery.[103, 112, 114, 115] Although these reports qualitatively describe a benefit to this approach the extent of the effect and impact on patient outcome has not been determined.

1.2.2.h Prognosis

As mentioned above the ability to surgically achieve a gross total resection appears to be the most significant factor in outcome for choroid plexus carcinomas.[81] Age at presentation is also felt to be an independent prognostic factor in addition to the more difficult prospect of achieving a complete surgical resection in these younger children. Strong evidence for adjuvant therapies is difficult to attain given the small numbers of this rare tumour presenting to individual units each year. As it stands the evidence would suggest survival advantage for both radiotherapy[109] and chemotherapy[110] following best surgical resection. Overall one, five, and ten year projected survival rates of choroid plexus carcinoma patients are 71, 41 and 35% respectively.[81]

1.2.3 Low grade astrocytomas

1.2.3.a Definition

Astrocytomas account for approximately 60% of all glial neoplasms.[35] The World Health Organisation categorises these tumours as low grade (WHO grades 1 and 2) and high grade (WHO grade 3 and 4).[69] Within the low grade tumours there are two major categories, the more common diffuse infiltrating lesions and the less common circumscribed lesion. The latter circumscribed lesions are generally seen in children and young adults and have a more favourable outcome.

1.2.3.b Epidemiology

Low grade astrocytomas account for 15% of adult and 25% of children's brain tumours. Age of presentation is biphasic with a childhood peak at 6-12 years and adult peak between the third and fifth decades with a median age of 35 years.[35] Overall incidence of diffuse low grade gliomas is 0.09 (95% CI 0.08-0.10) per 100,000.[36]

The most common form of circumscribed astrocytomas are pilocytic astrocytomas (WHO grade 1) which form 10-15% of all paediatric brain tumours and over 25% of posterior fossa tumours in children.[35] The annual incidence of pilocytic astrocytomas is 0.34 (95% CI 0.32-0.36) per 100,000.[36]

1.2.3.c Aetiology

Apart from ionizing radiation, which is the cause for a very limited number of brain tumours, there are no convincing environmental aetiological factors identified for patients with astrocytomas.[116] Within low grade astrocytomas

there is a recognised strong association with neurofibromatosis type 1 and the development of pilocytic astrocytomas, particularly tumours of the optic pathway/hypothalamus.[117]

1.2.3.d Molecular pathology

The only genetic alteration consistently associated with low grade gliomas is mutation of the *p53* gene.[118] The location of the *p53* gene is on 17p13.1 an area often deleted in astrocytomas of all grades and loss of heterozygocity of chromosome 17p is present in 50-60% of diffuse astrocytomas and up to 80% of gemistocytic tumours.[35] Increased expression of the platelet derived growth factor receptor alpha and its ligand PDGFα is also common particularly in tumours with loss of heterozygocity on chromosome 17p.[119] Mutations affecting codon 132 of the isocitrate dehydrogenase 1 gene (*IDH1*) are found in more that 60% of diffuse astrocytomas.[120] In contrast to oligodendrogliomas, combined loss of heterozygocity on chromosomes 1p and 19q is rare in diffuse astrocytomas.[121]

Pilocytic astrocytomas commonly carry either duplications of the *BRAF* oncogene on chromosome 7q34 or more rarely *BRAF* activating mutations. This leads to increased mitogen-activated kinase signaling.[122] Pilocytic astrocytomas in neurofibromatosis type 1 patients commonly carry allelic losses at the *NF1* gene locus on 17q11.2. This is rarely seen in spontaneous tumours.[123]

In comparison to diffuse astrocytomas, most pilocytic astrocytomas do not show allelic loss on chromosome 17p or mutations of the *p53* or *IDH1* genes.

1.2.3.e Histopathology

Microscopy shows a well differentiated tumour of astrocytic lineage with low to moderate cellularity and without necrosis, microvascular proliferation or high mitotic activity.[35] There are three histological subtypes of diffuse astrocytoma and four subtypes of circumscribed tumours.

1.2.3.e.i Fibrillary astrocytomas

This is the most common type of diffuse astrocytoma (Figure 1.7) and is composed of multipolar neoplastic astrocytes with scant cytoplasm and fine cell processes that go to build up a fibre-rich glial matrix.

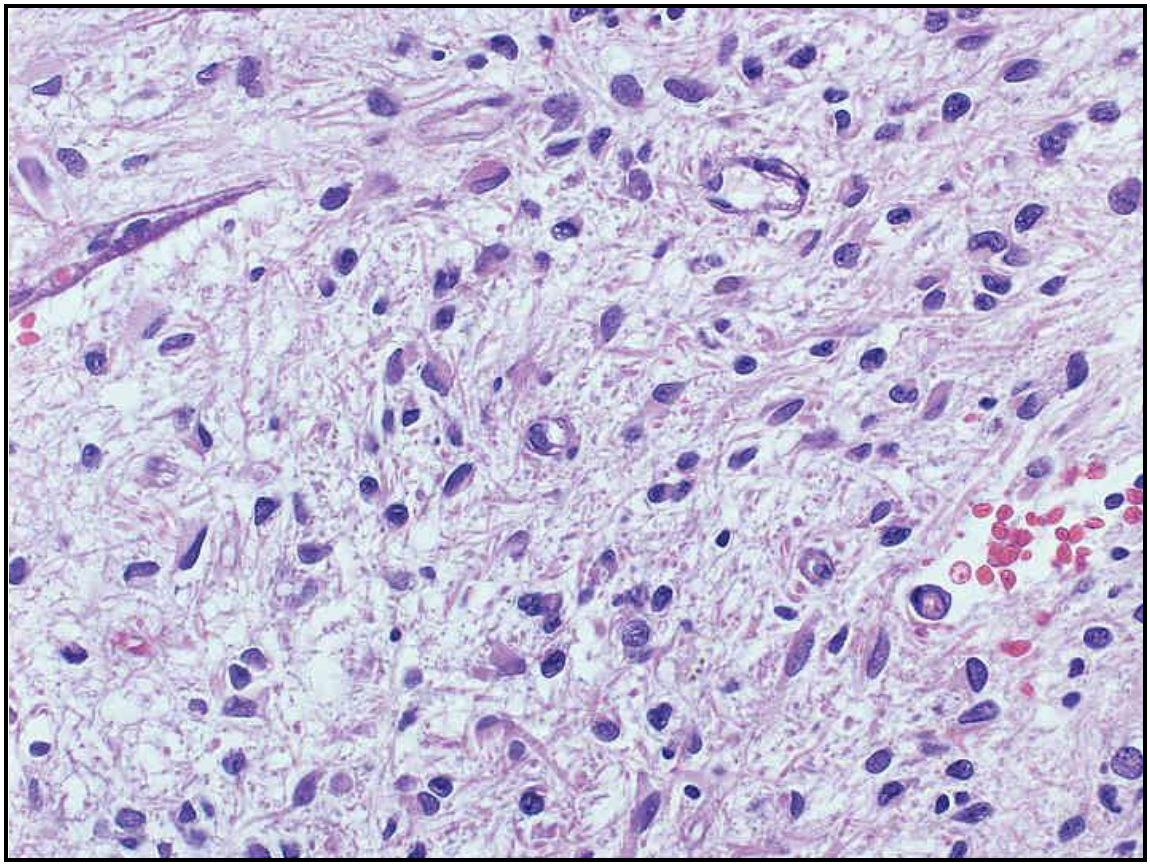


Figure 1.7 – Histology of a diffuse fibrillary astrocytoma. Conspicuous cytoplasmic processes, mild nuclear pleomorphism, and only modest hyperchromasia are evidenced by the cells of this well-differentiated astrocytoma. The absence of mitotic activity supports its classification as a low-grade lesion. Note the dyscohesive growth pattern. (Image courtesy of PathConsult - http://www.pathconsultddx.com)[70]

1.2.3.e.ii Gemistocytic astrocytoma

Gemistocytes are cells with an enlarged eosinophilic cytoplasm, eccentric nucleus and stout processes (Figure 1.8). To make the diagnosis of a gemistocytic astrocytoma there needs to be greater than 20% of the cells evident demonstrating the gemistocytic phenotype. It was previously thought that this subtype had a worse prognosis and be more considered a grade 3 rather than its actual grade 2 classification. However in recent years the evidence for this has been questioned.[124]

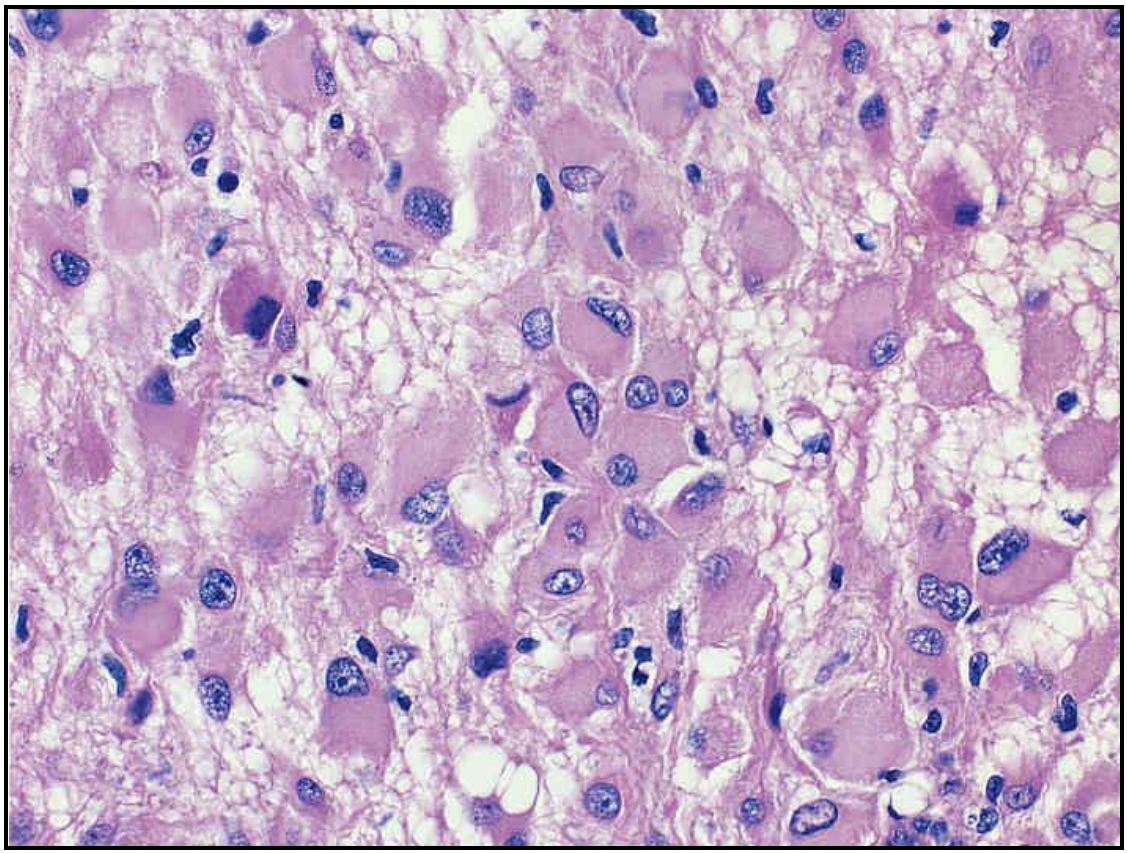


Figure 1.8 – Histology of a gemistocytic astrocytoma. Gemistocytic cells are plump with glassy pink cytoplasm. Radially arranged gemistocytes project short cytoplasmic processes towards centring blood vessels to form pseudorosettes. (Image courtesy of PathConsult - http://www.pathconsultddx.com)[70]

1.2.3.e.iii Protoplasmic astrocytoma

This is a rare variant of diffuse astrocytoma, composed of tumour cells with eosinophilic cytoplasm and a few flaccid processes embedded in a microcystic or mucoid matrix (Figure 1.9). There is a suggestion in the sparse literature that they may favour younger male patients with a fronto-temporal location and similar biological behaviour to fibrillary variants.[125]

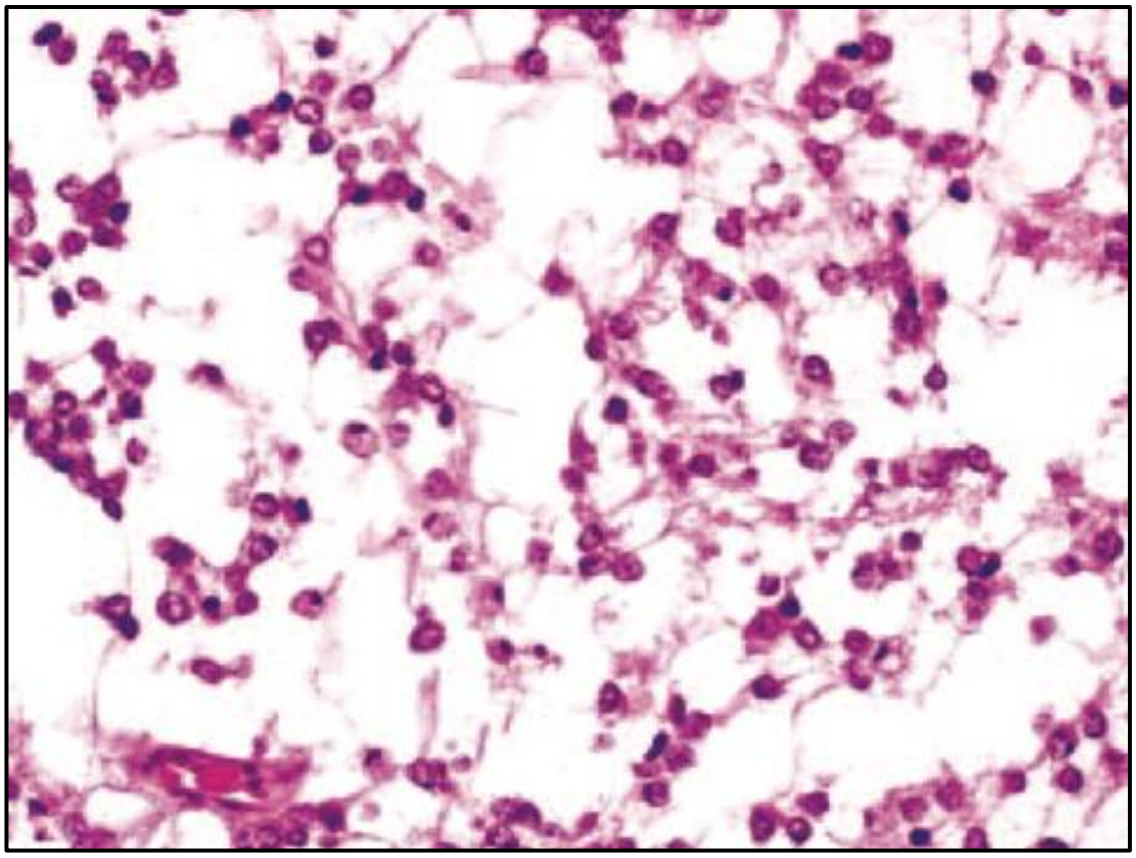


Figure 1.9 – Histology of a protoplasmic astrocytoma. The features consist of a relatively homogenous, small astrocytic cell population with short, delicate processes. (Image courtesy of PathConsult - http://www.pathconsultddx.com)[70]

1.2.3.e.iv Pilocytic astrocytoma

This is the most common variant of circumscribed low grade astrocytomas. The histological appearance is one of low to moderate cellularity with a biphasic pattern consisting of areas with bipolar (piloid) cells and multipolar (microcystic) tumour cells (Figure 1.10).[35] An important diagnostic feature of pilocytic astrocytomas is the presence of Rosenthal fibres. These are intracytoplasmic eosinophilic hyaline masses that are characteristic but not pathognomonic of pilocytic astrocytomas as they are also seen in gliosis and Alexander's disease.

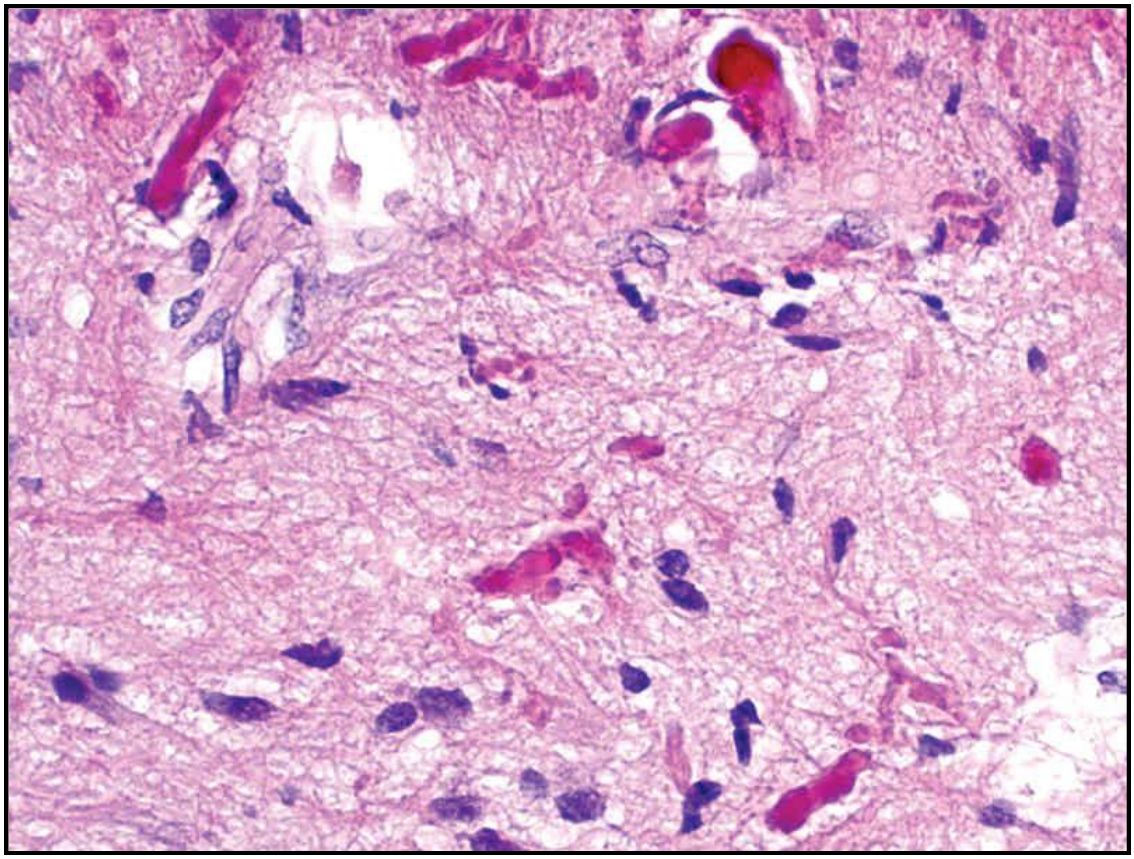


Figure 1.10 – Histology of a pilocytic astrocytoma. The biphasic cellular populations and architecture of the classic pilocytic astrocytoma are in evidence. The lesion's process-bearing spindle cell (piloid) constituents fashion a densely fibrillar matrix, whereas its process-poor (protoplasmic) elements aggregate in regions of myxoid change that often progress to microcyst formation. (Image courtesy of PathConsult - http://www.pathconsultddx.com)[70]

1.2.3.e.v Pilomyxoid astrocytoma

These are a variant of pilocytic astrocytomas that were first described in 1999[126] and have recently been added to the World Health Organisation classification of CNS tumours.[69] They are characterised by a monomorphic population of bipolar neoplastic astrocytes on a background of a myxoid matrix (Figure 1.11). In contrast to pilocytic astrocytomas, Rosenthal fibres are often missing. They are typically found in optic chiasm/hypothalamic region and have a higher risk of local recurrence and dissemination compared to pilocytic astrocytomas.[126] The World Health Organisation therefore classifies them as a grade 2 tumour.

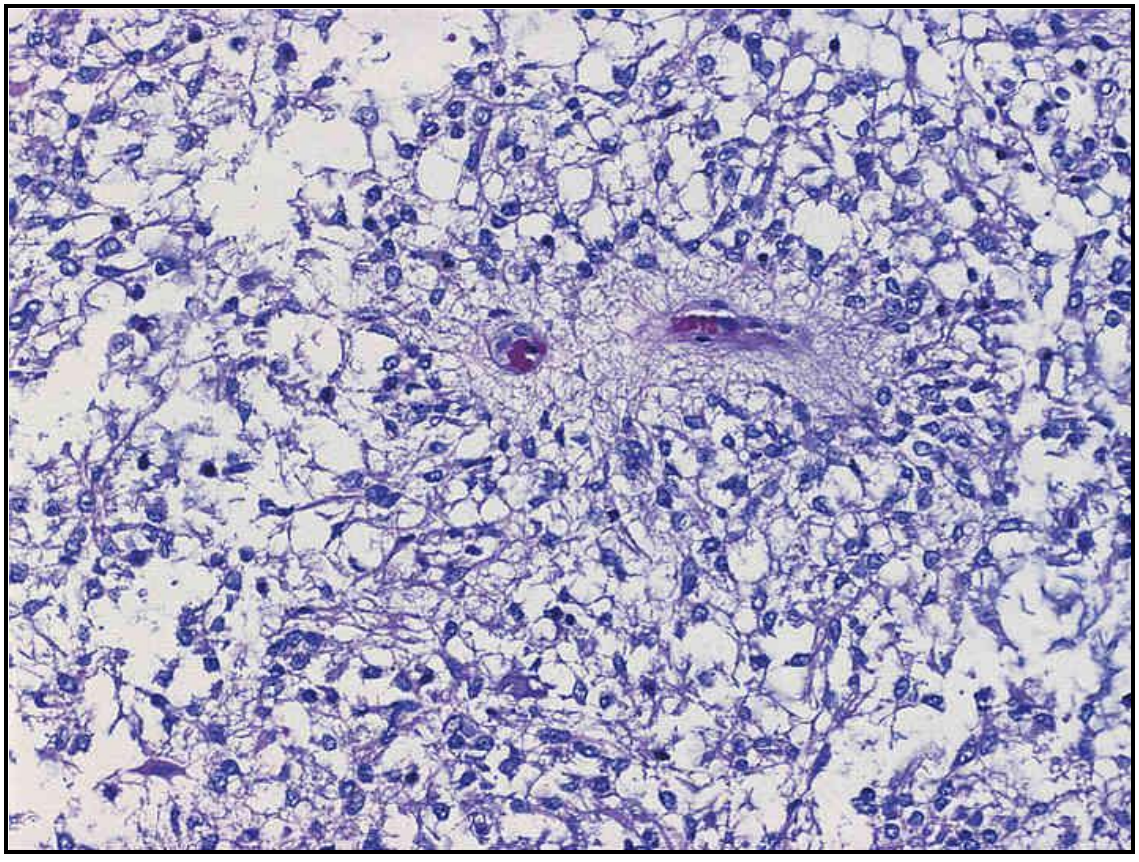


Figure 1.11 – Histology of a pilomyxoid astrocytoma. Spindled cytologic features, diffuse myxoid change, and focal perivascular pseudorosetting (centre top) characterize this emerging entity. (Image courtesy of PathConsult - http://www.pathconsultddx.com)[70]

1.2.3.e.vi Pleomorphic xanthoastrocytoma

These are another rare variety of generally circumscribed astrocytic tumours. They are often cystic and tend to occur peripherally in the cortex, extending into the leptomeninges with the temporal lobe being the most common location.[35] Histologically they show a fascicular growth pattern with the hallmark of bizarre, pleomorphic multinucleated giant cells (Figure 1.12). The tumours are classified as grade 2 by the World Health Organisation and have a reasonably favourable prognosis with a 10 year survival of greater than 70%.[127]

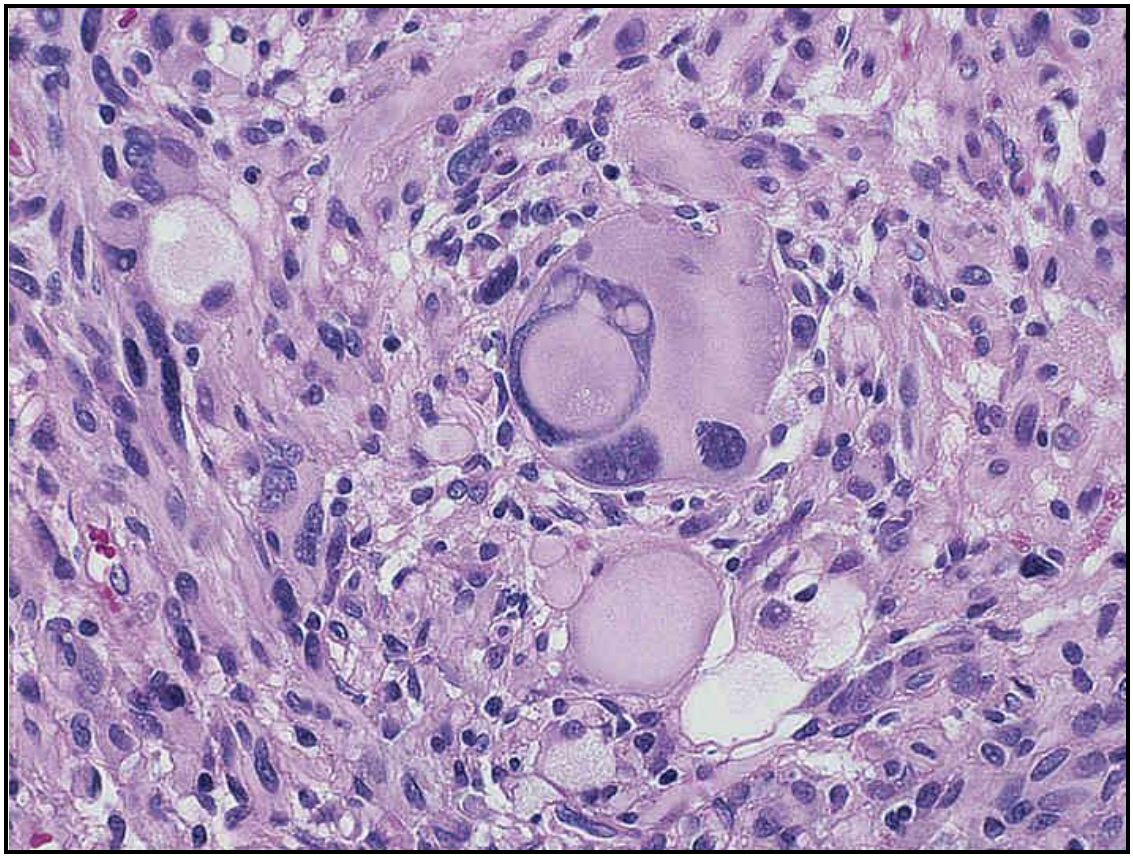


Figure 1.12 – Histology of a pleomorphic xanthoastrocytoma. Spindle and giant cells, including bizarre multinucleated forms, combine to give this relatively indolent neoplasm a most disturbing appearance. Note hyaline, granular, and vacuolar cytoplasmic alterations, the last attesting to lipid accumulation. (Image courtesy of PathConsult - http://www.pathconsultddx.com)[70]

1.2.3.e.vii Subependymal giant cell astrocytoma.

These are well circumscribed lesions typically arising from the ventricular wall at the foramen of Monroe. They are very closely associated with tuberous sclerosis although spontaneous cases have been described. It was initially thought that these cases were due to a forme fruste of tuberous sclerosis secondary to somatic mosaicism. More recently, cases have been described where a two hit loss of heterozygocity and mutation have been shown in one of the tuberous sclerosis genes only within the tumour itself ruling out a somatic moscaicism.[128]

Subependymal giant cell astrocytomas are moderately cellular tumours composed of pleomorphic large astrocytic cells with abundant glassy eosinophilic cytoplasm and round gangloid nuclei with distinct nucleoli (Figure 1.13).

Subependymal giant cell astrocytomas are classified as grade 1 tumours by the World Health Organisation and have a favourable clinical outcome. Recent studies have shown that the pathway activated in tuberous sclerosis involves the mammalian target of Rapamycin receptor and that Subependymal giant cell astrocytomas do respond to blocking of this pathway with Rapamycin therapy.[129]

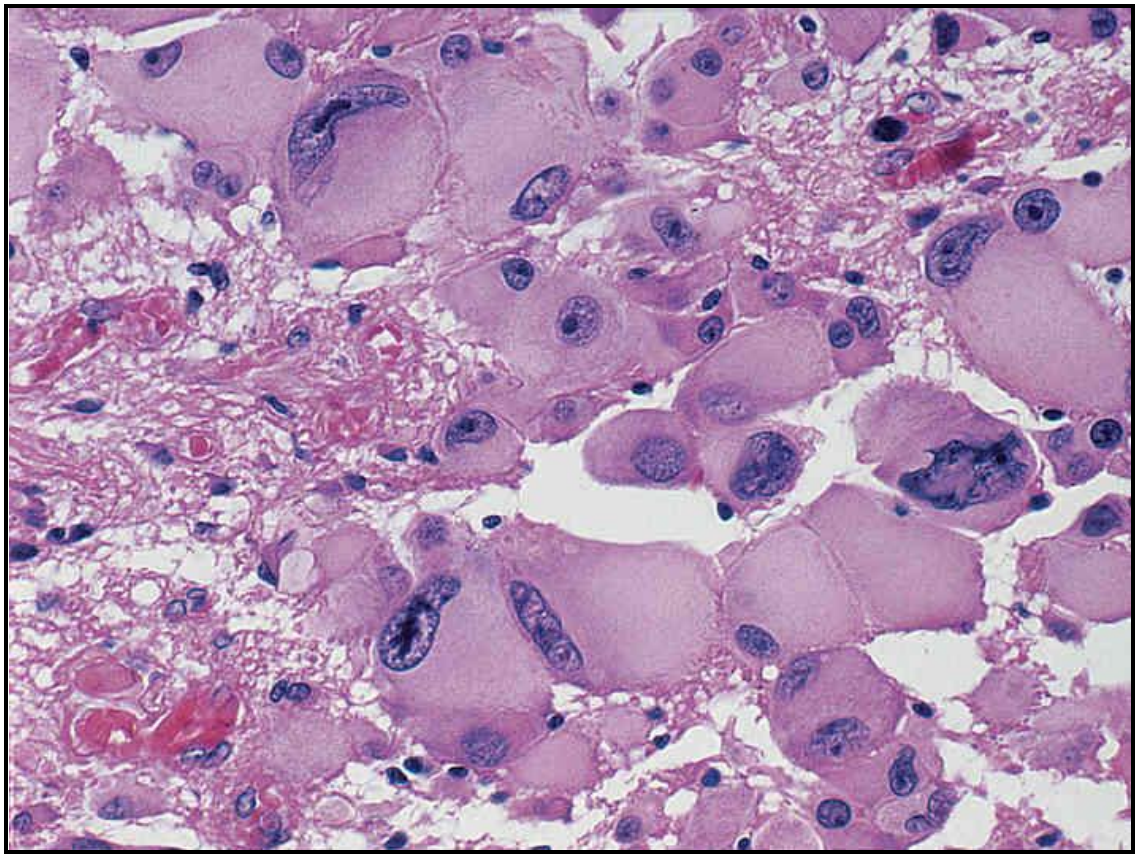


Figure 1.13 – Histology of a subependymal giant cell astrocytoma. Tumour cells that can achieve giant proportions, often polygonal in contour and closely apposed in lobular array, are responsible for this neoplasm's name (but not evident in all cases). (Image courtesy of PathConsult - http://www.pathconsultddx.com)[70]

1.2.3.f Macroscopic appearance and localisation

Diffuse astrocytomas predominantly grow in the cerebral hemispheres (Figure 1.14) although the brainstem is a recognised location in children. The lesions tend to be ill-defined masses that enlarge existing structures with blurred anatomical margins. There may be infiltration along white matter tracts and across the corpus callosum into the contralateral hemisphere.

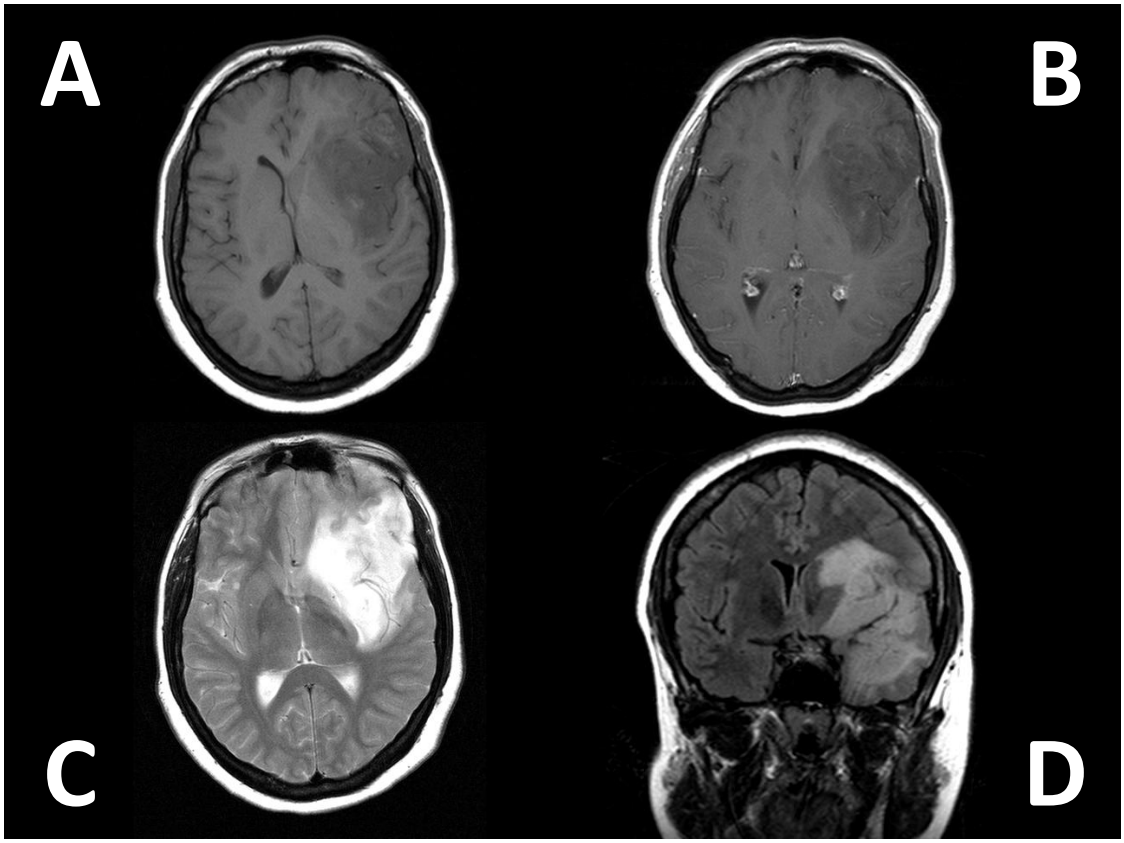


Figure 1.14 – MR scan of a patient with a grade II astrocytoma of the insular region. A – axial T1, B – axial T1 post contrast, C – axial T2 and D – coronal flair images showing an extensive left fronto-temporal lesion, low signal on T1 and high signal on T2 with no enhancement.

The posterior fossa is the most common location for pilocytic astrocytomas (Figure 1.15). They tend to be based in the cerebellar hemispheres and are

often cystic with a brightly enhancing mural nodule. The cyst wall may or may not itself enhance.[35]

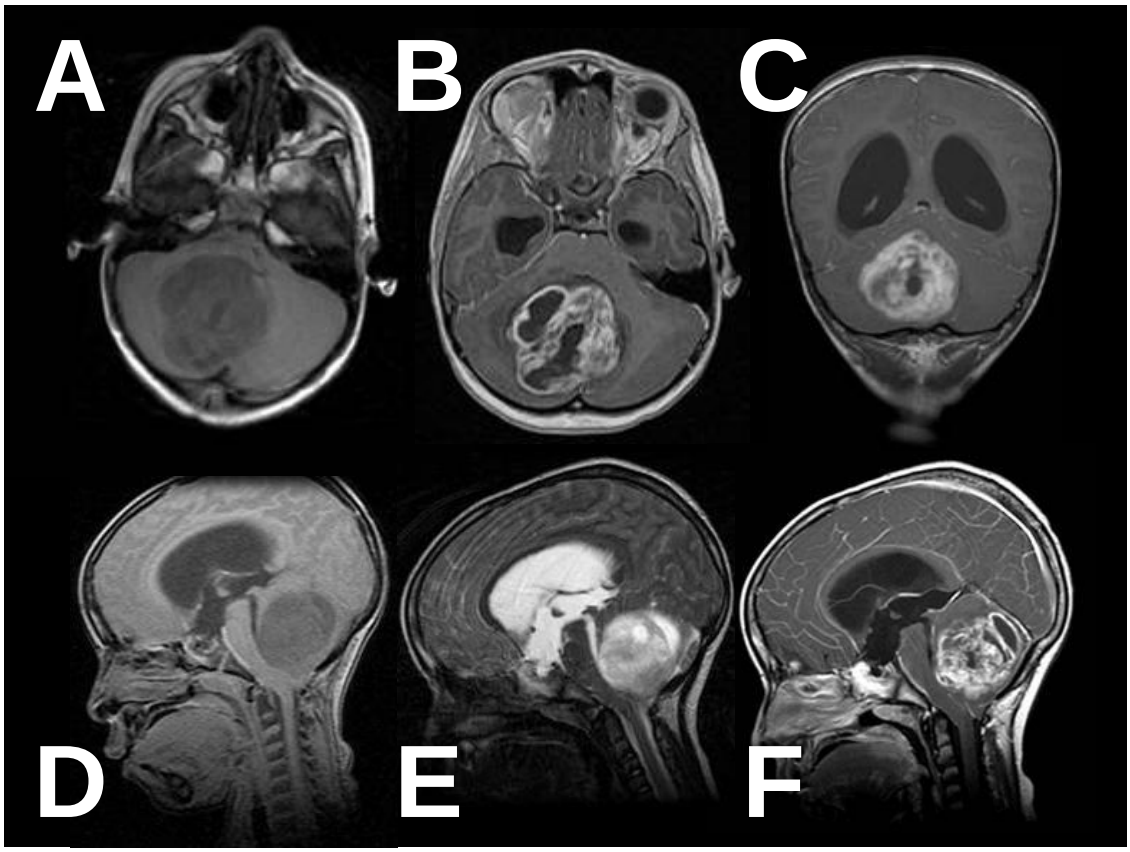


Figure 1.15 – MR scan of a patient with a pilocytic astrocytoma of the posterior fossa. A – axial T1, B – axial T1 post contrast, C – coronal T1 post contrast, D – sagittal T1, E – sagittal T2 and F – sagittal T1 post contrast images showing an enhancing, partly cystic lesion arising from the right cerebellar hemisphere.

1.2.3.g Treatment

1.2.3.g.i Observation

For low grade astrocytomas diagnosed without raised intracranial pressure or neurological deficit there is a management option to observe the patient, clinically and radiologically. There have been a variety of studies that have shown that a course of management such as this does not have any impact on overall survival.[130, 131] There is no doubt , however that undertaking

biopsies in patients with radiological low grade gliomas will show more aggressive histology a proportion of times. This, together with the psychological stress that the uncertainty of diagnosis may bring would advocate at least an initial biopsy at the time of presentation.[35] If during a period of observation there is evidence of growth or new enhancement within a tumour then this would generally mandate histological confirmation of the lesion with a biopsy/resection.

1.2.3.g.ii Surgery

Options for surgical intervention for patients with low grade astrocytomas include biopsy (either stereotactic or open) and open resection. Stereotactic biopsy of low grade astrocytomas is generally a safe, well tolerated procedure with morbidity and mortality rates less than 1%.[132] The issue with undertaking any form of biopsy is that a low grade lesion undergoing the start of malignant transformation would often be heterogeneous in histological appearance and there is therefore the possibility of being falsely reassured with a low grade result on biopsy. Therefore biopsies of multiple areas are advocated in these circumstances.[133]

The role of the extent of resection in diffuse low grade gliomas is a controversial topic. There are no randomised controlled trials examining this question and there are now unlikely ever to be such trials given the general lack of equipoise, limited numbers of patients and potential for long survival times.[35] There are many cohort studies that have tried to answer this question including more recent larger studies and systematic reviews.[134, 135] There is a general weighting of the evidence in favour of greater resection of

lesions to have a progression free and overall survival advantage. In a study of 216 patients from 2008, those with at least 90% extent of resection had 5- and 8-year overall survival rates of 97% and 91%, respectively, whereas patients with less than 90% extent of resection had 5- and 8-year OS rates of 76% and 60%, respectively. After adjusting each measure of tumour burden for age, Karnofsky performance score (KPS), tumour location, and tumour subtype, overall survival was predicted by extent of resection (hazard ratio = 0.972; 95% CI, 0.960 to 0.983; P < .001).[135] Various surgical adjuncts such as image guidance, intra-operative imaging and intra-operative monitoring are all utilised to try and achieve maximum surgical resection.[136-138]

For circumscribed low grade astrocytomas the surgical goal is to try and achieve a gross total resection. If this is accomplished for pilocytic astrocytomas then the 25 year survival rate is approximately 95%.

1.2.3.g.iii Radiotherapy

There have been several well conducted randomized controlled trials in this area of treatment. The dose response in two studies showed that lower dose treatment resulted in equivalent disease control with fewer complications to high dose treatments.[139, 140] As a result a standard dose of 45-50.4 Gy in 25-28 fractions of 1.8 Gy is accepted as standard practice in the UK and internationally.

In terms of the timing of radiotherapy in the disease course, another well conducted randomized controlled trial showed no benefit to overall survival

in patients receiving radiotherapy at the time of histological diagnosis compared with those receiving radiotherapy at the time of a malignant progression of their tumour.[141, 142] In this study there was an improvement in progression free survival from 3.4 to 4.8 years with initial radiotherapy at histological diagnosis, but this study generally supports an initial observational strategy in order to avoid earlier complications of radiation therapy in the disease course. However this approach is being readdressed with the availability of more advanced techniques for focally delivering radiotherapy that are now more generally available.[35]

Radiotherapy for circumscribed astrocytomas is generally used when there has been failure to control the disease surgically. In these circumstances there is reasonable expectation of local control with approximately 60% response at 10 years following radiotherapy.[143]

1.2.3.g.iv Chemotherapy

The most commonly used chemotherapy agents for diffuse low grade gliomas are temozolomide, procarbazine, CCNU and vincristine. The use of temozolomide is becoming the mainstay of current treatment with response rates from 31-61% and its use as an alternative to radiotherapy post surgical resection of low grade astrocytomas has been explored.[144, 145]

Chemotherapy for circumscribed astrocytomas would usually be reserved for disease that cannot be treated surgically where radiotherapy is contra-indicated, notably in the under 3 age group. For pilocytic astrocytomas the standard first line treatment internationally is vincristine and carboplatin with

radiographic response rates of 52% in patients with recurrent disease and 62% in newly diagnosed patients.[146-148]

1.2.3.h Prognosis

Malignant transformation is a well described phenomenon in low grade astrocytomas, with 13% to 86% of initially diagnosed low grade tumours reported to have recurred at a higher histological grade.[35] This means that although there is significant variation in the literature, most studies give a median survival for low grade astrocytomas of between 7 to 9 years.[149-153] This figure appears to have improved over recent decades. A study from Norway showed the median survival for low grade astrocytomas improved from the period 1970-1981 where it was 4.5 years (95% CI 3.4–5.7) to 9.1 years (95% CI 7.4–10.9) in the period 1982-1993. The most recent figures for survival for diffuse low grade astrocytomas are 1, 5 and 10 year survival rates of 73.5%, 46.9% and 37.7%.[36]

There are several clinical factors that have been shown to be associated with improved survival in low grade astrocytomas. These include age less than 40 at diagnosis, presence of seizures at diagnosis, absence of neurological deficits at diagnosis, Karnofsky Performance Score greater than or equal to 70 and mini mental state examination score greater than 26 out of 30.[140, 149, 154-159] In terms of tumour factors a pre-operative maximum diameter of greater than 5-6 cm and the presence of contrast enhancement are poor prognostic factors.[140, 149] The effect of surgical resection on survival is discussed above in section 1.2.3.f.ii.

For pilocytic astrocytomas gross total resection is the most important prognostic factor as discussed above. Subtotal resections do reduce progression free survival,[160, 161] however, there is a recognised incidence of tumour involution of between 14-45% with subtotal resections leaving small volumes of tumour.[162-164] Overall survival rates for pilocytic astrocytomas at 1, 5 and 10 years are 96.1%, 92.0% and 90.3%.[36]

1.2.4 Oligodendrogliomas

1.2.4.a Definition

Oligodendrogliomas are diffusely infiltrating, well differentiated gliomas that are preferentially located in the cerebral hemispheres of adult patients composed of neoplastic cells resembling oligodendrocytes.

1.2.4.b Epidemiology

Oligodendrogliomas represent 5% of all reported brain tumours. The median age of diagnosis is 41-49 with an annual incidence of 0.67 (95% CI 0.64-0.71) per 100,000 population.[36]

1.2.4.c Aetiology

As with astrocytomas there are no strongly associated aetiological factors in the development of oligodendrogliomas

1.2.4.d Molecular pathology

Oligodendrogliomas typically show allelic loss on chromosome arms 1p and 19q in approximately 80% of cases. In most instances this is due to an unbalanced t(1;19)(q10;p10) translocation.[165]. *IDH1* mutations are common

as with diffuse astrocytomas.[120] There is evidence that hypermethylation of the O^6-methylguanine methyltransferase (*MGMT*) gene is found in oligodendrogliomas, particularly those with 1p 19q loss.[166]

1.2.4.e Histopathology

There are two grades of oligodendroglial tumours, grade 2 and grade 3 (anaplastic).[69]

Grade 2 oligodendrogliomas are moderately cellular, diffusely infiltrating tumours consisting of isomorphic cells with round hyperchromatic nuclei (Figure 1.16).[35] Nodular areas of increased cellularity may be present but if the mitotic activity is low this does not automatically categorise them into grade 3. The cells suffer from artefactual swelling that results in clear cells with central spherical nuclei and well defined cell margins (fried egg appearance).

Anaplastic oligodendrogliomas show increased mitotic activity, microvascular proliferation and necrosis, with or without pseudopalisading. Otherwise the typical cellular architecture of oligodendrogliomas is still recognisable.

Oligoastrocytoma tumours exist with a mixture of distinct neoplastic cell types that may be either intermingled (diffuse variant) or in separate areas within the tumour (compact variant).[35] The tumours show distinct populations of both astrocytic and oligodendroglial lineage. They can show typical anaplastic features within both cellular components to be classified as grade 3 anaplastic oligodendrogliomas.

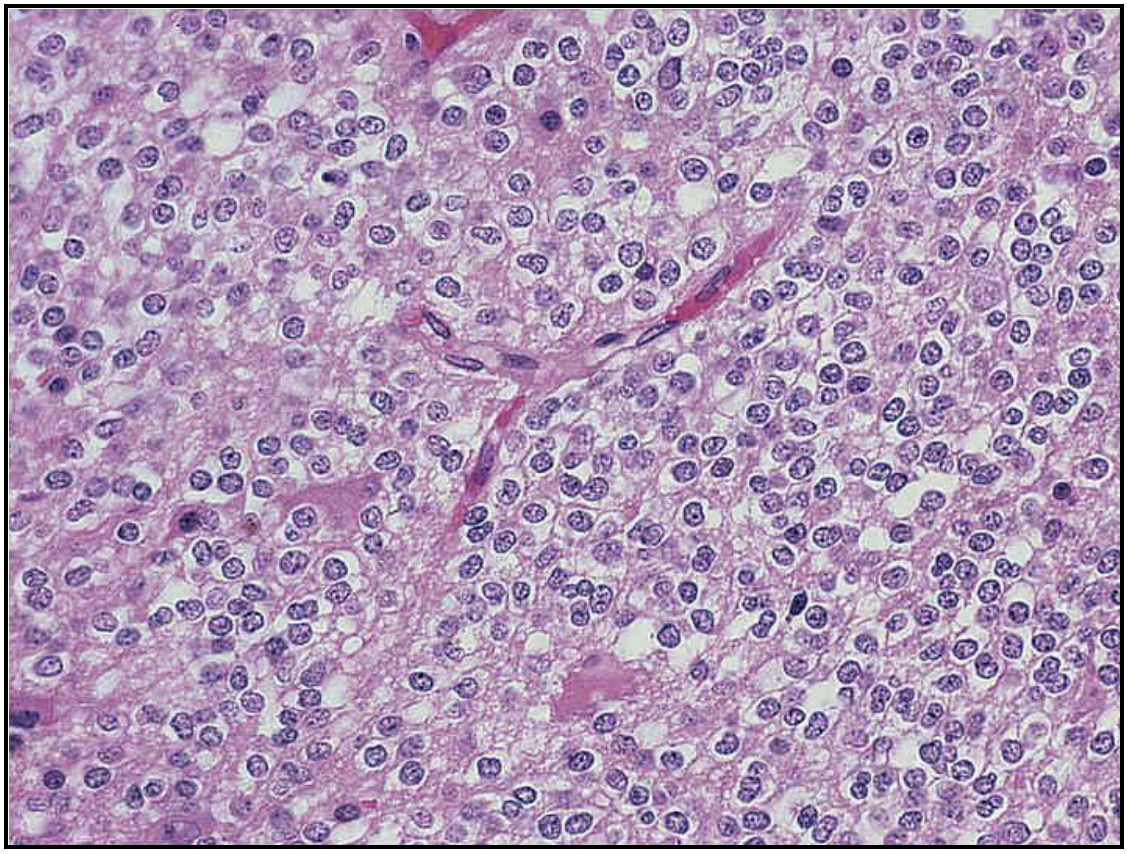


Figure 1.16 – Histology of an oligodendroglioma. Uniform, round nuclei and clear perinuclear halos (artefacts of delayed fixation) typify well-differentiated oligodendrogliomas. (Image courtesy of PathConsult - http://www.pathconsultddx.com)[70]

1.2.4.f Macroscopic appearance and localisation

The most common location for oligodendrogliomas is in the frontal lobes with the mass centred in the white matter and frequently invading into cortex (Figure 1.17). Rarely, they can develop in the cerebellum, brainstem or spinal cord. Calcification within the tumour is a common finding in oligodendrogliomas.[35]

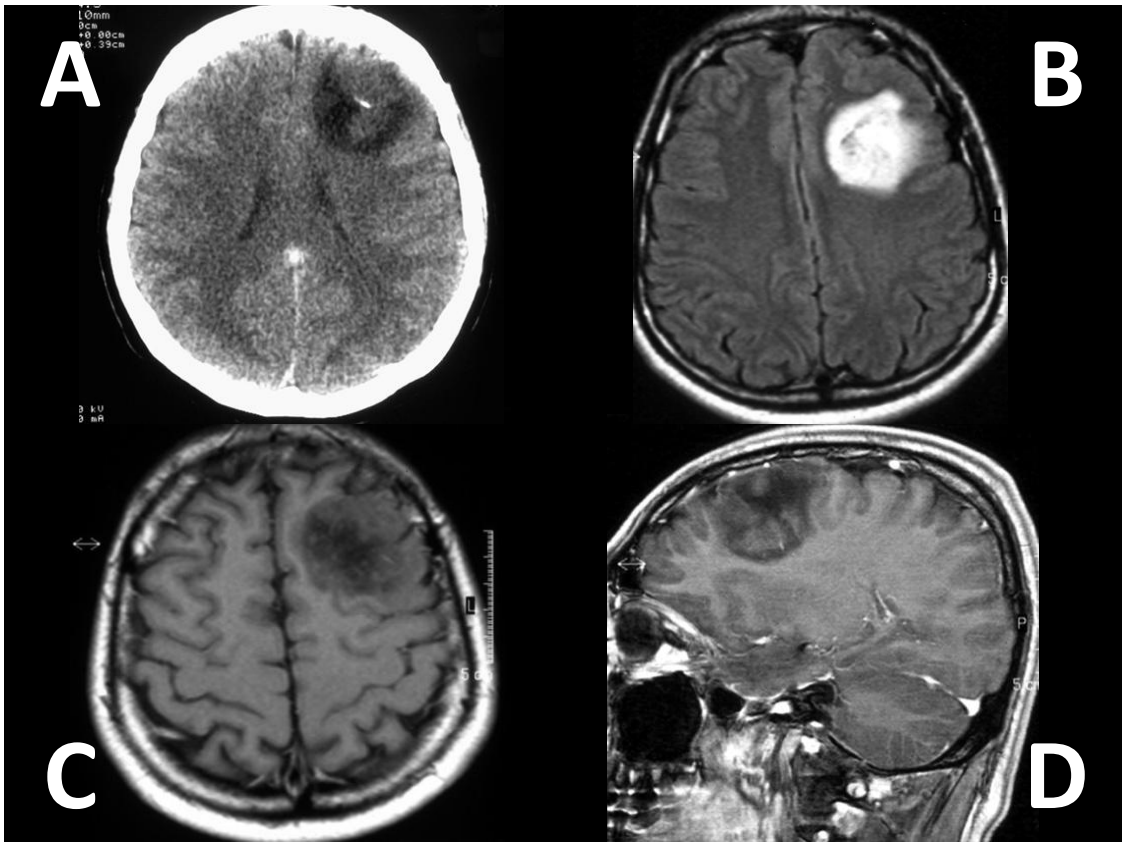


Figure 1.17 – MR scan of a patient with an oligodendroglioma. A – axial CT scan, B – axial flair, C – axial T1 post contrast and D – Sagittal T1 post contrast images showing a partly calcified non-enhancing lesion in the left frontal lobe.

1.2.4.g Treatment

1.2.4.g.i Surgery

The principles for surgical management of oligodendrogliomas are similar to those for astrocytomas (see 1.2.3.g.ii). One added factor in recent years has been the recognition of improved response to chemotherapy in oligodendrogliomas, particularly when there is loss of 1p 19q.[167] This may alter the surgical strategy, either by biopsying an apparent low grade glioma sooner or being less aggressive surgically with a know oligodendroglioma with 1p19q loss.

1.2.4.g.ii Radiotherapy

There is no randomised controlled trial comparing observation against radiotherapy for either low grade or anaplastic tumours. There is , however good evidence that radiotherapy at doses of 54-60 Gy given in fractions of 1.8-2 Gy provides durable control for patients with both low grade[142] and grade 3 oligodendrogliomas.[168]

It is recognised that up to 30% of patients with oligodendrogliomas will experience leptomeningeal seeding.[35] Even with this high rate craniospinal radiotherapy is not routinely given to these patients because of the side effects and likelihood of response to chemotherapy of disseminated disease.

1.2.4.g.iii Chemotherapy

The classic therapy for oligodendrogliomas is the PCV regimen of procarbazine, lomustine/CCNU and vincristine. This regimen had shown response rates of more than 50% and median survival times of 15-24 months in patients with recurrent oligodendroglial tumours.[169]

Temozolomide has generally replaced PCV as first line therapy for oligodendrogliomas for both primary[170] and relapsed tumours.[171]

The finding of improved survival with oligodendrogliomas was first described in 1998.[167] Subsequent studies have confirmed this finding but also found that there is no survival benefit without adjuvant treatment, particularly chemotherapy. It is therefore felt that this is a factor predictive of response to treatment rather than an independent prognostic factor in itself.[172]

1.2.4.h Prognosis

Given the generally good response to chemotherapy in the majority of oligodendrogliomas, survival rates are generally better than those for astrocytomas. Survival at 1, 5 and 10 years is 90.5%, 75.5% and 56.1% in grade 2 oligodendrogliomas and 77.4%, 50.4 and 33.8% in anaplastic oligodendrogliomas respectively.

1.2.5 High Grade Astrocytoma

1.2.5.a Definition

Tumours in this category would be WHO grade 3 anaplastic astrocytomas and WHO grade 4 Glioblastomas.[69]

Glioblastoma is the most common glial neoplasm which is preferentially located in the cerebral hemispheres of adult patients. Broadly they are categorised into primary glioblastomas that occur de novo with no pre-existing lesion and secondary glioblastomas which represent malignant change within a lower grade diffuse glial tumour.[35]

Anaplastic astrocytomas are astrocytic gliomas with increased cellularity, cytological atypia and increased mitotic activity. As with glioblastomas they can occur de novo or secondary to a lower grade astrocytoma.[35]

1.2.5.b Epidemiology

Glioblastoma comprises 80% of malignant gliomas and 18.5% of all brain tumours. Median age at diagnosis for glioblastomas is 64 years. Anaplastic astrocytomas form 10-25% of astrocytic gliomas and overall 2.7% of brain tumours. The median age of diagnosis for anaplastic astrocytomas is 51.[36]

Overall malignant gliomas comprise approximately 2% of all cancers with an annual incidence of 5 per 100,000 population. However, given the younger age of presentation and overall poor life expectancy, these tumours have a relatively greater impact on loss of years of working life than other more common tumours.[36]

1.2.5.c Aetiology

As with low grade gliomas, the only environmental aetiological factor that has been identified is therapeutic ionising radiation.[173] There are several genetic conditions that are predisposed to developing high grade astrocytomas. The autosomal dominant *p53* gene germline mutation Li-Fraumeni syndrome are known to develop malignant astrocytomas as one of tumours related to the condition.[88] Turcot's syndrome is a genetic condition involving familial polyposis of the colon with an increased risk of colon cancer. These patients have a higher rate of developing malignant astrocytomas.[174]. Low grade astrocytomas occurring in patients with tuberous sclerosis and neurofibromatosis type 1 and 2 have the potential to progress to malignant tumours.

1.2.5.d Molecular pathology

Genetic analysis has shown marked differences between primary and secondary gliomas.[175]

Primary glioblastomas more frequently demonstrate EGFR amplification, homozygous deletion of *CDKN2A* and $p14^{ARF}$, *CDK4* amplification, *MDM2* or *MDM4* amplification, *RB1* mutation/homozygous deletion, monosomy 10 and *PTEN* mutation.[176].

p53 mutations are more commonly associated with secondary glioblastomas, being present in over 60% compared to 30% of primary glioblastomas.

In secondary glioblastomas, *EGFR*, *MDM2* or *MDM4* amplification and *PTEN* mutation is rare. Allelic loss on chromosome 10 is generally confined to

markers on 10q. Allelic loss of 19q and 13q, promoter hypermethylation of the *RB1* gene and overexpression of *PDGFRA* are more common in secondary glioblastoma. IDH1 point mutations are also far more common in secondary glioblastoma.[120]

Although the genetic alterations in primary and secondary glioblastomas are very different, there are common pathways that both target, namely the p53 pRb1, Pten/Pi3k/Akt and mitogen-activated protein kinase pathways.[176, 177]

1.2.5.e Histopathology

Glioblastomas are highly cellular tumours composed of cells with a variety of morphologies (Figure 1.18). Nuclear atypia is common and mitotic activity is usually high. It is essential to see microvascular proliferation and or necrosis to make the diagnosis.[69]

There are two variants of glioblastoma in the WHO classification, giant cell glioblastoma and gliosarcoma.

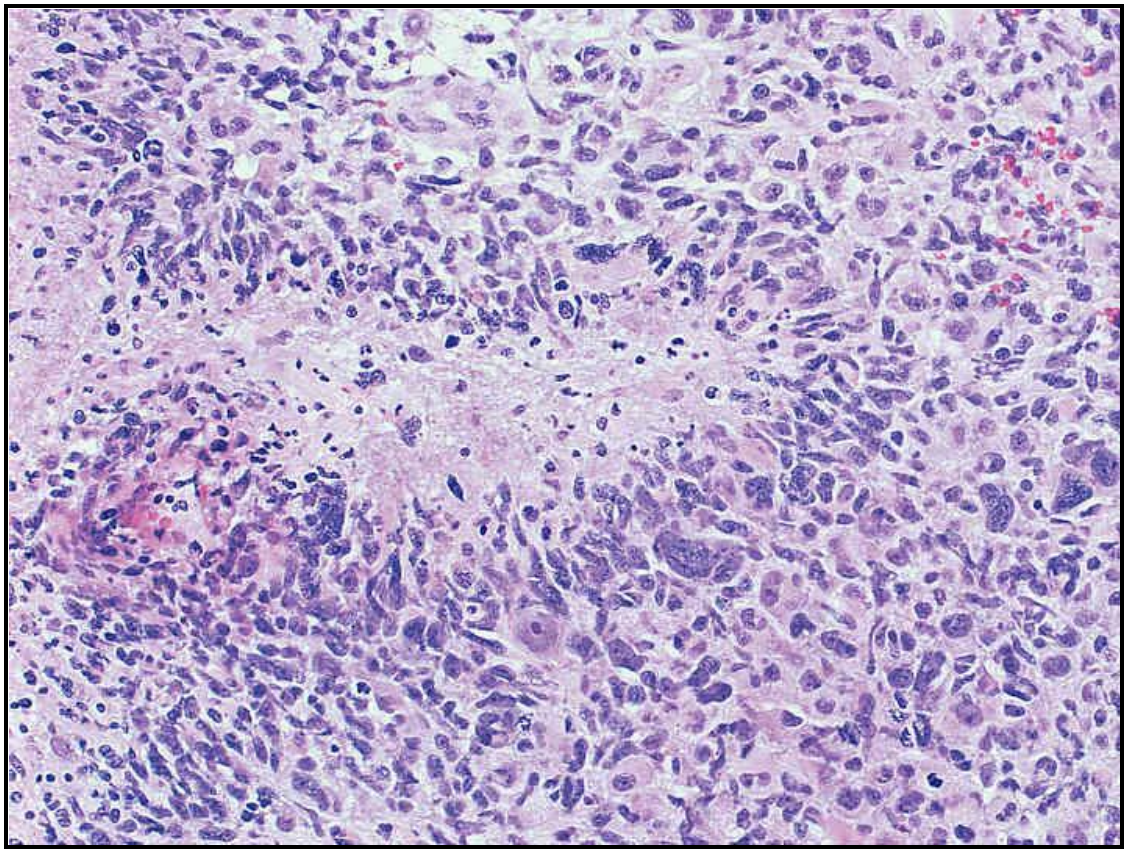


Figure 1.18 – Histology of a glioblastoma. Dense cellularity, striking pleomorphism, and zones of coagulative necrosis lined by 'palisading' tumour cells characterize the prototypical glioblastoma. Note the complex 'glomeruloid' quality of the microvascular proliferation at right. Astrocytic elements are seen on the left side. (Image courtesy of PathConsult - http://www.pathconsultddx.com)[70]

1.2.5.e.i Giant cell glioblastoma

This variant is characterised by numerous, pleomorphic, multinucleated giant cells. Some giant cell glioblastomas demonstrate a collagen and reticulin fibre-rich matrix. On imaging and clinically they tend to be well circumscribed lesions and this may be why there is some evidence that they can do better than classical glioblastomas.[35]

1.2.5.e.ii Gliosarcomas

These tumours show a biphasic pattern of gliomatous and sarcomatous elements. The gliomatous elements have the typical appearance of a glioblastoma and stain positive for glial fibrillary acidic protein (GFAP). The sarcomatous elements are rich in reticulin fibres and composed of GFAP-negative spindle cells. The outcome for gliosarcoma is felt to be slightly worse than a classical glioblastoma.[178]

1.2.5.f Macroscopic appearance and location

The vast majority of glioblastomas arise in the cerebral hemispheres (Figure 1.19). They appear as peripherally enhancing, centrally necrotic lesions with surrounding oedema on imaging. There is frequently invasion to adjacent structures and through the corpus callosum to the contralateral hemisphere.

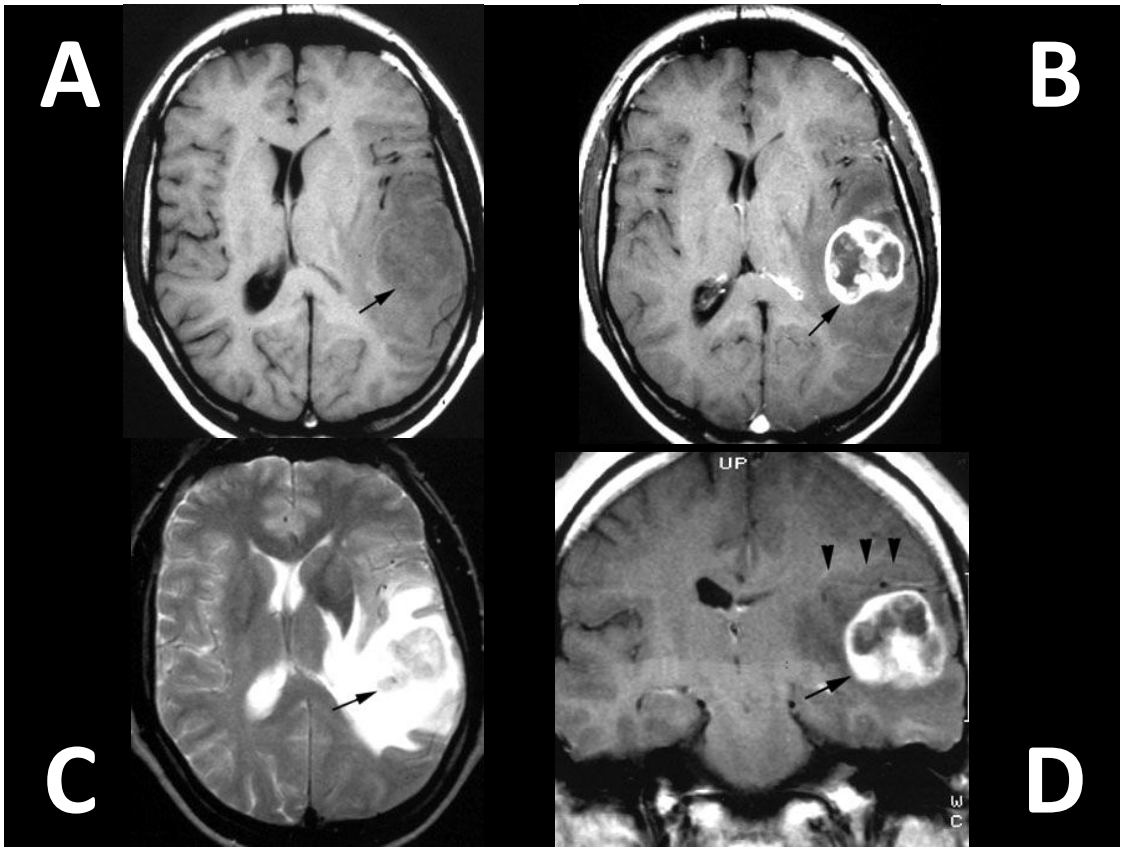


Figure 1.19 – MR scan of a patient with a glioblastoma. A – axial T1, B – axial T1 post contrast, C – axial T2 and D – coronal T1 post contrast images showing a left temporal peripherally enhancing lesion, which, as in this example, can have a well demarcated boundary (arrows with tails). There is a large amount of surrounding oedema (arrows without tails).

1.2.5.g Treatment

1.2.5.g.i Surgery

There is ongoing debate on the role and influence of surgical intervention in high grade astrocytomas/glioblastomas. There is consensus that histological confirmation of a lesion in those fit to undergo a biopsy is an appropriate management strategy. In terms of the effect of extent of resection in outcome of these tumours, there has never been a randomised controlled trial comparing biopsy with maximal surgical resection. There is however a growing body of evidence that suggests the greater the extent of resection then the better the outcome.[179, 180]

In addition to the potential benefits of surgical resection, there are new surgical technologies available that can deliver local therapies directly to the tumour. The currently most used example is Gliadel wafers, which are a polymer that provides a sustained release of carmustine (BCNU) directly to the resected tumour bed.[181] In a phase III trial the median survival has been shown to increase from 11.6 to 13.9 months with the use of Gliadel.[181]

1.2.5.g.ii Radiotherapy

There have been multiple trials showing a benefit to survival following fractionated radiotherapy for glioblastomas.[182] The standard dose is generally 54Gy to 60Gy in 30 fractions of 1.8Gy to 2Gy.

With its wider availability stereotactic radiosurgery is becoming increasingly utilised in the treatment of glioblastoma. This is particularly in the setting of nodular recurrence following previous fractionated radiotherapy. As yet

there is no strong evidence of a significant improvement in tumour control or survival.[183]

1.2.5.g.iii Chemotherapy

For many years patients with high grade gliomas were treated with systemic chemotherapy following radiotherapy or at the time of relapse. For glioblastoma there was no good clinical evidence to support this and for anaplastic astrocytoma there were only a few non-controlled trials showing a benefit. In 2001 a randomised controlled trial of chemotherapy following radiotherapy versus radiotherapy alone showed no benefit with chemotherapy for either glioblastoma or anaplastic astrocytoma.[184]

In recent years, however, temozolomide, an oral alkylating agent, given concomitantly with radiotherapy has been show to increase median survival by 2.5 months and give a 2 year survival of 26% compared to 8% in patients treated with radiotherapy alone.[185] The DNA repair gene O^6-methylguanine DNA methyltransferase (MGMT) plays an important role on the effect of temozolomide in glioblastomas. When it is epigenetically silenced by hypermethylation the benefits of temozolomide versus radiotherapy alone are significantly higher with an increase of 6.4 months in median survival to a median of 21.7 months. MGMT promoter methylation was evident in 45% of glioblastoma patients and the effect is so strong that all future trials involving temozolomide will need to stratify patients based upon their MGMT methylation status.[186]

1.2.5.h Prognosis

Despite recent advances, the overall prognosis for high grade astrocytomas remains poor. No therapy will reliably offer long term control of the tumours.

Proven factors related to prognosis are age and Karnofsky performance score at presentation.[35] Patients over 65-70 or who have a Karnofsky performance score of less than 70 will have a worse outcome.

Survival rates at 1, 5 and 10 years for anaplastic astrocytoma are 60.3%, 29.4% and 22.4% and for glioblastoma are 29.6%, 3.4% and 2.4% respectively.[36]

1.2.6 Medulloblastoma

1.2.6.a Definition

Medulloblastomas are a malignant embryonal tumour of the cerebellum, mainly occurring in children, with predominantly neuronal differentiation and a tendency to disseminate through CSF pathways.

1.2.6.b Epidemiology

Medulloblastomas are the most common malignant brain tumour in children. The annual incidence is approximately 0.5 per 100,000 with a peak age of presentation of 7 years. Infants and young adults can also be affected. There is generally a slight male predominance with approximately 65% of patients being male.[187]

1.2.6.c Aetiology

Most cases of medulloblastoma are sporadic but there have been several familial tumour syndromes with an association with medulloblastomas including Gorlin syndrome, Rubenstein-Tyabi syndrome, Li-Fraumeni syndrome, ataxia-telangiectasia, Turcot syndrome, neurofibromatosis and tuberous sclerosis.[35]

1.2.6.d Molecular pathology

In recent years there has been much discovered about the molecular pathways that are involved in the formation of medulloblastomas. The cell of origin of medulloblastomas is felt to be the granule cell progenitor.[188] Overactive sonic hedgehog signalling promotes granule cell progenitor proliferation and stimulates the development of medulloblastomas.

Although there is still some debate about the details, several groups have shown a reproducible molecular classification of medulloblastoma subtypes that have overlap with histological subgroups and strong associations with prognosis and survival.[189-192]

The most frequent genetic alteration in medulloblastomas is loss of chromosome 17p which is found in over 50% of cases and associated with gain of 17q, hence forming the isochromosome 17q. This genetic abnormality is associated with classic and large cell/anaplastic histological variants. Target genes that have been implicated on 17p are the *REN (KCTD11)* and *HIC1* genes.[193, 194]

WNT pathway activation is seen in 15% of medulloblastomas and is most frequently caused by somatic *CTNNB1* mutations indicated by nuclear accumulation of its gene product β–catenin. This molecular subtype is associated with a subgroup of the classic histological variant which lacks chromosome 17 alterations (otherwise typical in classical variant) but having chromosome 6q losses. This subtype is seen in older children and has a more favourable prognosis.

Desmoblastic histological variant medulloblastomas also often lack 17p losses but are associated with sonic hedgehog pathway (SHH) abnormailities.[195] These include mutations in the tumour suppressor gene *PTCH* on chromosome 9q. *PTCH* encodes a receptor component of the SHH pathway that control granule cell progenitor proliferation in cerebellar development. Inactivating mutations of *PTCH* or the Human Supressor of Fused *(hSUFU)* gene on chromosome 10 leads to uncontrolled activation of the SHH pathway and over-proliferation of granule cell progenitor cells. This is the mechanism by which germline mutations of the *PTCH* gene (Gorlin syndrome) or *hSUFU* gene predispose to medulloblastomas.[196, 197]

Amplification of the *MYC* gene (most commonly *MYCC* and *MYCN*) on chromosome 8q24 occurs in 5-10% of cases. This is associated with the large cell/anaplastic histological variants and an overall poor prognosis.[198-200]

1.2.6.e Histopathology

As already described the histological variants of medulloblastoma are closely associated with molecular profiles of the tumours. There are five subtypes of medulloblastoma in the WHO classification.[69, 201]

1.2.6.e.i Classic medulloblastoma

Medulloblastomas are composed of densely packed, small, round tumour cells with scant cytoplasm, round or carrot shaped nucleus and condensed chromatin (Figure 1.20). The classic variant occurs in approximately 70% of cases. There may be formation of Homer-Wright rosettes.

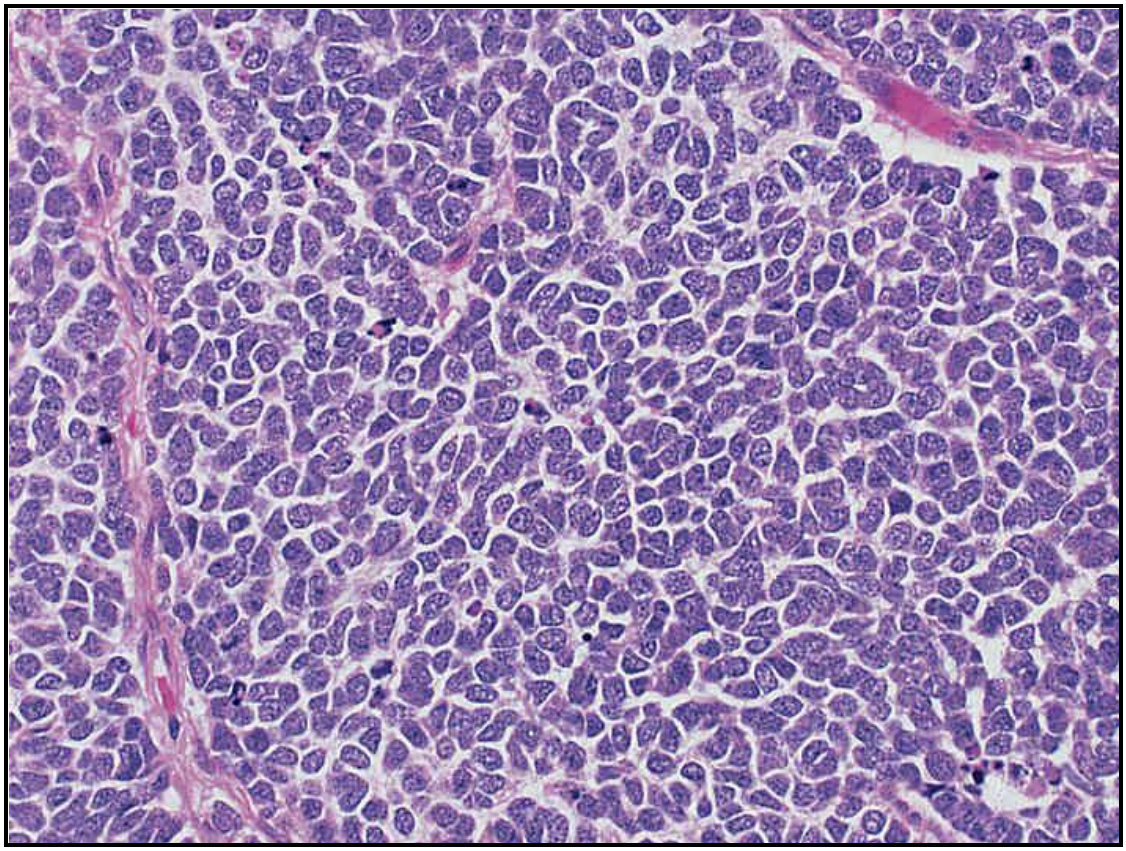


Figure 1.20 – Histology of a medulloblastoma. The classic medulloblastoma is a highly cellular neoplasm composed of diminutive, undifferentiated-looking elements possessed of little definable cytoplasm and prone to nuclear moulding. (Image courtesy of PathConsult - http://www.pathconsultddx.com)[70]

1.2.6.e.ii Desmoplastic/Nodular medulloblastoma

Desmoplastic medulloblastoma comprises of 10-20% of cases and is made up of islands of well differentiated cells surrounded by large amounts of reticulin and collagen. It generally carries a more favourable prognosis.[202]

1.2.6.e.iii Medulloblastoma with extensive nodularity

These tumours show large nodules and advanced neurocytic differentiation with smaller areas resembling desmoplastic medulloblastoma. It occurs in infants and with treatment the prognosis appears to be favourable.

1.2.6.e.iv Anaplastic medulloblastoma

Cases of medulloblastoma showing severe and diffuse anaplasia qualify for the diagnosis of anaplastic. This variant is associated with a poor outcome.

1.2.6.e.v Large cell medulloblastoma

This rare subtype is characterised by large tumour cells with eosinophilic cytoplasm, enlarged nuclei and single prominent nucleoli. Mitoses and apoptotic figures are abundant with large areas of necrosis commonly seen. Clinically this variant has very aggressive behaviour and is associated with a poor prognosis.

1.2.6.f Macroscopic appearance and localisation

Medulloblastomas typically arise in the midline of the posterior fossa dorsal to the fourth ventricle (Figure 1.21) although the desmoplastic variants are often more centred in a cerebellar hemisphere. On pre contrast CT scan they appear hyperdense as a consequence of their cellularity.

At the time of diagnosis 30% will have evidence of metastatic spread through the CSF pathways.

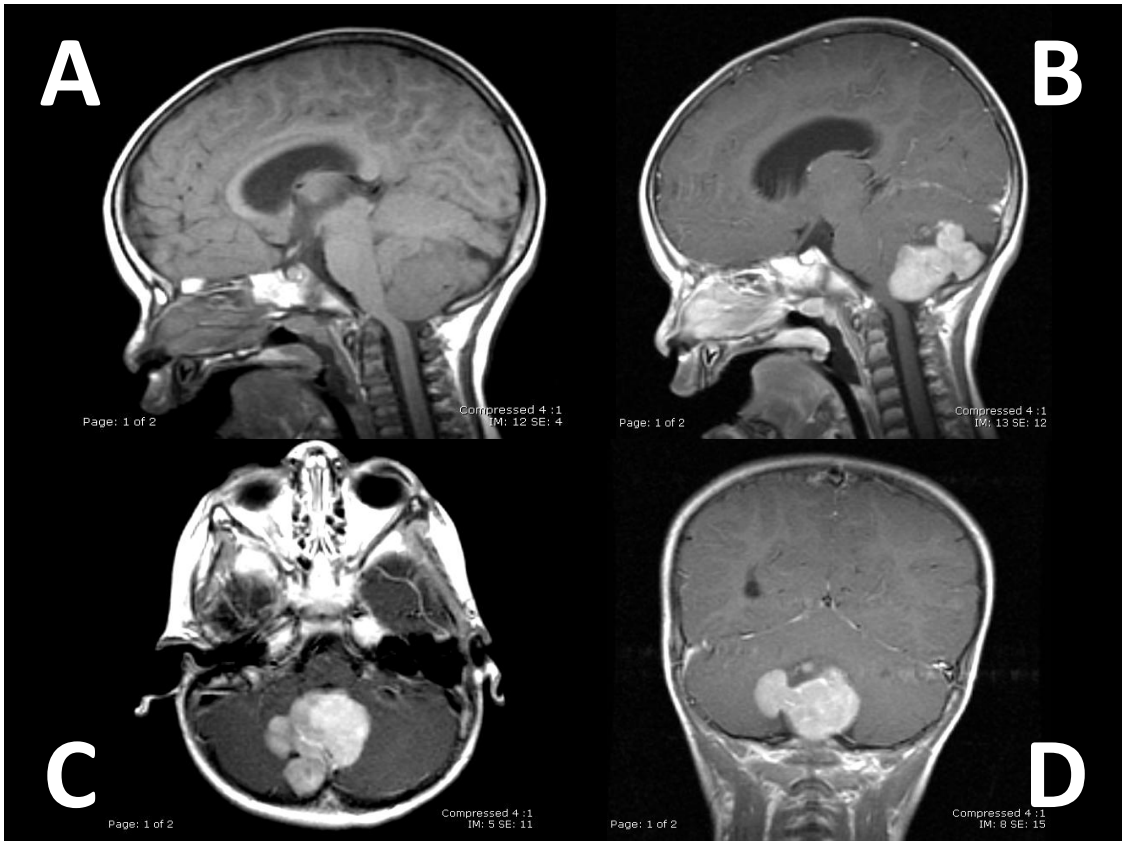


Figure 1.21 – MR scan of a patient with a medulloblastoma. A – sagittal T1, B – sagittal T1 post contrast, C – axial T1 post contrast and D – coronal T1 post contrast images showing a uniformly enhancing, somewhat nodular lesion arising from the cerebellar vermis, causing a secondary obstructive hydrocephalus. It is isointense on pre-contrast T1 images suggesting a cellular tumour.

1.2.6.g Treatment

It has been recognised for some years that the goal of surgical resection of medulloblastoma should be to achieve a gross total resection or be leaving less than 1.5cm^3 of tumour as anything more that this significantly worsened survival.[203]

Given the location of the tumour, obstructive hydrocephalus is a common presenting finding and overall about 20-30% of patients will require a permanent CSF diversion at some point of their treatment.[35]

1.2.6.h Radiotherapy

Radiotherapy has been shown to be beneficial for medulloblastomas but it is limited by its long term complications in younger children. Most children under 3 would not be treated with post operative radiotherapy but attempts would be made to hold the disease with chemotherapy until they had reached at least 3 years of age.

Standard doses would be 36 Gy in 20 fractions to the craniospinal axis and 54 Gy boost in 30 fractions to the posterior fossa. Attempts have been made to spare the dose as much as possible given the cognitive sequelae even in children over 3 years old. One study described a reduced dose of radiotherapy to the standard risk (age >3, <1.5cm^3 residual tumour post operation and no metastases at presentation) group of 23.4 Gy to the craniospinal axis, 36 Gy to the posterior fossa and 55.8 Gy to the tumour bed. High risk patients received 36-39.6 Gy to the craniospinal axis, with a 3D conformal boost totalling 55.8 Gy to the tumour bed. Survival at 5 years was 85% in the standard risk and 70% in the high risk group.[204].

As well as the cognitive side effects there is risk following radiotherapy of developing a second malignancy which has been reported as 10%.[205]

1.2.6.i Chemotherapy

The medical treatment of medulloblastoma is constantly evolving.[206, 207] However, there are a variety of different agents and regimens that have been trialled in multicentre settings that have not clearly shown great advantage over the others. With increasing knowledge of the molecular basis of medulloblastomas it is hoped that more individually targeted and effective therapies will be available in the future.

1.2.6.j Prognosis

Many of the important prognostic factors have already been discussed above and it is clear that the molecular characteristics of the tumours will have more influence in terms of prognosis and treatment in the future.

With improvements in survival rates the quality of survival of children must be carefully considered and assessment and support of development is essential in this vulnerable group.[208]

1.2.7 Ependymoma

1.2.7.a Definition

Ependymomas are glial tumours with cellular features of ependymal differentiation. They arise from radial glial-like stem cells[209] in the cerebral subventricular zone lining the fourth ventricle and within the spinal cord.

1.2.7.b Epidemiology

Ependymomas are the third most common CNS tumour in children following astrocytoma and medulloblastoma. They represent 5% of CNS tumours in adults, 10% in children under 15 years and 30% in children under 3 years of age. The overall annual incidence is approximately 0.34 (95% CI 0.32-0.34) per 100,000.[36]

1.2.7.c Aetiology

No environmental factors have been identified in the development of ependymomas. Approximately 2-5% of patients with neurofibromatosis type 2 develop ependymomas which usually occur in the spine.[35]

1.2.7.d Molecular pathology

The most common chromosomal change is the loss of chromosome 22 in 30-60% of cases.[210] The actual gene on chromosome 22 remains to be identified since the *NF2* gene is mutated in only a small subgroup of patients and *hSNF5/INI1* mutations are absent. p53 mutations and amplifications of *CDK4* and *EGFR* are usually absent in ependymomas. However expression of *ERBB2*, *ERBB4* and *EGFR* is often up-regulated and *EGFR* over-expression is linked to poor prognosis.[211, 212]

There are marked genetic differences in ependymomas depending upon their location with supratentorial ependymomas expressing elevated levels of *EPHB-EPHRIN* and *NOTCH* pathway members, whereas spinal ependymomas showed up regulated expression of *HOX* genes.[209]

Increased expression of the catalytic subunit of human telomere reverse transcriptase (*hTERT*) has a negative effect on survival. Five-year overall survival was 84% (SEM, 7%) and 41% (SEM, 7%) for hTERT-negative and hTERT-positive tumours, respectively (P = .001).[213, 214]

1.2.7.e Histopathology

The diagnostic hallmarks of ependymoma are perivascular pseudorosettes which are tumour cells extending radial fibrillary processes towards vessels and true ependymal rosettes which are canals and tubuli composed of a single layer of cuboidal tumour cells (Figure 1.22).

The World Health Organisation classifies ependymomas into four broad categories; grade 2 ependymomas, grade 3 anaplastic ependymomas, myxopapillary ependymomas and subependymomas.

The classic or benign grade 2 ependymomas are further split into four histological variants. Cellular ependymoma is characterised by a hypercellular appearance with narrow perivascular pseudorosettes, a fairly uniform cellular appearance but with a low proliferative index. Papillary ependymomas are rare tumours that contain tubulovillous architecture as their characteristic feature. Clear cell ependymomas have clear cytoplasm with a perinuclear halo which resembles oligodendrogliomas, neurocytomas,

clear cell carcinomas and haemangioblastomas. Its immunoreactivity to glial fibrillary acidic protein (GFAP) and features on electron microscopy would usually differentiate it from these other tumour types. Tanycytic ependymomas consist of elongated cells arranged in fascicles. Ependymal rosettes and perivascular pseudorosettes are poorly delineated with these tumours.

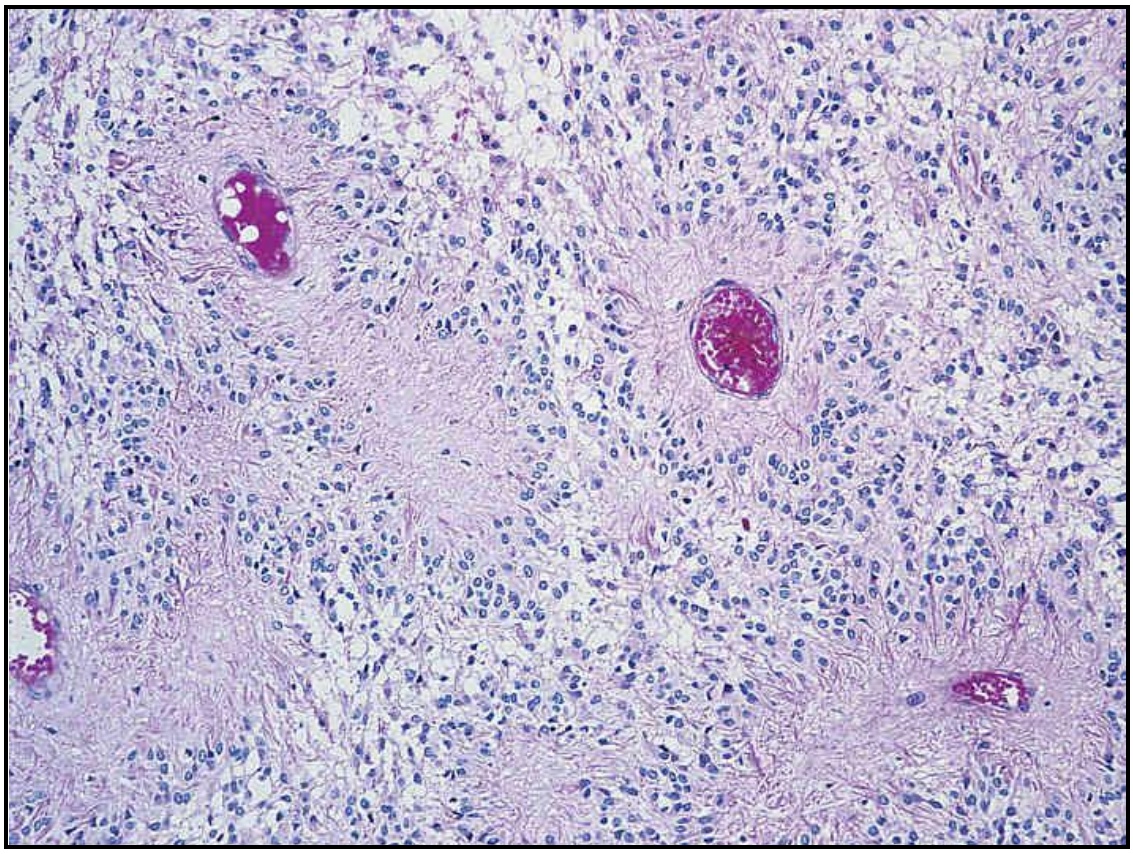


Figure 1.22 – Histology of an ependymoma. The cytoplasmic processes of ependymal tumour cells condense about blood vessels to form pseudorosettes. In addition, true ependymal rosettes are seen with ependymal cells forming tubular structures. (Image courtesy of PathConsult - http://www.pathconsultddx.com)[70]

Anaplastic (grade 3) ependymomas are malignant tumours with high cellularity, nuclear atypia, hyperchromatism, necrosis, vascular proliferation and high mitotic index. They are estimated to occur in 30% of

ependymomas. Historically the differentiation between these and grade 2 lesions has been controversial, leading to the finding of similar outcomes in both tumour grades.[215] This has particularly been when ependymomas in different locations have been analysed together, where there is now good evidence that they are biologically distinct lesions.[209]

Myxopapillary ependymomas almost exclusively occur at the conus, but have been reported throughout the cranial spinal axis. They are characterized by GFAP-expressing, cuboidal to elongated tumour cells radially arranged in a papillary manner around vascularised stromal cores. Mitotic activity is very low. A mucoid matrix material accumulates between tumour cells and blood vessels, and fills the tumour microcysts. They are classified as WHO grade 1 tumours and have a favourable prognosis, particularly with complete excision.

Subependymomas are typically well demarcated, non-enhancing lesions located in the walls of the ventricular system. They are characterized by clusters of isomorphic nuclei embedded in a dense fibrillary matrix of glial cell processes with frequent occurrence of small cysts. Mitoses are very rare or absent. They are classified as WHO grade 1 tumours and surgical excision is curative.

1.2.7.f Macroscopic appearance and localisation

Ependymomas can occur anywhere within the cranial spinal axis, usually related to the CSF pathways. The majority (>90%) are cranial with 65-70% of these being infratentorial.[35] Supratentorial ependymomas are equally

divided between the parenchyma and ventricular system (75% lateral and 25% third ventricle).

A typical lesion in the posterior fossa would show some calcification on CT scan and on MR scan would show cellular signal characteristics (isointense to grey matter on T1 and T2) with a fourth ventricular location and extension of the tumour out of the foramina of Luschka and Magendie. There is often some heterogeneous enhancement seen post contrast (Figure 1.23).

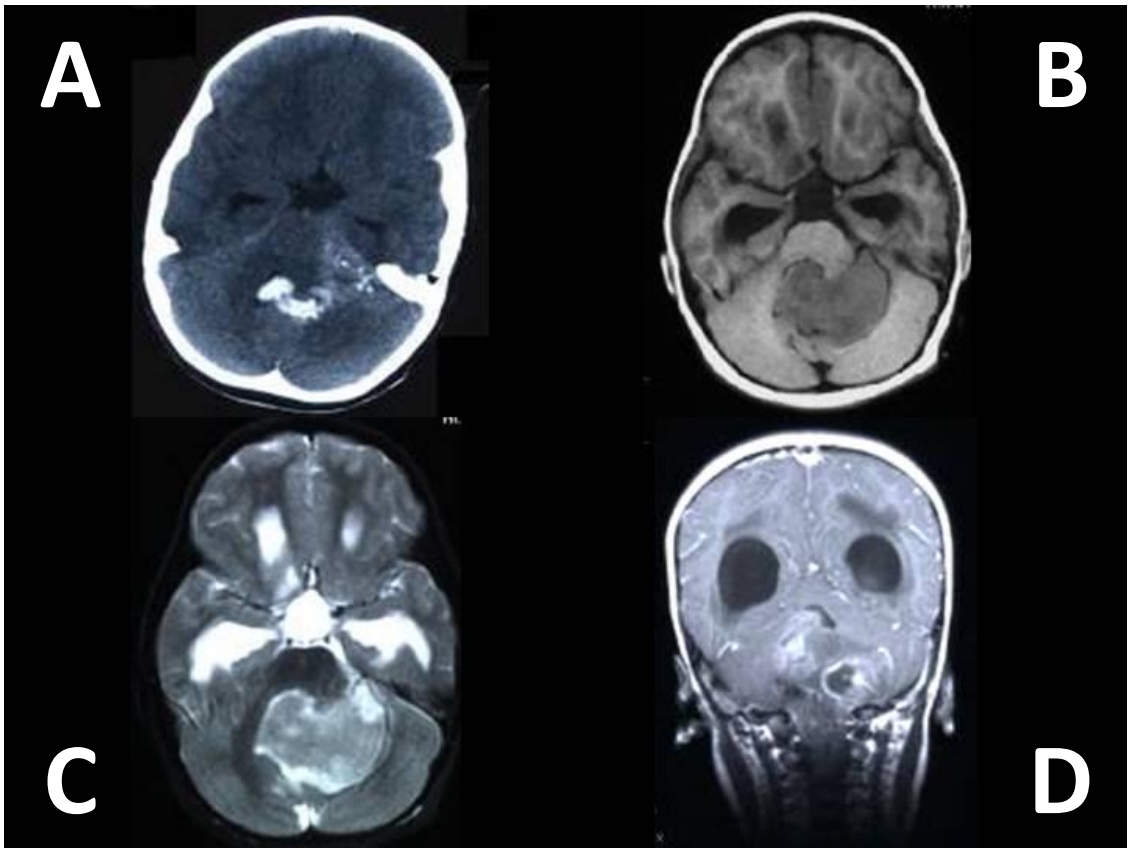


Figure 1.23 – CT and MR scans of a patient with a posterior fossa ependymomas. A – axial CT, B – MR axial T1, C – axial T2, D – coronal T1 post contrast images showing a partly calcified lesion located in the fourth ventricle, extending out into the left foramen of Luschka with cellular signal characteristics and patch enhancement.

1.2.7.g Treatment

1.2.7.g.i Surgery

There is consensus that gross surgical excision of ependymomas should be the surgical goal where possible with five year survival rates of 88% where this is achieved compared with 53% where only a subtotal resection was performed.[35] Complete tumour removal reduced the rate of spinal seeding from 9.5% to 3.3% with subtotal.

However, gross total removal of infratentorial lesions is only achieved in 50-70% of cases.[35]

Hydrocephalus is common at presentation of posterior fossa ependymomas and even with aggressive tumour removal 30-50% of patients will still require some form of CSF diversion.

1.2.7.g.ii Radiotherapy and radiosurgery

Intracranial ependymomas appear to be relatively radiation resistant tumours. The use of adjuvant radiotherapy following surgery is somewhat controversial for some indications but there is reasonable agreement that it is beneficial for patients with posterior fossa tumours, subtotally resected tumours, WHO grade 3 tumours and all patients presenting with disseminated disease. Standard doses of 54-59.4 Gy fractionated over a 5-6 week period to the tumour bed with a 1-2cm margin are usually given.

For children under 3 years of age there has been a general avoidance of radiotherapy because of the concerns over significant compromise to cognitive development. However a recent study has shown good tumour

control with conformal radiotherapy in the under 3 population, but long term cognitive consequences remain to be seen.[216]

1.2.7.g.iii Chemotherapy

The desire to avoid radiotherapy in young patients has led to the investigation of chemotherapy as an adjuvant therapy. This has been shown to be effective in delaying radiotherapy but the long term consequences of this management strategy have also not been fully investigated.[217] Overall response rated for most chemotherapy regimens in ependymomas have been in the order of 20%.

1.2.7.h Prognosis

One of the greatest problems with defining prognosis for ependymomas has been the difficulty with classification and the mixing of biologically different tumours within the analysis of various studies.

Overall survival at 1, 5 and 10 years is 88.5%, 72.5% and 65.0% respectively.[36] However age has a significant impact on survival with 1, 5 and 10 year survival rates of 86.1%, 53.4% and 47.1% for patients aged 0-14 years and 92.3%, 86.2% and 80.4% for patients aged 20-44 years.[36]

1.3 Vascular Endothelial Growth Factor (VEGF)

1.3.1 Introduction

The vascular system of the skin comprises the blood and lymphatic circulations, both of which are located in the dermis. Early in embryogenesis the blood vascular system evolves from the primary capillary plexus.[218] The development of the lymphatic vasculature during embryogenesis lags behind that of the blood vessels, suggesting that both processes are regulated by different signals.[219] Over one hundred years ago, Florence Sabin proposed a theory on the venous origin of the lymphatic vasculature;[220] it is only in recent years that studies have provided experimental evidence in support of Sabin's model.[221, 222] VEGF signalling often represents a critical rate-limiting step in angiogenesis - the formation of new blood vessels from a pre-existing vascular bed.[223, 224] Substantial evidence implicates VEGF as a primary angiogenic factor and mediator of pathological angiogenesis.[225]

1.3.2 VEGF: a historical perspective

The observation that tumour growth was accompanied by increased vascularity was made more than a century ago by several investigators, including the celebrated pathologist Rudolf Virchow, in German-language publications.[224] In 1939, Ide et al. postulated the existence of a tumour-derived blood vessel growth-stimulating factor.[226] In 1945, Algire et al. advanced this concept, proposing that "the rapid growth of tumour transplants is dependent upon the development of a rich vascular supply".[227]

Algire also suggested that vascular proliferation was a critical step in tumourigenesis, because it was likely to confer a growth advantage on the tumour cells relative to normal cells.[227] In 1968, the first experiments to test the hypothesis that tumours produce angiogenic factors were performed by Greenblatt and Shubi[228] and Ehrmann and Knoth.[229] They demonstrated that tumour angiogenesis was mediated by diffusible factor(s) produced by the tumour cells. In 1971, Folkman proposed that anti-angiogenesis might be an effective approach to treat human cancer[230] and attempted to isolate a "tumour angiogenesis factor" from human and animal tumours. Subsequently, the angiogenic effects of various factors, including epidermal growth factor (EGF), transforming growth factor (TGF)-α, TGF-β, tumour necrosis factor (TNF)-α and angiogenin were reported.[231]

Independent and unrelated lines of research converged toward the identification of VEGF. In 1983, Senger et al. described the partial purification of a protein able to induce vascular leakage in the skin, which was named "tumour vascular permeability factor" (VPF).[232] In 1989, Ferrara and Henzel reported that they had isolated a diffusible endothelial cell-specific mitogen from medium conditioned by bovine pituitary follicular cells.[233] They named the isolated protein "vascular endothelial growth factor" (VEGF) to reflect the restricted target cell specificity of this molecule. Subsequently, cDNA cloning of the genes for both VPF[234] and VEGF,[235] also in 1989, demonstrated that they were the same molecule.

It is now understood that VEGF belongs to a family of closely related vascular growth factors that have a unique role in controlling growth and differentiation of multiple anatomic components of the vascular system (Figure 1.24). In addition to VEGF (also known as VEGF-A) the VEGF-family also includes placental growth factor (PlGF);[236, 237] VEGF-B,[238, 239] VEGF-C,[240, 241] VEGF-D[242, 243] and a viral form, VEGF-E.[244] VEGF-C and VEGF-D primarily regulate lymphangiogenesis.[221, 245] In the remainder of this thesis VEGF-A will be referred to as VEGF.

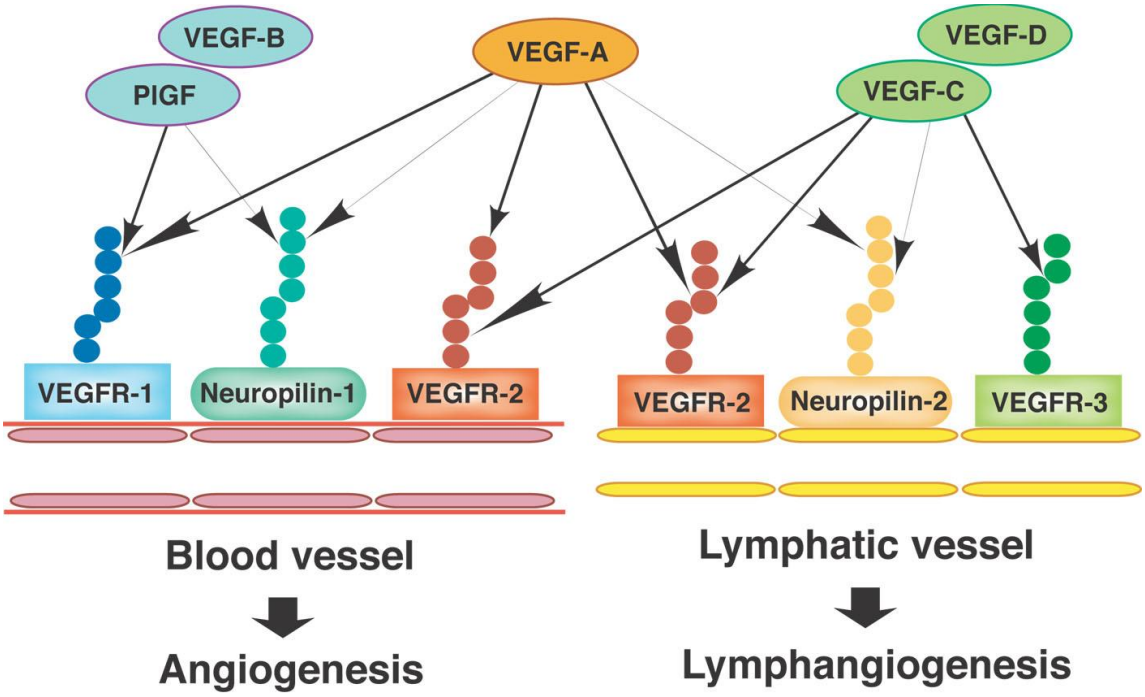


Figure 1.24 - VEGF-A is the founding member of a family of closely related growth factors and is well established as a pro-angiogenic cytokine. The VEGF receptors (VEGFRs) are expressed on the endothelial cells of both blood and lymphatic vessels. VEGFR-1 is exclusively expressed on blood-vessel endothelial cells (BECs), VEGFR-3 is exclusively expressed on lymphatic endothelial cells (LECs); and VEGFR-2 is expressed on both BECs and LECs. (Courtesy of Professor MJ Detmar, Boston, MA, USA.)

1.3.3 Angiogenesis

1.3.3.a The angiogenic pathway: molecular regulation of angiogenesis

The adult blood vasculature is usually quiescent but retains the ability to initiate a rapid physiological or pathological angiogenic response if the balance between endogenous inhibitors and stimuli is altered - the "angiogenic switch".[246] New vessel growth and maturation are highly complex and coordinated processes, requiring the sequential activation of a series of receptors by numerous ligands in endothelial cells.[247-249] The sequence of events involved in angiogenesis includes: increased vascular permeability and leakage; degradation of basement membrane; endothelial cell proliferation and migration through the surrounding extra cellular matrix (ECM) and maturation and stabilisation of the newly formed vessel bed.

The first stage of angiogenesis includes capillary vasodilation and hyperpermeability with subsequent extravasation of plasma proteins, including prothrombin and fibrinogen, into the surrounding ECM. Fibrin derived from this cascade provides a provisional scaffold to support endothelial cell adhesion and migration.[250] Local enzymatic degradation of the confining basement membrane is required for endothelial cells to egress from the parent vessel and sprout into the surrounding tissue.[251] This process involves secretion and activation of matrix metalloproteinases (MMPs),[252, 253] zinc-dependent extracellular endopeptidases, growth factors (e.g. VEGF) or cell-matrix interactions.[254] MMPs known to be secreted by endothelial cells include MMP-1, MMP-2, MMP-9 and membrane-type-

MMP.[254, 255] MMPs possess the capacity to degrade all components of the ECM and appear to be important for neovascularisation. The evidence for this includes: MMP-2-deficient mice which have reduced angiogenic responses; and specific MMP inhibitors which prevent new blood vessel formation both in vitro and in vivo.[256, 257] MMPs are likely to contribute to neovascularisation pathways in several ways including the facilitation of movement of proliferating vascular sprouts through the surrounding stroma and liberation of stimulatory angiogenic factors normally sequestered in inactive form within the ECM.

Movement of endothelial cells is further mediated through interactions with the matrix via integrins. These integrins include $\alpha_v\beta_3$ which is constitutively expressed at low levels on quiescent blood vessels but up regulated on the surface of endothelial cells of newly formed capillaries following exposure to stimuli such as VEGF.[258, 259] The interaction between VEGF and $\alpha_v\beta_3$ activates a calcium-dependent signalling pathway which promotes endothelial cell migration.[260] Disruption between matrix and integrin using $\alpha_v\beta_3$ antagonists results in endothelial cell apoptosis and termination of the angiogenic response.[259] $\alpha_v\beta_3$ also binds directly to MMP-2 on the surface of proliferating, invading endothelial cells thereby facilitating cell surface localisation of this enzyme and potentiating its ECM matrix degradative effects.[261] A non-catalytic carboxy-terminal haemopexin-like domain of MMP-2 (known as PEX) is also generated during angiogenesis. PEX is responsible for blocking binding of $\alpha_v\beta_3$ and MMP-2 and hence produces a

negative feedback system to down-regulate new blood vessel formation after the initial stimulus[262].

Pericytes, pluripotential perivascular cells of mesenchymal origin, are involved in the final step in angiogenesis - the assembly of endothelial cells into tubes and remodelling of the immature vascular bed. Interactions between pericytes and endothelial cells, via long cytoplasmic processes, are of key importance in the stabilisation of newly formed, leaky blood vessels.[263] The function of pericytes in these pathways of maturation and maintenance are in turn under the influence of the BB isoform of platelet-derived growth factor (PDGF)[264] and angiopoietin (Ang)-1 and -2.[265]

The angiopoietins are produced by mesenchymal cells and are expressed at sites of blood vessel proliferation and remodelling. The Ang family of proteins appears to play complementary and co-ordinated roles in vascular development with VEGF. They bind to the Tie (tyrosine kinase with immunoglobulins and EGF homologous domains) receptors, of which two have been described: Tie-1 and Tie-2.[266] Although a ligand for Tie-1 has not yet been confirmed, Ang-1 binds to Tie-2 leading to increased permeability and sprouting of endothelial cells and pericyte recruitment to the vascular bed.[267] Ang-2, which exhibits 60% homology with Ang-1, also binds to Tie-2 but acts as a natural antagonist to Ang-1.[268] Ang-2 de-stabilises mature blood vessels by displacing Ang-1 from its receptor Tie-2. Inter-relationships between the VEGF and angiopoietin families are further demonstrated by studies where disruption of a stable vascular bed by Ang-2, in the absence of

VEGF, leads to vessel regression; if VEGF is highly expressed, however, further neovascularisation occurs.[269] This model may be of clinicopathological relevance in situations where VEGF levels are elevated, Ang-2 is highly expressed and active neovascularisation is occurring. In addition, strategies to block VEGF might not work solely by inhibition of its direct angiogenic effects but perhaps by allowing Ang-2-induced regression to proceed uninterrupted. Fiedler et al have found that Ang-2 is a key cytokine which can up regulate both inflammation and angiogenesis – two processes thought to involve relatively few common molecular mechanisms.[270, 271] The study findings suggest that on blood-vascular endothelial cells (BECs), Ang-2 operates as a counterbalance to the anti-inflammatory Ang-1.[270] Not only does Ang-2 destabilise mature vessels, it also promotes inflammation and VEGF-induced angiogenesis. The study demonstrates that the effects of both Ang-1 and Ang-2 are mediated through the same receptor, Tie-2. Activation of Tie-2 by Ang-1 seems to down-regulate inflammation (paracrine regulation) by blocking nuclear transcription factor kappa B (NF-κB;[272] and prevents the uncontrolled onset of inflammatory reactions induced by minor amounts of TNF-α.[270] However, the study illustrates that TNF-α induces production and secretion of Ang-2 by the endothelium itself (autocrine regulation).[270] Thus, competitive binding of Ang-2 to Tie-2 blocks the anti-inflammatory function of Ang-1 and sensitises the endothelium to the TNF-α signals. Furthermore, Ang-2 also sensitises endothelial cells to VEGF, thus promoting angiogenesis, vascular rearrangement, endothelial cell migration and proliferation.[270, 271]

1.3.3.b Anti-angiogenic regulators

1.3.3.b.i Thrombospondins

Angiogenesis is under the control of both stimulatory and inhibitory regulators. The best characterised of the inhibitory molecules are the thrombospondins (TSPs) 1-5 of which TSP-1 and -2 have been the most extensively studied. TSP-1 is a multifunctional matrix protein produced by various cell types including dermal microvascular endothelial cells.[273] It accumulates in the basement membrane of quiescent vessels but is absent in actively forming endothelial sprouts.[274] It has been shown to inhibit angiogenesis both in vitro and in vivo and appears to inhibit endothelial cell migration by binding to matrix proteins in addition to endothelial cell surface receptors and integrins such as $\alpha_v\beta_3$.[275, 276] TSP-2, similar to TSP-1 in structure, also exerts powerful anti-angiogenic effects.[277]

1.3.3.b.ii Tissue inhibitors of metalloproteinases

MMP activity is inhibited by specific tissue inhibitors of metalloproteinases (TIMPs) 1- 4 which bind and inactivate MMP in a 1:1 stoichiometric fashion and possess anti-angiogenic properties.[278, 279] TIMP-1 and TIMP-2 have been the most completely characterised molecules of the group and appear to inhibit the activity of most MMPs, although TIMP-1 preferentially inhibits MMP-1 and TIMP-2 blocks MMP-2.[280] A newly described family with anti-angiogenic properties, METH-1 and METH-2, which contain metalloproteinase and thrombospondin domains, specifically inhibits human endothelial cell proliferation in vitro although their mode of action is at present unknown.[281]

1.3.3.b.iii Protein fragments

Negative regulators of new blood vessel formation can be stored in the ECM, often as cryptic segments within larger proteins which are not themselves inhibitors. Examples of such molecules include: angiostatin,[282] a 38 kilo Daltons (kDa) fragment of plasminogen; and endostatin,[283] a 20kDa carboxy-terminal fragment of collagen XVIII. These factors exert their angioinhibitory effects by reducing endothelial cell migration and proliferation and by promoting apoptosis.[282, 284]

1.3.3.c Vascular structure and function in brain tumours

Normal brain structure is highly specialised and brain tumours disrupt this structure. As well as endothelial cells and pericytes, astrocytes form part of the structure that selectively restricts the exchange of molecules between the intracerebral and extracerebral circulations. This is termed the blood brain barrier (BBB).

Tight junctions between endothelial cells prevent any hydrophilic molecule over 500kDa from passively entering the brain. In addition to this there are several active transport proteins that exclude exogenous compounds from the brain such as the P-glycoprotein/multi-drug resistance proteins (P-gp/MDR) that contribute to drug resistance in brain tumours.[285] It is felt that this is why there is an increased incidence of brain metastases in extracranial tumours treated with new biological agents, where this barrier creates a sanctuary for the tumour cells.[286]

Brain tumour vessels show typical neoplastic features of marked angiogenesis with endothelial proliferation.[287] The blood brain barrier is

disrupted in large areas of the tumour vasculature but there is preservation of its function within some of the tumour vessels with less transvascular transport than in the same tumour types grown subcutaneously.[288] The abnormal leakiness of tumour vessels adds to tumour tissue extracellular fluid accumulation causing oedema and an increase in tumour tissue fluid pressure which has been shown to be a cause of reduced drug delivery to tumours.[289]

1.3.4 Activities of VEGF

1.3.4.a Role of VEGF in physiological angiogenesis

Angiogenesis, the formation of new blood vessels from a pre-existing vascular bed, is important for a number of physiological processes such as tissue repair, reproduction[290] and endocrine gland function.

1.3.4.a.i Embryonic and postnatal development

In 1996, two studies demonstrated an essential role of VEGF in embryonic vasculogenesis, angiogenesis and early haematopoiesis in mice and that inactivation of a single VEGF allele resulted in embryonic lethality between 11 and 12 days.[291, 292] Further investigations identified a critical VEGF gene-dosage dependence during development. For instance, conditional VEGF gene inactivation in VEGF loxP mice, using a Nestin promoter-driven Cre-recombinase (causes recombination), showed that severe reductions in the dosage of VEGF from neural progenitor cells led to decreases in vascularity and subsequent hypoxia, resulting in the specific degeneration of the cerebral cortex and neonatal lethality.[293, 294] In contrast, even modest

increases in VEGF gene expression, achieved by the insertion of a LacZ cassette in the 3'-untranslated region of the VEGF gene, result in severe abnormalities in heart development and embryonic lethality at embryonic day 12.5 – 14.[295] VEGF-C also plays an essential role in development, as its inactivation results in embryonic lethality due to defective lymphatic development and fluid accumulation in tissues[221]. However, by comparison, inactivation of placental growth factor (PlGF)[296] or VEGF-B[297] does not result in major development abnormalities.

In early postnatal life VEGF is required not only for proliferation but also for survival of endothelial cells.[298] VEGF has a key role in neonatal renal development[299] although in adult mice,[298] rats[300] and in juvenile primates[301] VEGF neutralisation has no significant effect on glomerular function.

1.3.4.a.ii Skeletal growth and endochondral bone formation

Endochondral bone formation is a fundamental mechanism for longitudinal bone growth during which cartilage, an avascular tissue, is replaced by bone in the process of endochondral ossification.[302] It is reported that a VEGF gradient is needed for directional growth and cartilage invasion by metaphyseal blood vessels[298, 303] and VEGF blockade in developing mice and primates is accompanied by almost complete arrest of this process together with impaired trabecular bone formation and marked expansion of the hypertrophic chondrocyte zone.[298, 301] An important corollary is that cessation of anti-VEGF treatment is followed by capillary invasion, restoration of bone growth, and normalisation of the growth plate architecture.

1.3.4.a.iii Angiogenesis in endocrine glands

Angiogenesis is key for normal cyclical ovarian function. Selection of a dominant follicle in monovular species,[304] follicular growth, and the development of the corpus luteum are dependent on the proliferation of new capillary vessels.[305] Angiogenesis associated with corpus luteum development also plays a key role in the delivery of cholesterol to luteal cells for progesterone biosynthesis.[306] Subsequently, the blood vessels regress, suggesting the coordinated action of inducers as well as inhibitors of angiogenesis in the course of the ovarian cycle.[268, 307] Previous studies have observed that the VEGF mRNA expression is temporally and spatially related to the proliferation of blood vessels in the ovary[308, 309] and that administration of VEGF inhibitors delays follicular development[310] and suppresses luteal angiogenesis in rodents[311, 312] and primates.[301, 313-315]

Studies have identified a novel angiogenic factor, endocrine gland derived VEGF (EG-VEGF), which is selectively expressed in steroidogenic tissues and which plays a cooperative role with VEGF in the regulation of angiogenesis in the human ovary.[316] EG-VEGF is not structurally related to VEGF but belongs to a unique gene family having distant homology to Dickopf, an inhibitor of Wnt signalling.[317, 318]

Investigators have also demonstrated that VEGF is required for the formation of a dense network of fenestrated capillaries in some, but not all, pancreatic islets. In addition, glucose tolerance tests reveal that the VEGF-induced capillary network is not strictly required for blood glucose control but is essential for fine tuning blood glucose regulation.[319]

1.3.4.b Role of VEGF in physiological lymphangiogenesis

VEGF is well established as a pro-angiogenic cytokine. However, depending on the target tissue, it may also induce lymphangiogenesis[320, 321] and support the growth of isolated LECs.[322] At least some of the effects of VEGF on lymphatic vessels might be indirect, secondary to oedema or to recruitment of inflammatory cells that produce VEGF-C and VEGF-D.[323, 324] In addition, several other molecules are known to be important for later stages of lymphatic development. These include Ang-2;[325] podoplanin, a cell surface glycoprotein;[326, 327] Net, a member of the Ets transcription factor family;[328] and the integrin α9β1 complex which might be involved in lymphatic vessel stabilisation.[329] Interestingly, the vascular expression pattern of neuropilin (NP)2 resembles that of VEGFR-3. VEGF-C binds to NP2, suggesting that NP2 could act as a co-receptor for VEGFR-3.[330]

1.3.4.c Mitogenesis and endothelial survival

VEGF is a survival factor for endothelial cells, both in vitro and in vivo.[331-337] In vitro, VEGF prevents endothelial apoptosis by mechanisms mediated by the phosphatidylinositol 3-kinase (PI3 kinase) /Akt pathway[336, 338] and also induces expression of the anti-apoptotic proteins Bcl-2, A1,[335] X-linked inhibitor of apoptosis (XIAP),[339] and survivin[340] in endothelial cells. In vivo, the ability of VEGF to prolong the survival of endothelial cells is dependent on developmental stage. For instance, VEGF inhibition results in extensive apoptotic changes in the vasculature of neonatal, but not adult, mice.[298] It has also been shown that VEGF stimulates surfactant production by alveolar Type II cells, resulting in a protective effect from respiratory distress

syndrome in mice.[341] Studies in amyotrophic lateral sclerosis have illustrated the potential role of VEGF as a neuronal protective factor.[342]

1.3.4.d Effects of VEGF on bone marrow cells and haematopoiesis

VEGF has been shown to promote monocyte chemotaxis[343] and to have haematopoietic effects.[344] Delivery of VEGF to adult mice has been reported to inhibit the development of dendritic cells[345, 346] and it has been suggested that in this way VEGF facilitates tumour growth through escape from the host immune system. VEGF also increases production of B-cells and the generation of immature myeloid cells.[347]

1.3.4.e Enhancement of vascular permeability and haemodynamic effects

VEGF, through mechanisms which involve nitric oxide (NO),[348-350] plays a key role in the regulation of vascular permeability and has been shown to induce endothelial fenestration in some vascular beds.[351] There has been much debate whether there is a correlation between vascular permeability and angiogenesis. Some investigators have suggested that an increase in microvascular permeability is necessary for angiogenesis as it permits extravasation of fibrin, which can act as a scaffold for endothelial cell proliferation and migration.[352, 353] However, others[354] have reported that members of the Src family are differentially involved in mediating VEGF-dependent permeability and that enhanced vascular permeability is not a requirement for VEGF-dependent angiogenesis.

VEGF induces vasodilatation in vitro in a dose-dependent fashion[355, 356] and has a tonic homeostatic role in the regulation of blood pressure. The

mechanism is likely to involve endothelial NO synthase, but remains to be fully elucidated. Hypotension has been observed to be a dose-limiting side effect in human trials in which VEGF was systemically administered[357] and conversely, administration of anti-VEGF monoclonal antibodies to cancer patients results in blood pressure elevation.[358]

1.3.5 VEGF Isoforms

The human VEGF gene is localised on chromosome 6p21.3[359] and has nine possible exons.[360, 361] As a result of alternative exon splicing a number of different VEGF isoforms, of varying amino acid length, can be generated (Figure 1.25).[225] Commonly occurring VEGF isoforms include - $VEGF_{121}$, $VEGF_{165}$, $VEGF_{189}$, $VEGF_{206}$.[360, 361] However, less frequent isoforms have also been described including - $VEGF_{145}$,[362] $VEGF_{183}$,[363] and $VEGF_{162}$.[364] Substitution of exon 8 for exon 9 by alternate splicing is reported to convert pro-angiogenic forms into inhibitory forms such as $VEGF_{165b}$.[365] Mouse and rat isoforms are shorter by one amino acid.

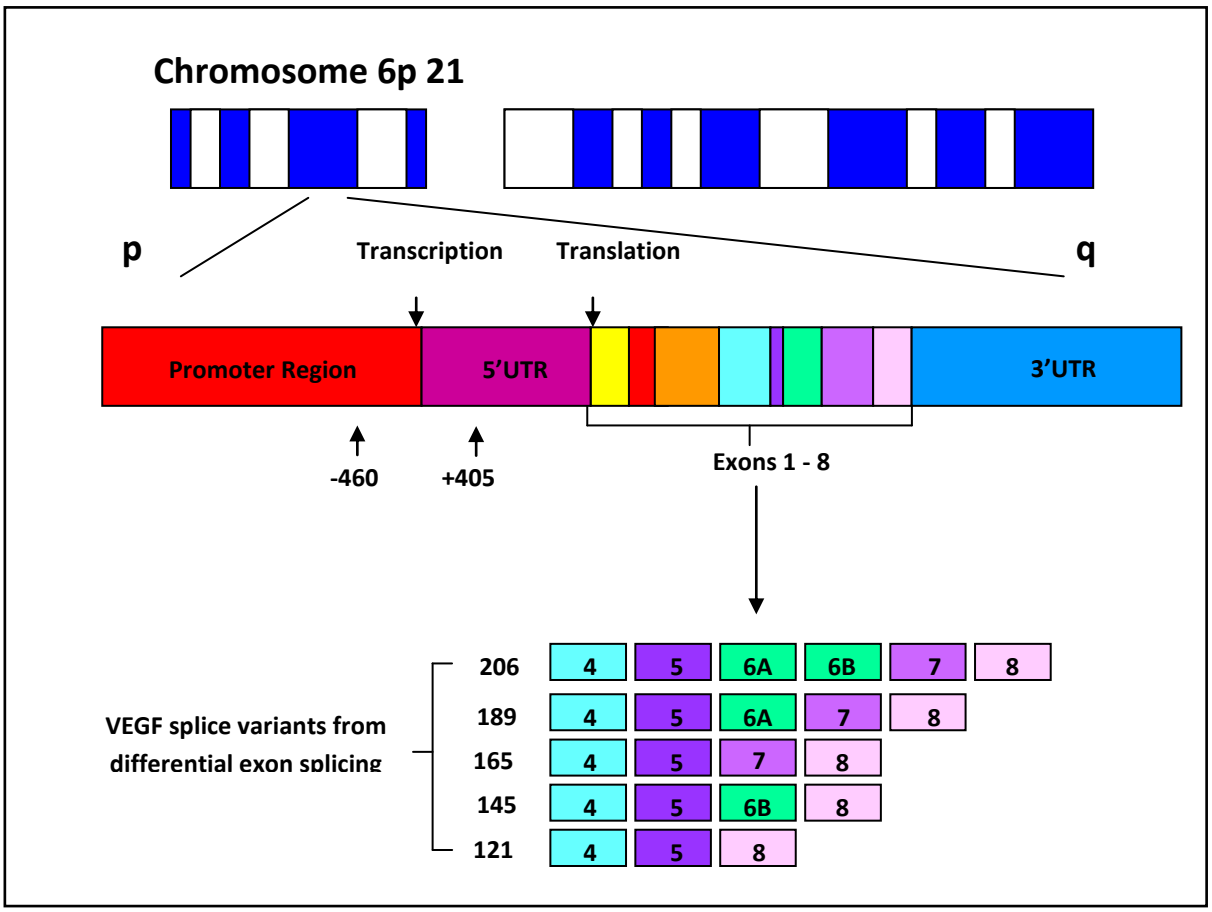


Figure 1.25 - The VEGF gene is located on chromosome 6 - at 6p21 - and is highly polymorphic. The two polymorphisms occurring at highest frequency in the promoter and 5 prime untranslated regions of the gene are situated at positions -460 and +405 (numbering from transcription start). As a result of alternative exon splicing a number of different VEGF isoforms, of varying amino acid length, can be generated. Commonly occurring VEGF isoforms include - $VEGF_{121}$, $VEGF_{165}$, $VEGF_{189}$, $VEGF_{206}$.

$VEGF_{165}$ is the major VEGF isoform[366] and is a heparin-binding homodimeric glycoprotein of 45kDa[233] which structurally forms an antiparallel homodimer (two VEGF monomers oriented side-by-side and head-to-tail) covalently linked by two disulphide bridges between cystine-51 and cystine-60.[367] The dominant feature within the VEGF monomer is the cystine knot motif that is found in other growth factors of the "cysteine-knot superfamily", whose members are characterised by a common motif of eight spatially conserved cysteines, which are involved in intra- and intermolecular disulfide bonds.[367,

[368] $VEGF_{165}$ has optimal characteristics of bioavailability and biological potency because although it is secreted, a significant fraction remains bound to the cell surface and ECM.[369] In contrast, $VEGF_{121}$ is an acidic polypeptide which fails to bind heparin and thus remains freely diffusible.[225, 366] $VEGF_{189}$ and $VEGF_{206}$ are highly basic and therefore bind heparin with high affinity and are almost completely sequestered in the ECM.[366] The ECM-bound isoforms may be released in a diffusible form by heparin or heparanase, which displaces or liberates them from their binding to heparin sulphate moieties. Loss of the heparin-binding domain results in a reduction in the mitogenic activity of VEGF[370] and other studies have demonstrated that the heparin-binding VEGF isoforms provide essential stimulatory cues for the initiation of vascular branch formation.[371]

1.3.6 VEGF Receptors

VEGF binds to two, specific, structurally related, high- affinity type III receptor tyrosine kinases (RTKs), VEGFR-1 (also known as flt-1; fms-like tyrosine kinase) and VEGFR-2 (also known as KDR; kinase-insert-domain-containing receptor), triggering signal transduction pathways that mediate the angiogenic and permeability modulating responses.[372] VEGF-C binds not only to VEGFR-2 to induce angiogenesis,[240, 373] but also to a third, structurally related, receptor, VEGFR-3 (also known as flt-4), on lymphatic endothelium to induce lymphangiogenesis.[240, 374] VEGFR-3 also binds VEGF-D.[374] Both VEGFR-1 and VEGFR-2 have seven Ig-like domains in the extracellular region, a single-transmembrane component, and a consensus tyrosine kinase sequence that is interrupted by a kinase insert domain.[375-377]

Neuropilins (NPs), cell surface glycoproteins important in neuronal development, have been identified as a family of co-receptors for VEGF.[225]

VEGF, VEGFR-1 and VEGFR-2 represent a regulatory system essential for both normal and pathological angiogenesis[378-382] and both receptors are almost exclusively expressed within endothelial cells and preferentially within proliferating endothelium.

1.3.6.a VEGFR-1

VEGFR-1 is expressed in two forms:[381, 383] a full-length, membrane bound receptor capable of transducing signal, and a truncated, soluble receptor capable of sequestering ligand or dimerising with full-length receptor and preventing signal transduction – soluble (s)VEGFR-1.[381] Gene-targeting studies have demonstrated the essential role of this molecule during embryogenesis. VEGFR-1 knockout mice die in utero at approximately the ninth day.[379, 384] In these mice endothelial cells develop but fail to organise in vascular channels. VEGFR-1 binds VEGF, PlGF[385] and VEGF-B.[238] The binding site for VEGF (and PlGF) has been mapped primarily to the second Ig-like domain.[333, 386, 387] VEGFR-1 has a greater affinity for VEGF than VEGFR-2,[388-390] yet VEGFR-2 is phosphorylated approximately 10-fold more efficiently upon ligand binding.[382, 391]

VEGFR-1 expression is up-regulated by hypoxia via a hypoxia-inducible factor (HIF)-1 dependent mechanism.[392] High levels of sVEGFR-1 reportedly occur in plasma during pregnancy and in patients with essential hypertension.[393-395] Significantly lower levels have been observed in the plasma of patients with

cardiovascular disease and in smokers.[396, 397] In contrast to sVEGFR-1, which is an alternatively spliced cellular product, circulating sVEGFR-2 is thought to derive from shedding of the receptor from endothelial cell membrane into the circulation. Thus, circulating levels of both VEGF receptors could be regarded as surrogate markers of endothelial cell activity – with increased endothelial cell turnover likely to result in shedding of membrane bound receptor into the plasma.

Although VEGFR-1 was the first RTK to be identified as a VEGFR,[391] the precise function of this molecule is still the object of debate. Park et al. proposed that VEGFR-1 may be a "decoy" receptor that functions to limit VEGF/VEGFR-2 mediated angiogenesis by sequestering VEGF ligand and rendering it less available to VEGFR-2.[385] Gille et al. identified a repressor motif in the juxtamembrane region of VEGFR-1 that impairs PI3 kinase activation and endothelial cell migration in response to VEGF.[398] However, there is evidence that the tyrosine kinase domain of VEGFR-1 does play an angiogenic role and other studies have indicated that VEGFR-1 is able to interact with various signal-transducing proteins and generate, in some circumstances, a mitogenic signal.[399, 400]

Induced VEGFR-1/VEGFR-2 heterodimers can transduce signal.[380] VEGF-induced NO release appears to be mediated by VEGFR-1, and this NO release in turn acts as a molecular switch, inhibiting VEGFR-2 mediated proliferation and affecting de-differentiation of endothelial cells into capillary-like structures.[378] Autiero et al. have proposed that PlGF regulates inter- and

intramolecular cross-talk between the VEGF RTKs.[401] Activation of VEGFR-1 by PlGF resulted in transphosphorylation of VEGFR-2, thus amplifying VEGF-driven angiogenesis through VEGFR-2.[401] Experimental findings have also suggested that a key function of VEGFR-1 signalling in the vascular endothelium is not the regulation of angiogenesis but, rather, the paracrine release of tissue-specific growth/survival factors, possibly in a vascular bed-specific fashion.[402] Other studies suggest that at least in some circumstances, VEGFR-1 may transmit a pro-survival signal in endothelial cells, possibly mediated by induction of the anti-apoptotic gene survivin.[403]

1.3.6.b VEGFR-2

VEGFR-2 has a key role in developmental angiogenesis and haematopoiesis. In VEGFR-2 null mice, defective vasculogenesis leads to a failure to develop blood islands and organised blood vessels which results in intrauterine death between day 8.5 and day 9.5.[404] The VEGF binding site has been mapped to the second and third Ig-like domain[405] and VEGFR-2 undergoes dimerisation. It has been suggested that VEGFR-2 is the major mediator of the mitogenic, angiogenic, and permeability-enhancing effects of VEGF. VEGFR-2 activation by VEGF results in PI3 kinase/Akt-dependent activation of several integrins[406] and also induces endothelial cell growth by activation of the Raf-Mek-Erk pathway. VEGFR-2 activation has been shown to be required for the anti-apoptotic effects of VEGF for human umbilical vein endothelial cells with the pro-survival effect mediated by the PI3 kinase/Akt pathway.[336]

1.3.6.c VEGFR-3 (Flt-4)

In adults, the expression of VEGFR-3 is confined to the lymphatic endothelium.[407] VEGF-C and VEGF-D, which bind to, and activate, VEGFR-3, induce lymphangiogenesis (e.g. in the differentiated chick chorioallantoic membrane and when delivered to mouse skin using adenoviruses or by transgene expression.[408-410] Conversely, inhibition of these ligands by the expression of a soluble VEGFR-3–Ig fusion protein in mice starting at embryological day 15 caused regression of the developing lymphatic vessels by endothelial cell apoptosis.[411]

1.3.6.d Neuropilin (NP)1 and 2

Some tumour and endothelial cells were reported to express cell surface VEGF-binding sites distinct in affinity and molecular mass from the VEGF RTKs.[412] It was also observed that $VEGF_{121}$ failed to bind these sites, indicating that exon 7-encoded basic sequences were required for binding to this putative receptor.[412] Subsequently, Soker et al. identified NP1, a molecule previously implicated in neuronal guidance, which enhanced the binding of $VEGF_{165}$ to VEGFR-2.[413] NP1 has also been shown to bind directly with VEGFR-1, suggesting that one of the mechanisms by which VEGFR-1 functions as a negative regulator of VEGF activity is competing for NP1 binding.[414] Other studies have linked another of the NP family, NP2, to lymphatic vessel development.[415]

1.3.7 Role of VEGF in Pathological Conditions

VEGF has been implicated in the pathogenesis of a large number of pathological conditions including solid tumours, haematological malignancies, intraocular neovascular syndromes, cerebral oedema and diseases of the female reproductive tract.[290] VEGF up-regulation has also been implicated in various inflammatory disorders including wound healing, rheumatoid arthritis and psoriasis.[416, 417] Reinders et al. provided evidence for a role of VEGF as a proinflammatory mediator in allograft rejection. VEGF was found to be functional in the trafficking of human T-cells into skin allografts in vivo in the humanised severe combined immunodeficiency (SCID) mouse.[418]

Whereas tissue repair and tumour growth are predominantly associated with sprouting angiogenesis (i.e., the outgrowth of new capillaries from pre-existing vessels[419, 420]), the predominant type of angiogenesis observed during inflammation consists of vascular enlargement of pre-existing vessels rather than the formation of new blood vessels.[421] However, endothelial cell proliferation and vascular hyperpermeability are shared by both types of angiogenesis, and enlarged and hyperpermeable dermal microvessels are also a consistent feature of the skin inflammation associated with delayed-type hypersensitivity (DTH) reactions.[422]

Lymphatic vessels provide one of the main routes for tumour metastasis, especially for tumours of the breast, lung and gastrointestinal tract, which frequently colonise draining regional lymph nodes. Compared to the blood vasculature, relatively little is known about the biology of lymphatic vessels in tumours, the regulation of tumour lymphangiogenesis or the mechanisms

that determine the interactions of tumour cells with the lymphatic vessels. Although peritumoural lymphatic vessels contribute to tumour metastasis, opposite views exist as to whether intratumoural lymphatics have any role in tumour metastasis.[423] Many human tumours express VEGF-C, and increased VEGF-C expression correlates with lymph node metastasis in, for example, thyroid, prostate, gastric, colorectal and lung cancers.[424, 425] In breast cancer, VEGF-C expression correlates with lymph node positive tumours, whereas VEGF-D showed expression predominantly in inflammatory breast carcinoma.[426] Interestingly, although there are no lymphatic vessels in brain tumours, VEGF-C and VEGFR-3 have both been shown to be expressed in haemangioblastomas and glioblastomas.[427] Another study showed correlation of VEGFR-3 and tumour grade in grade 2 to 4 gliomas.[428] The mechanisms regulating VEGF-C or VEGF-D expression in tumours are not fully understood. Although VEGF-C is commonly expressed in cancer, it is not known to what extent tumour cells are directly responsible for the secretion of lymphangiogenic growth factors, such as VEGF-C and VEGF-D.[429]

Studies using various rodent models have provided evidence that tumour lymphangiogenesis facilitates lymphatic metastasis. Similarly, overexpression of VEGF-C or VEGF-D in murine tumours increased the number of peri- and/or intra-tumoural lymphatic vessels and enhanced metastasis to regional lymph nodes.[245, 430-432] The secreted, soluble VEGFR-3–Ig fusion protein produced by transfected human breast or lung carcinoma cells that have a high VEGF-C expression, or delivered by a systemic route

using adenoviruses, inhibits tumour lymphangiogenesis and lymph node metastasis in immunodeficient mice, further supporting the role of lymphatics in tumour development.[430, 433]

1.3.8 VEGF in brain tumours

VEGF and its receptors have been shown to be expressed in brain tumours and it is felt to play a major role in tumour angiogenesis and formation of peri-tumoural oedema (Figure 1.26).[434-436] VEGF has been shown to be expressed in gliomas,[436] haemagioblastomas,[437] meningiomas,[438] medulloblastomas[439] and ependymomas.[440]

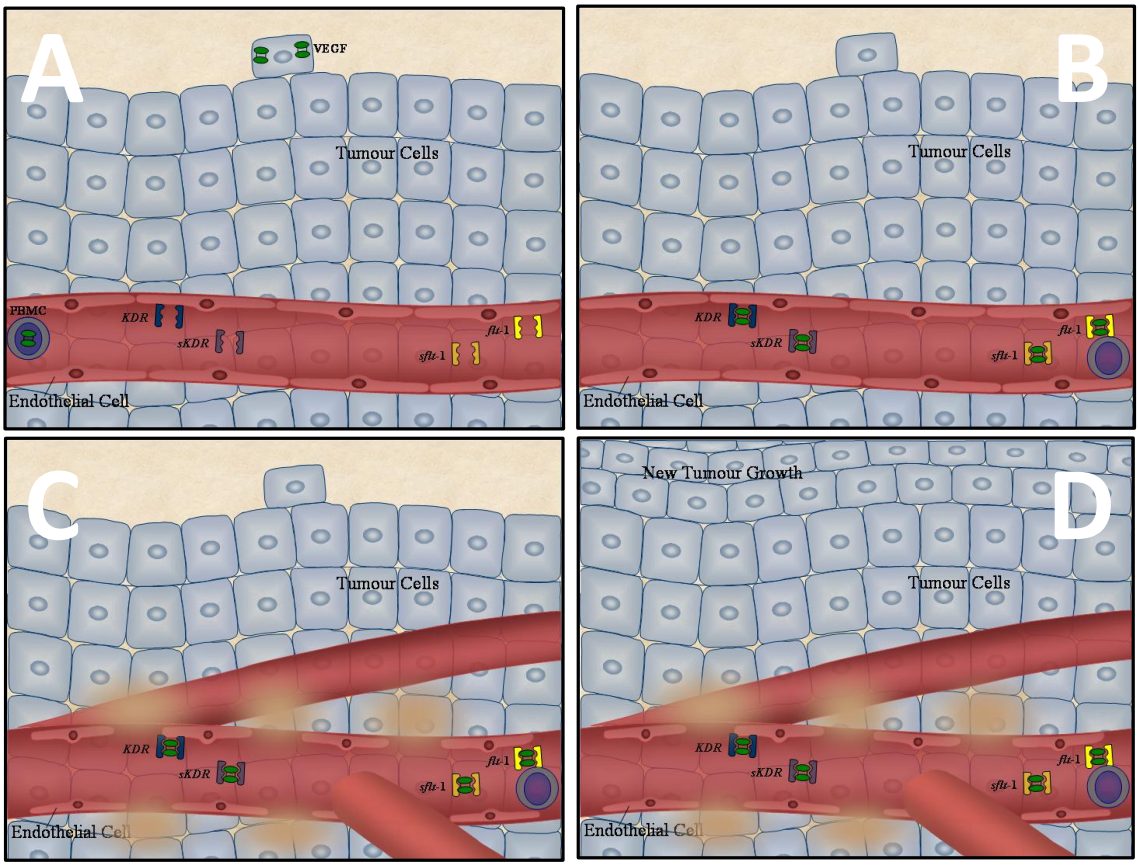


Figure 1.26 – The biology of VEGF in brain tumours. A - VEGF is a multifunctional cytokine that is stimulated to be produced by tumour and peripheral blood mononuclear cells by various triggers including hypoxia and hypoglycaemia. B - VEGF binds to two receptors *flt-1* and *KDR* which are present in both membrane bound and soluble forms. C - Following receptor binding, signalling pathways cause both new vessel formation and increased vascular permeability. D - New vascular supply allows delivery of the necessary substrates for tumour growth and progression.

1.3.8.a Astrocytomas

Low grade astrocytomas have a vascular pattern that is similar to normal brain, whereas glioblastomas are amongst the most vascular solid tumours seen.[441] Thus it is felt that in order for a low grade tumour to progress, there is a need for an "angiogenic switch".[12, 434]

In glioblastomas, VEGF expression is 50 times that of normal brain.[436] This expression is spatially restricted to perinecrotic (palisading) cells[442, 443] suggesting that hypoxia is able to induce VEGF in vivo. There is a positive

correlation of VEGF levels and general vascularity of tumours.[444] Abnormal vessel morphometry (e.g. vessel shape, length, compactness and branching), which is also associated with VEGF levels[445] has been shown to correlate with grade of astrocytoma and survival.[446] In addition cerebrospinal fluid levels of VEGF have been shown to correlate with tumour grade in astrocytomas.[447]

VEGFR-2 is confined to vascular cells of high grade gliomas, whereas VEGFR-1 is expressed in low and high grade gliomas, whilst neither are expressed to any significant level in normal brain. The levels of the receptors correlate with VEGF levels in tumours suggesting a co-ordinated up regulation as a component of tumour progression, possibly mediated via autocrine action of VEGF itself.[442, 448] In addition to this as well as tumour grade VEGF also correlates with survival in astrocytomas.[449-452]

Although vascular proliferation is a hallmark for malignant progression in low-grade astrocytomas, pilocytic astrocytomas seem to be an exception. Leung et al. found high level of VEGF transcripts with up-regulation of its receptors VEGFR-1 and VEGFR-2 in 14 pilocytic astrocytomas.[453] These findings suggest that VEGF expression in pilocytic astrocytoma may be responsible for the pronounced vascular proliferation and cyst formation commonly observed in these tumours. As hypoxia in low-grade astrocytomas has been not shown, it is still speculative, how VEGF is regulated in these tumours. A proposed mechanism to explain the expression of VEGF mRNA in low-grade astrocytomas is loss of function of

tumour suppressor gene p53.[454] However, immunohistochemical studies have failed to show a correlation between p53 accumulation (the mutant protein has an increased half-life) and VEGF expression.[442, 455]

1.3.9 VEGF and Therapeutic Angiogenesis

"Therapeutic angiogenesis" may be beneficial for conditions characterised by inadequate tissue perfusion as, at present, there are no effective alternatives to surgical reconstruction procedures. Early studies indicated that intra-arterial or intramuscular administration of $VEGF_{165}$ could significantly augment perfusion and development of collateral vessels in a rabbit model of chronic hind limb ischaemia.[456, 457] Arterial gene transfer with cDNA encoding VEGF also led to revascularisation in the same rabbit model to an extent comparable to that achieved with the recombinant protein.[458, 459]

In clinical trials, arterial gene transfer of naked plasmid DNA encoding $VEGF_{165}$ resulted in angiographic and histological evidence of angiogenesis after 4 weeks in a single patient with severe limb ischaemia.[460] However, a placebo-controlled phase II study (174 patients) in which recombinant human $VEGF_{165}$ was administered as a single intracoronary infusion, followed by three iv injections, did not demonstrate clinical benefit after 60 days, although some improvement in angina class was measured at a later time point.[357] Another controlled trial has reported an increase in vascularity following adenovirus-mediated delivery of $VEGF_{165}$ in limb ischaemia patients.[461] Several groups are exploring the possibility that more persistent

exposure to VEGF may achieve better results than in earlier trials. In this context, studies using a conditional VEGF switch showed that early cessation of the VEGF stimulus is followed by regression of newly formed vessels in the heart and liver but, after a critical duration of exposure, vessels persisted for several months and improved organ perfusion was observed.[462]

Other studies have shown that both recombinant[463] and adenovirus-delivered[464] VEGF leads to enhanced blood vessel formation and ossification in models of bone damage - findings which may have future clinical implications.

1.3.10 Anti-VEGF therapy in Brain Tumours

VEGF inhibition has been shown to suppress pathological angiogenesis in a wide variety of models, including genetic models of cancer, leading to the clinical development of a variety of VEGF inhibitors. This was particularly attractive in brain tumours given the high degree of endothelial proliferation and pro-angiogenic growth factor expression seen.[416]

Initial encouraging phase II results were followed by setbacks, such as the lack of efficacy of SU5416 (a VEGFR-2 tyrosine kinase inhibitor, Semaxanib®; developed by SUGEN Inc. a subsidiary of Pfizer Inc., New York, NY, USA) in a phase III study in metastatic colorectal carcinoma in combination with chemotherapy, or the lack of survival benefit in patients with refractory metastatic breast cancer treated with a humanised anti-VEGF

monoclonal antibody (bevacizumab; Genentech, South San Francisco, CA, USA) plus chemotherapy as a third-line therapy.[465]

However, a large phase III study in colorectal carcinoma was the first to provide unequivocal evidence that VEGF inhibition, using bevacizumab in combination with chemotherapy, may provide a substantial clinical benefit, including increased survival.[466] In terms of side-effect profile hypertension was more common in the bevacizumab-treated group but was readily managed in all cases with oral anti-hypertensitive agents.[466] An increased incidence of thrombosis and proteinuria was observed in a phase II but not in a subsequent phase III study. Bevacizumab (Avastin) was approved by the US FDA on 26th February 2004 as a first-line treatment for metastatic colorectal carcinoma.

Subsequent to this there have been various studies on bevacizumab in glioblastoma, and as a consequence of two particular studies, the FDA approved its use for glioblastomas on May 6th 2009.[467, 468] The first trial randomly assigned 167 patients with recurrent glioblastoma to bevacizumab therapy with or without irinotecan.[467] Response rates were reported to be between 28% and 38%, and 6 month progression free survival rates ranged from 43% to 50%. As had been reported in previous studies, most patients reduced their corticosteroid doses by 50% or more due to the marked antipermeability effect of bevacizumab. Adverse events were infrequent, with 8 (4.9%) intracerebral haemorrhages reported, the majority of which were not life-threatening, and 23 (14.1%) thromboembolic complications noted.

The other phase 2 trial evaluated by the FDA involved bevacizumab monotherapy in 48 heavily pre-treated patients with recurrent GBM.[468] The radiographic response rate was 35%, and the 6 month progression free survival rate was 29%. In addition to hemorrhagic and thromboembolic complications, common toxicities observed in these studies included hypertension, proteinuria, fatigue, and wound-healing complications.

As well as targeting VEGF, small molecular inhibitors of VEGFR-2 are currently being investigated. A phase II trial of cediranib which inhibits all known subtypes of VEGFR was undertaken in patients with recurrent glioblastomas.[469] Results were comparable to those reported for bevacizumab, with a response rate of 56% and a 6 month progression free survival rate of 26%.

Other vascular inhibitors include PDGFR, because of its role in pericyte recruitment.[470] The integrins $\alpha_v\beta_3$ and $\alpha_v\beta_5$ are highly expressed by tumour endothelial cells and facilitate angiogenesis as described earlier. Cilengitide (EMD121974) inhibits these integrins and appears promising in GBM patients with methylation of the *MGMT* gene promoter.[471]

1.3.11 Genetics

1.3.11.a Genetic mapping of complex disease

Over the last 20 years it has become possible to identify the genes underlying many monogenic or Mendelian diseases through development of techniques such as genetic linkage and positional cloning methodology. However, detection of the genetic aetiology of complex or multifactorial diseases is more complicated as by definition these conditions also have contributory environmental components. However, recent advances in the definition of haplotype structure and linkage disequilibrium (LD) within the human genome have provided new tools for the study and mapping of complex diseases. In common complex diseases there may be numerous susceptibility genes reported and replication of findings may be difficult. In different populations, a variety of genetic loci might confer genetic susceptibility to a particular disease. This phenomenon is called locus heterogeneity and may arise from difficulty in defining disease phenotype - complex diseases typically vary in severity of symptoms and age of onset. This problem may be diminished by stringent application of clinical diagnostic criteria and through the sub grouping of patients.[472-474]

1.3.11.b Allelic structure of complex diseases

Two models have been proposed to explain the genetic basis for complex diseases – the common disease/common variant (CD/CV) hypothesis and the genetic heterogeneity hypothesis. The CD/CV hypothesis suggests that for complex diseases, the genetic risk is conferred by relatively high frequency disease-predisposing alleles present at a small number of loci. The

alternative, genetic heterogeneity hypothesis, proposes that numerous loci have rare alleles, each of which can cause the disease.[472, 475, 476] Neither model has been confirmed but the CD/CV model has more support based on statistical models of human population expansion and differences in the kinetics of rare and common alleles.[477-479]

The mutation rate is approximately the same for both rare and common alleles. Rare alleles causing Mendelian disease mutations are highly penetrant and are usually under very strong selection pressure due to their adverse effect.[477-479] Susceptibility variants in complex disease appear to have moderate or low penetrance and therefore are much less prone to selection and thus able to reach higher frequencies. Common alleles are likely to be ancestral in origin, whereas rare alleles have relatively rapid turnover due to selection thus allele frequency remains low. In designing a genomics study, knowledge regarding the allelic structure of the disease to be investigated is key. Association studies are more likely to be successful if there are a few predominating alleles. Allelic heterogeneity can be accommodated by linkage analysis although locus heterogeneity may explain unsuccessful replication of linkage loci in genome scans.[478, 480]

There are, however, only a few examples of successful susceptibility gene mapping for complex diseases such as the APOEε4 allele in Alzheimer's disease. Although these disease variants may have higher penetrance and simpler allelic architecture than other complex diseases,[478] the susceptibility

alleles are fairly prevalent in the population thus supporting the CD/CV hypothesis.

1.3.11.c Single nucleotide polymorphisms

Single nucleotide polymorphisms (SNPs) are single base pair variations in genomic DNA for which different alleles exist in normal individuals and where the least frequent allele has a frequency of 0.01 or greater in the general population (Figure 1.27). In addition to bi-allelic polymorphism the term is sometimes also incorrectly used to describe tri- and tetra-allelic polymorphisms together with insertion and deletion variants. Bi-allelic variants comprise 4 different types, with the cytosine / thymine (C/T) - guanine / adenine (G/A) transition accounting for approximately two-thirds of SNPs.[481] It has been estimated that the total number of SNPs in the human genome may be about ten million,[482] with about 3 million of these accounting for common SNPs (minor allele frequency > 20%) or one in every 1 kilo bases (kb).[482-484] Region-specific differences in SNP density occur with SNPs being most frequent in non-coding regions rather than in coding regions. Those SNPs which do occur within coding regions tend to be synonymous SNPs, which do not result in alteration of the amino acid sequence and are probably a result of selection against deleterious alleles.[472, 475]

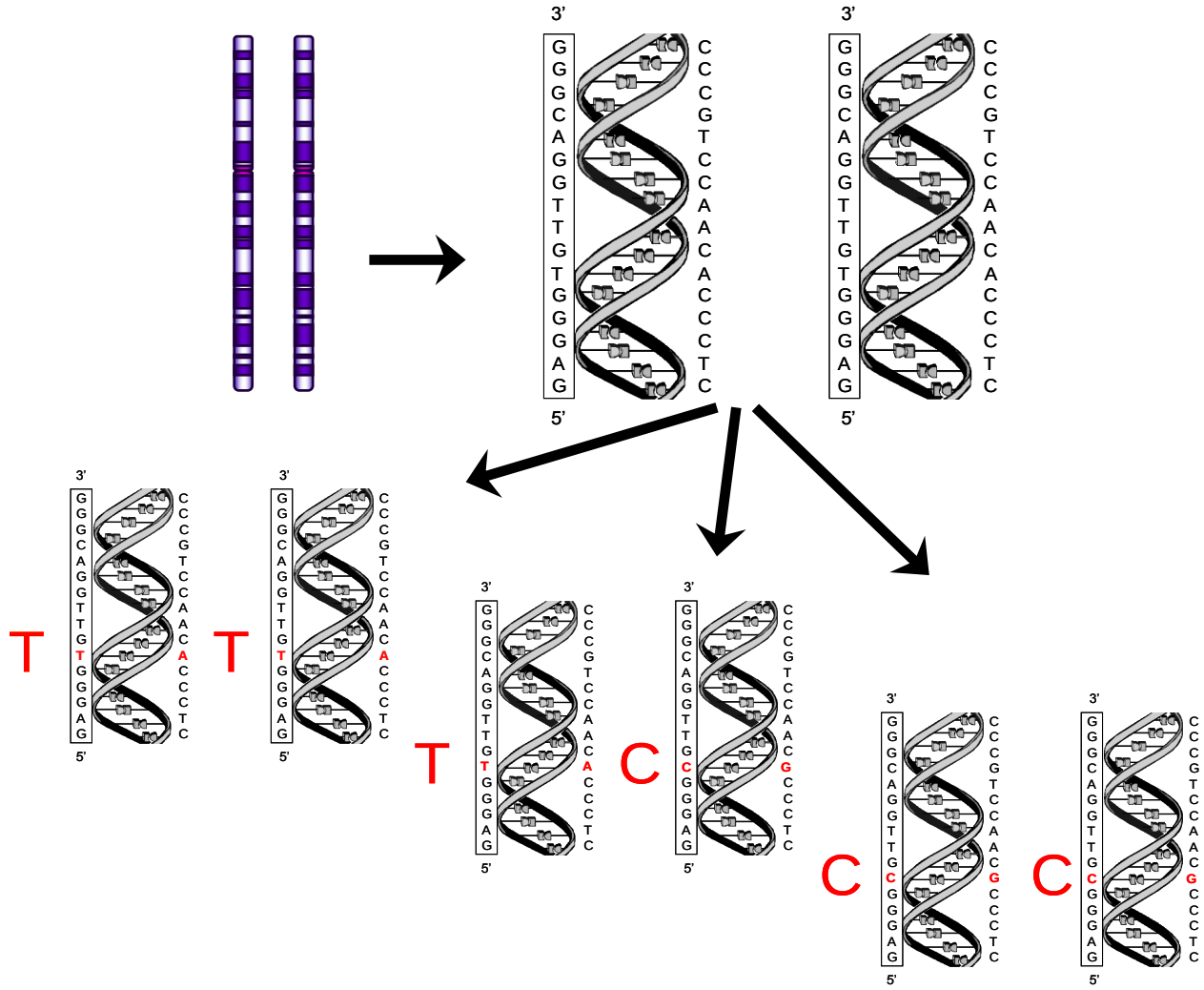


Figure 1.27 - Single nucleotide polymorphisms (SNPs). These are single base pair variations in genomic DNA for which different alleles exist in normal individuals. Bi-allelic variants comprise 4 different types, with the cytosine / thymine (C/T) - guanine / adenine (G/A) transition accounting for approximately two-thirds of SNPs. For bi-allelic polymorphisms individuals can have one of three possible genotypes. The SNP illustrated above is a C/T transition and possible genotypes include TT, TC or CC.

The random nature and low mutation rate of base-changing events confer reasonable stability to SNP alleles.[485] Consequently over 80% are common to all human populations (but with different allele frequencies).[483] Due to the frequency within the genome of SNPs and because it is possible to use high-throughput methods to rapidly genotype them, common SNPs are thought to be good markers for genome-wide mapping of complex diseases.

1.3.11.d Association studies

Association studies are effective tools in the study of complex diseases due to their greater statistical power than linkage analysis when there is locus heterogeneity.[473, 474] Most association studies in complex disease have been performed as candidate-gene studies. Candidate genes are usually selected on the basis of their possible biological function in disease pathogenesis and allelic variants of the candidate gene tested for association.[486, 487] To date, most association studies have used non-synonymous coding SNPs of candidate genes although SNPs within promoter regions or other important regulatory elements may also be disease-causing variants.

The case-control study design is the most commonly employed strategy in association studies. However, study design is key and patient and control groups need to be selected carefully and adequately matched otherwise statistically significant associations may reflect differences between the cases and controls rather than the actual influence of the studied allele. As any systematic allele frequency differences between cases and controls can appear to be an association,[486, 488] isolated populations have been suggested as a good case-control sample set because of the homogenous background.[489] However, population substructure must then be considered.[490] Control ascertainment may also be improved by using a prospective study cohort, as this will also allow monitoring of environmental factors. Furthermore, sample size should be sufficiently large to detect significant results and findings should always be replicated in other populations.[488]

To reduce the effect of population stratification family-based, as opposed to population-based, controls can be used in association analysis. The transmission disequilibrium test (TDT) is the most commonly used family-based association test.[491, 492] However, family-based testing is less powerful statistically than case-control studies and generally requires much larger sample sizes. Indeed, as disease onset is often late in complex disease, parents of the index case may be deceased.[488]

1.3.11.e Genome-wide linkage analysis

Genetic linkage means that alleles from two loci segregate together rather than independently in meiosis because of their close proximity on a chromosome. The extent of linkage between two loci is measured by the recombination fraction (θ), which is the fraction of meiotic events that show recombination between the loci of all possible meioses. For unlinked loci θ = 0.5 and for completely linked loci θ = 0.[493, 494] If the loci are syntenic, i.e. they lie on the same chromosome, then they might be expected always to segregate together, with no recombinants. However, during prophase of meiosis I, pairs of homologous chromosomes synapse and exchange segments. Only two of the four chromatids are involved in any particular crossover. A crossover will create two recombinant chromatids and leave two non-involved chromatids non-recombinant. Thus one crossover generates 50% recombinants between loci flanking it.[495]

Linkage analysis is used to locate a disease gene based on its close proximity to a segregating marker allele on the same chromosome. The standard tools for polymerase chain reaction (PCR) linkage analysis are microsatellites;

these are mostly (CA)*n* repeats. Tri- and tetranucleotide repeats are gradually replacing dinucleotide repeats as the markers of choice because they give cleaner results - dinucleotide repeat sequences are peculiarly prone to replication slippage during PCR amplification.[495] Morton (1955) demonstrated that logarithm ($\log_{10}$) of the odds of linkage (LOD) scores represent the most efficient statistic for evaluating pedigrees for linkage, and derived formulae to give the LOD score (as a function of θ) for various standard pedigree structures.[496] The LOD score is calculated as the ratio between the alternative assumptions that the loci are linked or are not linked as a function of the recombination fraction. LOD scores may then be calculated for a range of θ values and the most likely recombination fraction is the one that produces the highest positive LOD score. In a set of families, the overall probability of linkage is the product of the probabilities in each individual family.[493, 494, 496]

Standard LOD score analysis is a parametric linkage approach where the inheritance pattern, penetrance of the trait and gene frequency must be known or correctly estimated. If these parameters are incorrectly defined, the results can be skewed. In complex diseases, parametric analysis often uses both recessive and dominant inheritance models with different gene frequencies and penetrance values to see which model gives the highest LOD score. However, when performing multiple tests, the threshold for a significant linkage score has to be increased accordingly.

Non-parametric linkage analysis is the method of choice for mapping complex disease genes since prior knowledge of the parameters that define the mode of inheritance is not required.[493] Non-parametric linkage analysis is based on the higher than expected sharing of alleles by affected individuals. Affected-sibling pair (ASP) analysis is the predominant method used and involves monitoring of alleles which are identical by descent (IBD).[493, 497] However, the major disadvantage of this methodology is loss of statistical power because it is often difficult to determine whether two alleles are identical by state (IBS) or IBD. Consequently more families are needed than for parametric analysis[493] and the analysis is limited to pedigrees of moderate size since computational time increases exponentially with the number of individuals included.[498]

For simple Menedelian traits, a parametric LOD score >3 in two-point analysis has traditionally been used as significant evidence of linkage and this corresponds to a 5% significance level for a specific locus and a 9% genome-wide significance level. To minimise false-positive linkage results in complex diseases, more stringent criteria have been suggested. According to the widely accepted criteria of Lander and Kruglyak, the linkage results are classified into the following three categories: suggestive of linkage, significant evidence of linkage and highly significant evidence of linkage.[499] These would be expected to occur 1, 0.05 and 0.001 times in a genome scan respectively. These criteria have been questioned and only replication of a significant linkage result in a further sample can be interpreted as confirmed linkage.[500]

1.3.11.f Linkage disequilibrium (LD) mapping

LD is where alleles at two or more linked loci on the same chromosome occur together more often than by chance. LD is disrupted by recombination, mutation and gene conversion events. Mean LD declines with chromosomal distance, but there is large variation between different chromosomal regions. Recombination will rarely separate loci that lie very close together on a chromosome, because only a crossover located precisely in the small space between the two loci will create recombinants.[495] Therefore sets of alleles on the same small chromosomal segment tend to be transmitted as a block through a pedigree. Considerable variation exists in the size of the blocks in different genomic regions (from 1kb to 200kb). Such a block of alleles is a haplotype and these mark recognisable chromosomal segments which can be tracked through pedigrees and populations.[495] The European and Asian haplotypes are almost identical[479, 501] - characteristics in agreement with the "out of Africa" theory.[502]

The allelic structure of haplotype blocks is promising for genome-wide LD mapping as they can be treated for mapping purposes as alleles at a single highly polymorphic locus.[495] Over 80% of the haplotypes of a block are defined by less than 10% of the total SNPs of the block. Therefore only 2 or 3 SNPs per block, known as tag SNPs, may be needed to identify a block. However, the haplotype map for the whole genome must first be constructed – involving the typing of millions of SNPs in order to identify the tag SNPs. Subsequently, a much smaller subset of SNPs will need characterisation for whole genome LD mapping.[501, 503, 504]

1.3.11.g Use of population isolates

Population isolates have proven useful for mapping and cloning Mendelian disease genes. They offer the advantages of common environment and culture thus helping to reduce some of the background environmental noise surrounding complex diseases. Some population isolates such as Finland, have the advantage of good genealogical records.[489] Nevertheless, the population history and structure of genetic isolates together with the expected disease allele frequency are key considerations when choosing a population isolate for complex disease genetic study.[505] In young population isolates (<100 generations), such as Finland and Iceland, allelic heterogeneity is reduced, but due to a relatively large number of founders it may still be too high for successful mapping of a common complex disease. Very young isolates (<20 generations), such as sub-isolates in the Netherlands and French Canada have study advantages as they have experienced a very narrow bottleneck following rapid genealogical expansion. In these populations the allelic heterogeneity is reduced and LD is thought to extend over longer sections of DNA compared with young isolates.[489, 506]

1.3.12 The VEGF gene

1.3.12.a General considerations

The gene for VEGF is located on chromosome 6p.21 (Figure 1.25).[359, 507] The VEGF gene is highly polymorphic (Figure 1.28) with at least 15 SNPs described in the promoter region and 5'-untranslated region.[13, 508, 509] It is

therefore likely that this area of the VEGF gene represents a genetic "hot spot" and may be a region of key biological importance.[510] In a Manchester, UK based population of 115 mixed race healthy individuals, Watson et al identified the presence of 15 polymorphic sequences within the VEGF gene after screening 1262 base pairs (bp) of the VEGF promoter and all of exon 1 using PCR-single-stranded conformation polymorphism (SSCP) analysis.[13] Eight of the polymorphisms identified either created a new restriction endonuclease recognition site or destroyed an existing site.[13] PCR-restriction fragment length polymorphism (RFLP) typing strategies were developed for ten of the polymorphisms described including the two most common polymorphisms at -460 and +405.[13]

The two most common promoter/5'-UTR SNPs are a C→T transition at position –460 (Genebank accession number rs833061) in the promoter region and a G→C transversion at position +405 (Genebank accession number rs2010963) in the 5'-UTR. These SNPs are useful for association studies as they occur at highest frequency in this area of the gene and have been implicated as candidate SNPs in diseases with a putative angiogenic basis.[511, 512] Watson et al have previously documented significant linkage disequilibrium between the -460 and the +405 SNPs in the VEGF gene.[13] To ascertain haplotypes for the two polymorphisms at -460 and +405 they developed a combined sequence specific priming (SSP) PCR typing system which identified the cis/trans orientation of each allele. The -460 C/+405 G haplotype was found to be the most commonly observed haplotype in the

normal population. The -460 C/+405 C haplotype was very rare and only observed in one out of the 230 chromosomes analysed (frequency=0.004).[13]

Figure 1.28 - The gene for vascular endothelial growth factor (VEGF) is highly polymorphic. A small section of the VEGF gene sequence showing a large number of polymorphisms (highlighted in yellow).

1.3.12.b Regulation of VEGF gene expression

VEGF gene expression is tightly regulated, mostly at the transcriptional level, but also at the translational and post-translational levels.

Hypoxia is the major regulator, via binding of hypoxia inducible factor (HIF)-1 and HIF-2, each consisting of two subunits (α and β) to the hypoxia-responsive element (HRE) located in the VEGF promoter.[513, 514] Other mechanisms regulating VEGF transcription include several growth factors [e.g., epidermal growth factor (EGF), platelet-derived growth factor (PDGF), insulin-like growth factor (IGF)-1, tumour necrosis factor (TNF)-α, transforming growth factor (TGF)-α, TGF-β] and inflammatory cytokines (e.g., IL-1a, IL-1b, IL-6, IL-10 and IL-13).[372]

Hormones are also important regulators of VEGF gene expression. Thyroid stimulating hormone (TSH) has been shown to induce VEGF expression in several thyroid carcinoma cell lines.[515] Adrenocorticotrophic hormone (ACTH) is able to induce VEGF expression in cultured human fetal adrenal cortical cells[516] and several studies have implicated sex steroids as an important stimulus for VEGF regulation in hormone-sensitive tissues. The gonadotropins have been shown to be potent inducers of VEGF transcription in the ovary, both in vivo[311, 443] and in vitro.[517] Oestradiol has been shown to directly activate VEGF transcription through a variant oestrogen response element located 1.5kb from the transcription start[518] and progestins have also been reported to induce VEGF gene transcription in endometrial carcinoma cells.[519]

1.3.12.b.i Transcriptional factors

There have been a number of potential binding sites identified in the 5'-flanking region of the VEGF gene with the most important being specific protein-1 (Sp1), hypoxia-inducible factor 1 (HIF-1), signal transducer and activator of transcription-3 (Stat-3) and activator protein-1 (AP-1).[520]

Via phosphorylation modification, the transcriptional activity of Sp-1 can be regulated by affecting its DNA binding ability.[521] There have been four possible Sp-1 binding sites identified in the -38 to -109bp relative to the transcriptional start site.[520] These binding sites are essential for basal transcription of the VEGF gene and TNF-α dependent promoter activation in a human gliomas cell line.[522] Other factors may have an influence on Sp-1 activity including tumour suppressor genes von Hippel Lindau (*VHL*), *p53*, *p73* and oncogenes such as *Ras*, *Src* and *HER2/neu*. These genes can also have an effect on the transcriptional activity of HIF-1.[520] Stat3 binding sites have been localised to -842 to -849bp and it is activated by a variety of factors including EGF, PDGF, VEGF and IL-6.[523] There are a putative four AP-1 binding sites in the promoter region of the VEGF gene and the signalling occurs through the protein kinase C (PKC) and MAP kinase pathway.[520]

1.3.12.c Biological effect of VEGF single nucleotide polymorphisms

As mentioned above the VEGF gene is highly polymorphic and there have been many studies looking at the various polymorphisms in relation to disease associations, functional significance and prognosis in relation to both individual SNPs and more functionally the associated haplotypes (Table 1.3).

As can be seen from the table there is a huge amount of variation in results with contradictory findings in many studies.

Table 1.3 – The major papers describing VEGF SNP biology. The table summarises the available evidence on the topic of VEGF SNP biology. Nomenclature of SNPs varies in the literature with some groups defining the distance of the SNP from transcription start and others from translation start. The equivalent SNPs are: -1540 C→A (Genebank accession number rs699947) = -2578; -460 C→T (rs833061) = -1498; -116 G→A (1570360) = -1154; +405G→C (rs2011063) = -634). The +936 C→T is in the 3' untranslated region and is consistently named.

Study	-460 Associations	+405 Associations	Genotype Functional	Haplotype	Haplotype Functional	Other SNPs & Prognostic
Watson 2000[13]			Stimulated VEGF Production +405 GG>GC>CC			
Renner 2000[524]			+936 T allele decreased plasma VEGF in healthy subjects			
Awata 2002[511]		+405 CC diabetic retinopathy	+405 CC increased basal serum VEGF in health subjects			
Shahbazi 2002[525]			-1540 CC & -116 GG increased stimulated VEGF			-1540 C Allele & -116 G Allele increases renal allograft failure
McCarron 2002[526]						-116 AA Prostate Cancer
Howell 2002[527]				-1540/-116/+405 CAC melanoma less advanced disease		-116 AA genotype melanoma less advanced and thinner disease
Lambrechts 2003[342]		+405 GG amyotrophic lateral sclerosis	+405 G Allele impairs IRES B translation -116 A Allele reduces VEGF Transcription	-1540/-116/+405 AAG & AGG amyotrophic lateral sclerosis	-1540/-116/+405 AAG & AGG low VEGF Production	-116 AA amyotrophic lateral sclerosis
Stevens 2003[528]					-1540/-460/-160/-152/-116/+405 ACTAGG>ACCAAG=wild type CTCGGC Basal & stimulated VEGF Production	
Chen 2003[529]	-460 T allele calcium oxylate kidney stones					
Yang 2003[530]			-2549 18bp DD genotype increased VEGF transcription			-2549 18bp DD genotype diabetic nephropathy
Lin 2003[531]	-460 T allele Prostate Cancer					
Ray 2004[532]	-460 C allele proliferative diabetic retinopathy					
Hsieh 2004[533]	-460 T allele increased risk endometriosis					
Boiardi 2004[534]		+405 C allele giant Cell arteritis	-2549 18 bp Insertion/Deletion polymorphism II genotype increased stimulated VEGF production			-2549 18bp Insertion giant cell arteritis
Cooke 2004[535]		+405 G allele retinopathy of prematurity				
Young 2004[417]		+405 CC Psoriasis				
Papazoglou 2004[536]						+936 T allele severe pre-eclampsia
Papazoglou 2004[537]						+936 T allele pre-term delivery
Salvarani 2004[538]		+405 C allele Bechet's disease	-2549 18bp II increased stimulated VEGF in healthy adults			-2549 18bp I Bechet's disease +936 T allele uveitis

Study	-460 Associations	+405 Associations	Genotype Functional	Haplotype	Haplotype Functional	Other SNPs & Prognostic
Szeto 2004[539]						-1540 A allele worse survival for peritoneal dialysis patients -1540 CC genotype high serum & low peritoneal dialysate VEGF levels
Kariyazono 2004[540]		+405 G allele coronary artery lesions in Kawasaki's				
Koukourakis 2004[541]			+405 GG decreased VEGF expression and vascular density in non-small cell lung cancer (NSCLC)			-1540 CC decreased VEGF expression & vascular density in NSCLC -116 GG increased VEGF expression in NSCLC
Wolf 2004[542]						+936 C allele increased FDG PET uptake in breast cancer
Lu 2005[543]						+405 GG genotype worse overall survival -460/+405/+936 TCC haplotype better overall survival in breast cancer
Jin 2005[544]						+405 CC genotype & -1540/+405 CC haplotype large tumours and high grade in breast cancer
Kim 2005[545]						-1540 CC genotype poor grade in bladder cancer
Medford 2005[546]						+936 T allele ARDS & increased APACHE scores
Bhanoori 2005[547]		+405 GG endometriosis		-460/+405 T/C lower in endometriosis		
Tzanakis 2006[548]						+405 CC genotype larger, higher grade tumours & worse survival in gastric cancer
Rueda 2006[549]						-116 G & +405 C alleles -116/+405 GC haplotype increased risk of Henoch-Schönlein purpura with nephritis
Sfar 2006[550]		+405 C allele prostate cancer				-116 A & +405 C alleles development and higher grade in prostate cancer
Hefler 2007[551]						-1540/-116/+405 CGC homozygous haplotype worse overall survival in ovarian cancer
Kim 2007[552]						+936 T allele worse survival in all gastric cancer -460 C allele worse survival in stage 0 & 1 gastric cancer
Kong 2007[553]						+405 CC genotype better survival in hepatocellular cancer
Langsenlehner 2007[554]						+405 C allele small tumour size in breast cancer
Kim 2008[555]						+936 T allele better survival in acute myeloid leukaemia (AML) -1540/-460/+405 CTG haplotype worse survival in AML
Kim 2008[556]						+405 GG genotype & +936 T allele &

Study	-460 Associations	+405 Associations	Genotype Functional	Haplotype	Haplotype Functional	Other SNPs & Prognostic
						-1540/+405/+936 CGC haplotype worse survival in colorectal cancer
Lurje 2009[557]						+405 C allele worse progression free survival in stage II colon cancer
Maltese 2009[558]				-1540/-460/+405 ACG & CCC haplotypes increased risk of colorectal cancer		-1540 AA genotype increased risk of colorectal cancer
Masago 2009[559]						-1540 AA genotype -460 CC genotype -116 A allele worse survival in advanced non-small cell lung cancer
Pastuszczak 2009[560]						+405 GG genotype Worse 30 day mortality following coronary artery bypass grafting
Smerdel 2009[561]						-460 CT & +405 CG genotypes better survival in ovarian cancer
Lambrechts 2009[562]				-1540/-116/+405 AAG & AGG no longer associated in amyotrophic lateral sclerosis meta analysis		-1540 AA genotype Amyotrophic lateral sclerosis in males only
Bradbury 2009[563]						+936 C allele & -460/+405/+936 CGC worse survival in oesophageal cancer
Dassoulas 2009[564]						-1540 A & +936 T alleles trend to association with colorectal cancer -1540 A, +405 C & +936 T alleles worse survival in colorectal cancer
Steffensen 2010[565]				-1540 C allele -460 T allele +405 C allele increased serum VEGF in ovarian cancer		-1540/-460/-116/ +405/+936 ACGGC Improved survival in ovarian cancer
Hansen 2010[566]						-1540 CA, -460 CT & +405 CG genotypes Response to chemotherapy in colorectal cancer
Kim 2010[567]						-1540 C & -460 C allele and -1540/-460/+405 ACG homozygous haplotype disease progression in chronic myeloid leukaemia
Hansen 2010[568]			-460 T allele & +405 C allele increased VEGF expression in normal but not cancerous colorectal tissue			
Riuz 2010[569]						-116 GG genotype local invasion in head and neck cancer
Lorenzen 2010[570]						+936 T allele worse event free survival oesophageal cancer

There are a variety of oncological and non-oncological diseases where positive findings have been made, with the common theme of the diseases being a putative link with angiogenesis.

From a functional perspective, a significant correlation has been observed between VEGF protein production and the VEGF +405 polymorphism in both healthy[13] and disease states.[342, 511, 532] Watson et al observed a significant correlation between +405 genotype and LPS-stimulated peripheral blood mononuclear cell (PBMC) VEGF protein production in healthy subjects, with lowest VEGF protein production observed for CC homozygotes and highest production for GG homozygotes.[13] Further work from the Manchester group using a promoter readout assay to analyse the effect of the VEGF -460/+405 polymorphisms on VEGF gene expression, showed that a complex haplotype containing the VEGF -460C and +405G alleles, is associated with 71% higher basal VEGF promoter activity when compared with wild type.[528] The study describes two promoter haplotypes containing the VEGF -460C and +405G alleles, which differ in activity - those containing the -160 C→T (minor allele frequency 0.017) or the -116 G→A (minor allele frequency 0.3) polymorphisms.[528] This suggests that one or both of these SNPs may be functionally influential in VEGF production in a haplotype which contains the VEGF -406C and +405 G alleles.[512, 528]

Elevated serum VEGF levels have also been associated with the +405 C allele in patients with retinopathy and Type 2 diabetes.[511] However, it is arguable that it is inappropriate to measure VEGF in serum, as platelets represent a

large source of stored VEGF which can be released on serum collection.[571] Elevated plasma levels of VEGF have been documented in association with the +405 C allele in patients with amyotrophic lateral sclerosis[342] and the +405 G allele was observed to reduce both internal ribosome entry site mediated VEGF expression and translation of the large (L)-VEGF isoform.[342]

The 5'-UTR of VEGF contains two internal ribosome entry sites, (IRES)-A at nucleotide +744 to +1037 and IRES-B at nucleotide +90 to +482, both of which are involved in enhancing the translation of adenine-uracil-guanine (AUG)-initiated VEGF.[572] However, the predicted secondary structure of the IRES-B sequence is significantly remodelled for the +405 G allele and it has been suggested that this could make the IRES-B site less optimal for IRES-dependent VEGF translation.[342] L-VEGF is 205 amino acid residues longer than the AUG-initiated VEGF forms due to initiation of VEGF translation at the first (out of four) cytosine-uracil-guanine (CUG) codons which is situated at position +498 in the 5'-UTR.[573] Analysis of the secondary structure of the 5'-UTR shows that this initiation codon is located in the immediate vicinity of +405 G→C. Thus sequence variation at this site could significantly alter the structure of the translation initiation codon and could result in reduced production of VEGF.[342] L-VEGF is proteolytically cleaved at the peptide signal sequence, converted to the shorter isoforms ($VEGF_{121}$, $VEGF_{165}$ and $VEGF_{189}$) isoforms and secreted into the extracellular milieu.[573] The role of L-VEGF is not clear although it may serve as a reservoir for generation of the shorter isoforms.[572] Therefore, a reduction in both internal ribosome entry site

mediated VEGF expression and translation of the large L-VEGF isoform could result in lower plasma levels of VEGF in patients with the +405 G allele.[342]

Chapter 2 – Prognostic factors relating to sex steroid receptors in meningiomas

2.1 Clinical Material and Methods

All patients with a histologically confirmed diagnosis of Meningioma operated on between 1988 and 1998 in the Department of Neurosurgery, Royal Preston Hospital were recruited for this study. Clinical information was obtained from the hospital case notes together with special files prospectively kept for the Meningioma follow up clinic run in the department. Where patients were lost to follow up, an attempt was made to contact their General Practitioner to confirm their current status. Recurrence was defined as either reappearance at the site of a previously fully resected tumour or enlargement in size of the residual tumour on follow up imaging.

2.1.1 Steroid Receptor Immunohistochemistry

The tumours were evaluated for presence of progesterone receptors (PR) and oestrogen receptors (ER) using immunohistochemistry.

The antibodies used were a polyclonal rabbit anti-human antibody (Dako UK Ltd., Ely, Cambridgeshire) and a monoclonal mouse anti-human oestrogen receptor α, clone 1D5 (Dako UK Ltd., Ely, Cambridgeshire).

The archival formalin fixed paraffin embedded tissue blocks of the meningiomas were sectioned at 5µm, mounted on glass slides and dewaxed in xylene and graded alcohols. Epitope retrieval was achieved by microwaving in a pH 9 citrate buffer for 20 minutes. The primary antibodies

were applied to consecutive levels of the Meningioma for 30 minutes at room temperature. The Dako duet kit solution (Dako UK Ltd., Ely, Cambridgeshire) containing biotinylated goat anti-mouse/rabbit immunoglobulin was applied followed by the streptavidin label and the chromogen diaminobenzidine which ultimately stains the receptors brown. The sections were counterstained with haematoxylin, dehydrated, mounted and examined by light microscope. A positive result was registered when more that 10% of the cells were stained brown. Known positive controls of ductal breast carcinoma were run in each batch. Negative controls were prepared by omitting the primary antibody and replacing it with normal serum.

The sections were examined by two histopathologists and a neurosurgeon assessing for nuclear and cytoplasm positivity for both progesterone and oestrogen receptors. In addition the meningioma histological subtype was determined by both the histopathologists.

Steroid hormone receptors are found in the cytoplasm of cells but are only functionally active in the nucleus. The immunohistochemical analysis of the receptors has the advantage over cytosol assays in that the receptors can be localised to the biologically active nucleus.

2.1.2 Statistical Analysis

The Chi squared or Fisher's exact test with the Mantel-Haenszel estimate of common odds ratio were undertaken to compare differences in proportions.

Kaplan-Meier survival analysis with log rank testing and the Cox proportional hazards model were used to estimate differences in survival.

2.2 Results

There were 102 patients in total during this time period with 74 females and 28 males. Mean age at presentation was 60 years 9 months (range 27 to 87). Mean follow up was 7 years 7 months (maximum 17 years 2 months).

2.2.1 Histological Subtypes

The distribution of histological grade was typical with 88 (87%) being grade 1. Overall 11 (10%) were grade 2 or atypical and 3 (3%) were grade 3 or anaplastic (Figure 2.1).

Distribution of Meningioma Histological Subtypes (n=102)

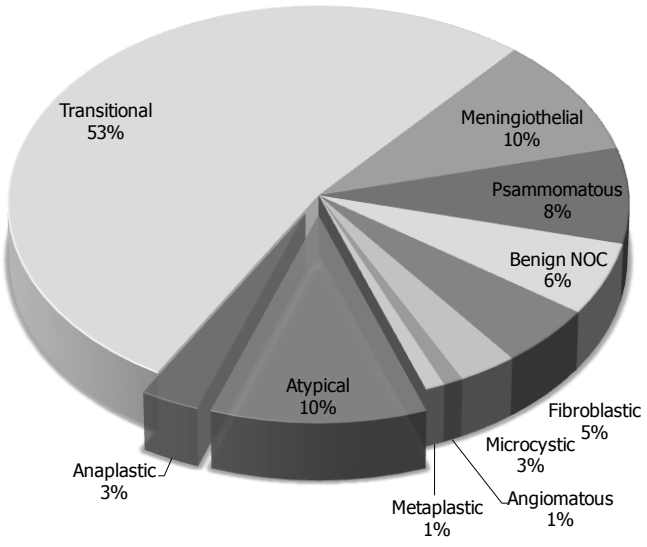


Figure 2.1 - Distribution of Meningioma histological subtypes. The majority of tumours were grade 1. Grade 2 (atypical) and grade 3 (anaplastic) tumours are exploded on the pie chart

2.2.2 Steroid Receptor Status

There was 100% Agreement in designation of tumour receptor status between the 3 independent assessors (Figure 2.2).

Nuclear progesterone staining was positive in 56 (55%) of all tumours. Cytoplasmic progesterone receptor staining was positive in 90 (88%) of tumours including 35 out of the 46 nuclear progesterone receptor negative patients. Nuclear oestrogen receptor staining was very rare with only 1 case (1%) positive in the whole cohort. Oestrogen receptor cytoplasmic staining was more frequently seen in 54 (53%) of all tumours.

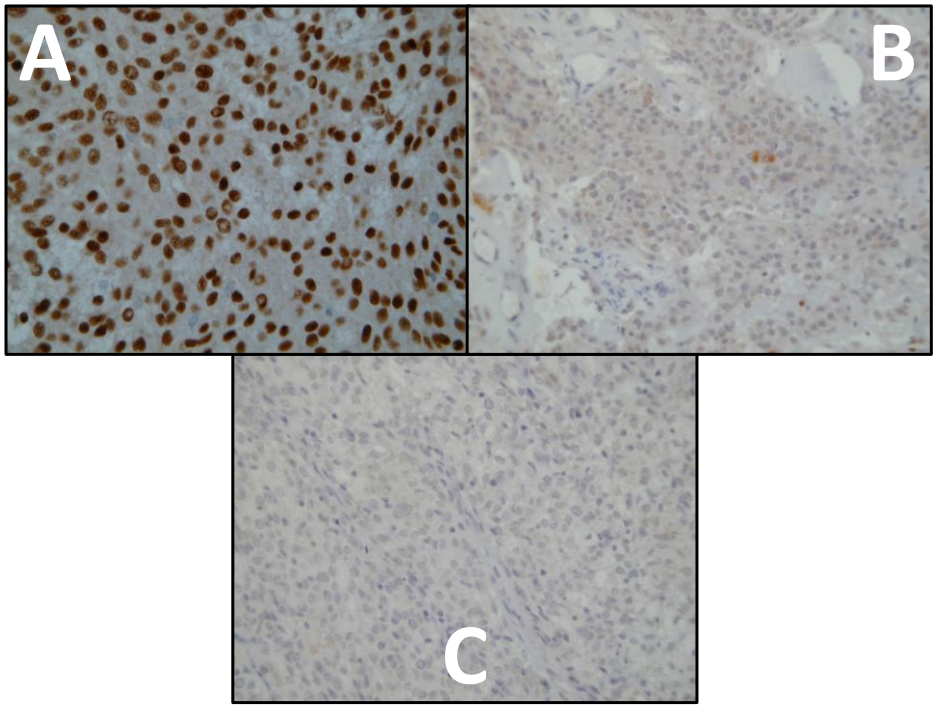


Figure 2.2 – Immunohistochemistry results of steroid receptors. Sections showing immune-staining for tumours that are: A – nuclear and cytoplasm receptor positive; B – cytoplasm receptor positive and C – nuclear and cytoplasm negative.

There was a significantly higher proportion of males that were progesterone receptor negative compared to females (Figure 2.3).

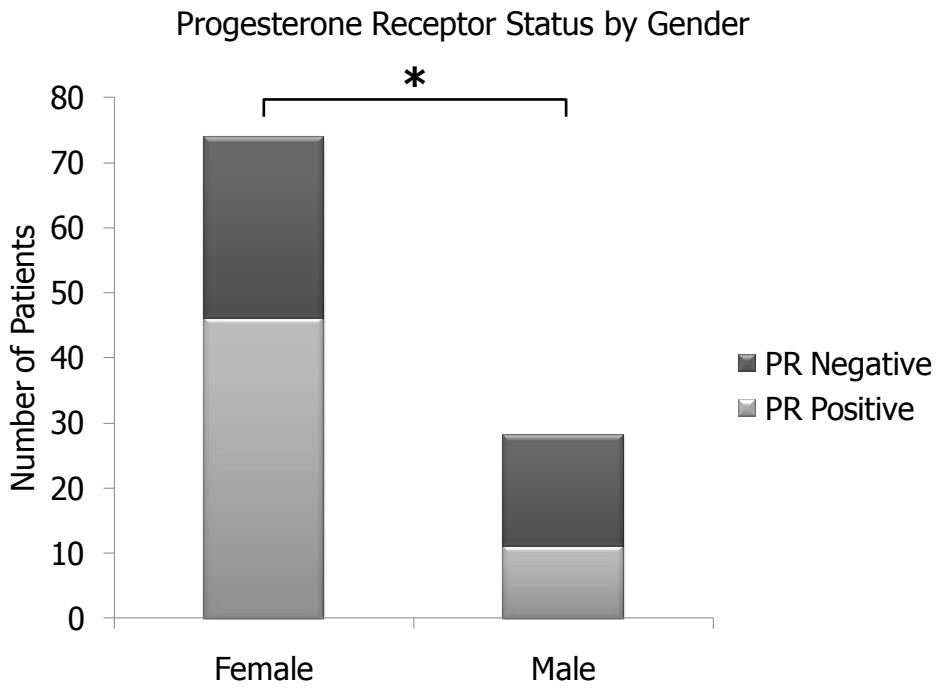


Figure 2.3 - Progesterone receptor status by gender. Meningiomas in males are significantly more likely to be progesterone receptor negative; males 60.7% females 37.8% PR negative ($\chi^2 = 4.312$, df = 1, p = 0.038)

There was a strong association with progesterone receptor status and tumour grade. All of the 14 grade 2 or 3 Tumours were progesterone receptor negative (Figure 2.4).

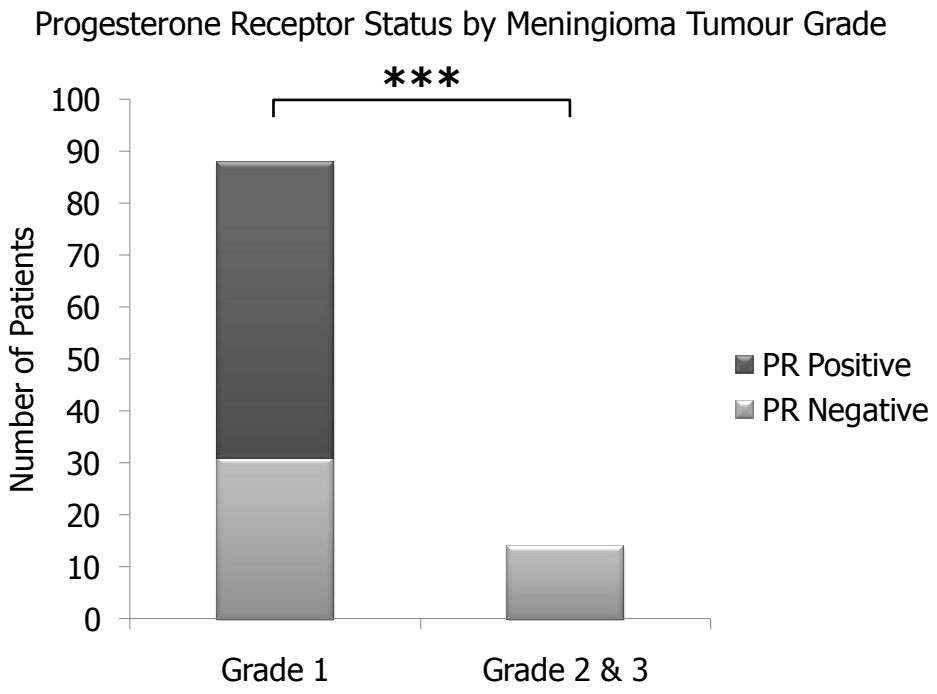


Figure 2.4 - Progesterone receptor status by meningioma tumour grade. There is a strong association with higher grade of tumour and negative PR status (χ^2 = 20.555, df = 1, p = 0.000003)

2.2.3 Recurrence

There were 9 patients that recurred at a mean time of 3 years 4 months and median time of 2 years 3 months from initial diagnosis (Range 1 year to 8 years 9 months) (Table 2.1).

		Non-Recurrent (%)	Recurrent (%)	Total	Odds Ratio	Fisher's/ χ^2 P value
All Patients		93 (91)	9 (9)	102		
Gender	Male	27 (96)	1 (4)	28	NS	0.438
	Female	66 (89)	8 (11)	74		
Progesterone Receptors	Nuclear +ve	55 (96.5)	2 (3.5)	57	5.066	0.041*
	Nuclear -ve	38 (84)	7 (16)	45		
	Cytoplasm +ve	83 (92)	7 (8)	90	NS	0.286
	Cytoplasm -ve	10 (83)	2 (17)	12		
Oestrogen Receptors	Nuclear +ve	1 (100)	0 (0)	1	NS	1.000
	Nuclear -ve	92 (91)	9 (9)	101		
	Cytoplasm +ve	48 (89)	6 (11)	54	NS	0.495
	Cytoplasm -ve	45 (94)	3 (6)	49		
WHO Tumour Grade	Grade 1	84 (94.5)	4 (4.5)	88	11.667	0.002**
	Grade 2 & 3	9 (64)	5 (36)	14		
Simpson Grade	Grade 1	27 (96)	1 (4)	28	NS	0.100
	Grade 2	26 (96)	1 (4)	27		
	Grade 3	6 (86)	1 (14)	7		
	Grade 4	20 (77)	6 (23)	26		
	Grade 5	2 (100)	0 (0)	2		
Dichotomised Simpson Grade	Grade 1 & 2	53 (96)	2 (4)	55	6.623	0.025*
	Grade 3,4 & 5	28 (80)	7 (20)	35		

Table 2.1 – Factors relating to recurrence of meningiomas. There are significant associations with recurrence and progesterone receptor negativity, higher WHO grades and decreased resection as categorized by a dichotomised Simpson grade.

There was a strong association with atypical/anaplastic grade tumours (grade 2 and 3) and recurrence (Figure 2.5).

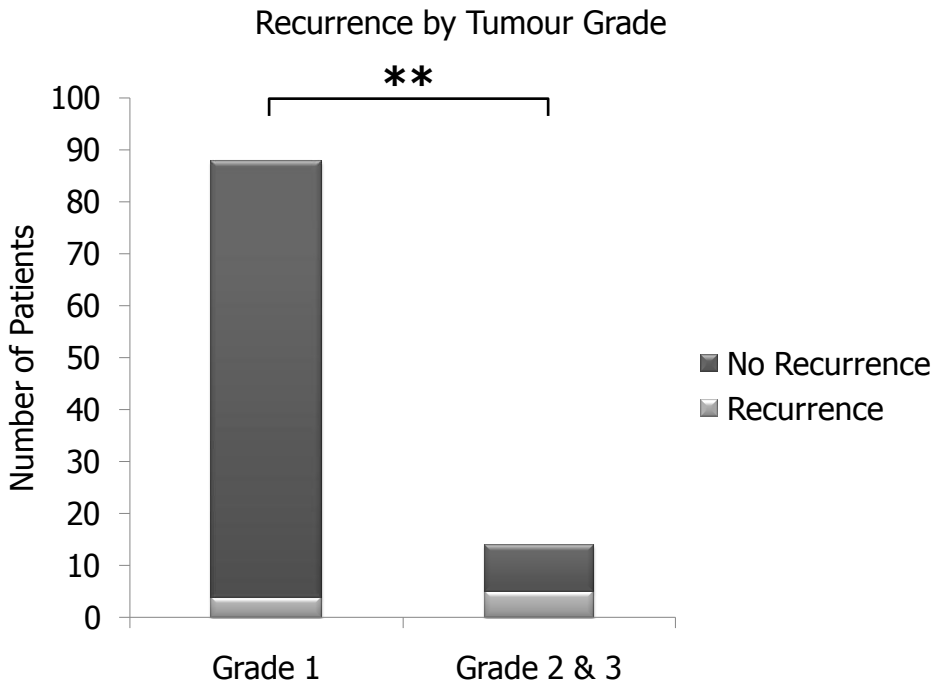


Figure 2.5 - Recurrence by tumour grade. There was a significantly higher risk of recurrence with grade 2 or 3 histology (χ^2 = 14.586, df = 1, Fisher's Exact 2-sided p = 0.002; Odds ratio = 11.667, 95% CI 2.646 to 51.438)

Kaplan-Meier plots show a significantly worse progression free survival for patients with atypical or anaplastic tumours (Figure 2.6).

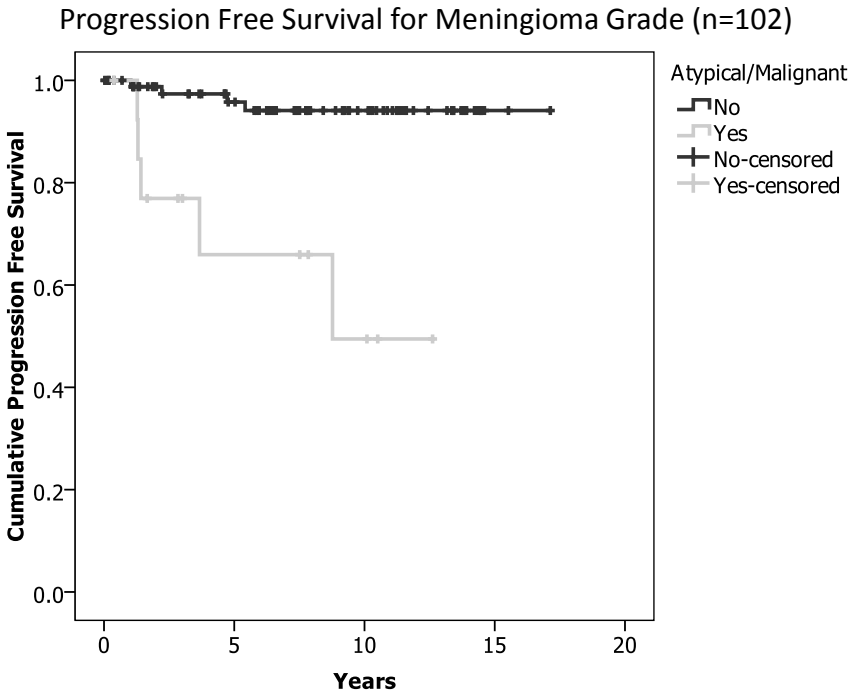


Figure 2.6 - Kaplan-Meier plot of progression free survival by tumour grade (grade 1 vs. grade 2 & 3). There was a significantly higher recurrence rate in the grade 2 & 3 patients (Log rank = 17.127, p = 0.000035)

There was no difference in recurrence between males and females (Figures 2.7 & 2.8)

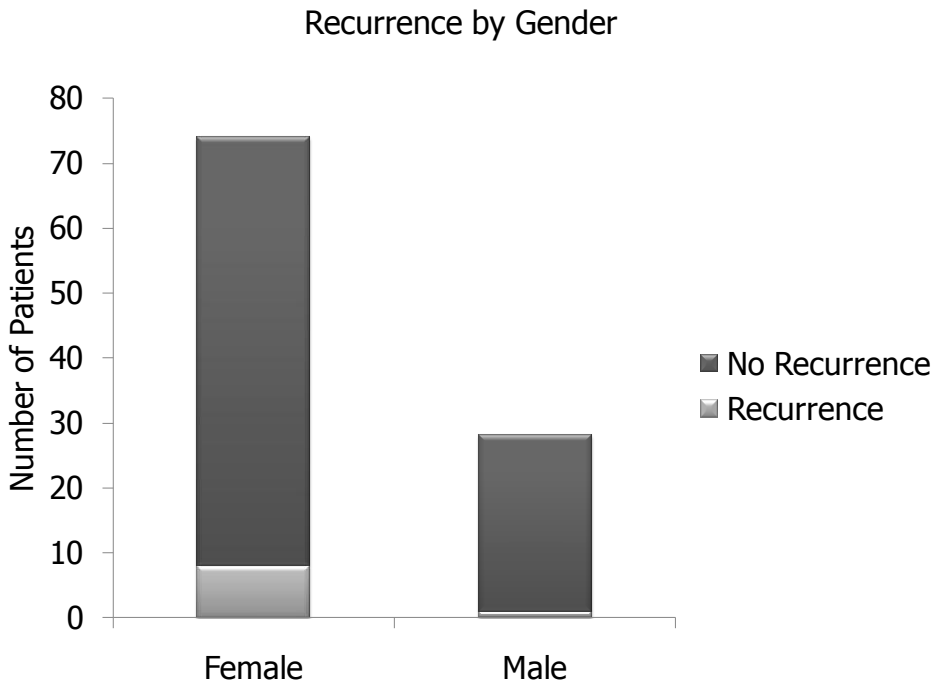


Figure 2.7 - Meningioma recurrence rate by gender. There was no difference in recurrence rate between males and females (χ^2 = 1.323, df = 1, Fisher's Exact 2-sided p = 0.438)

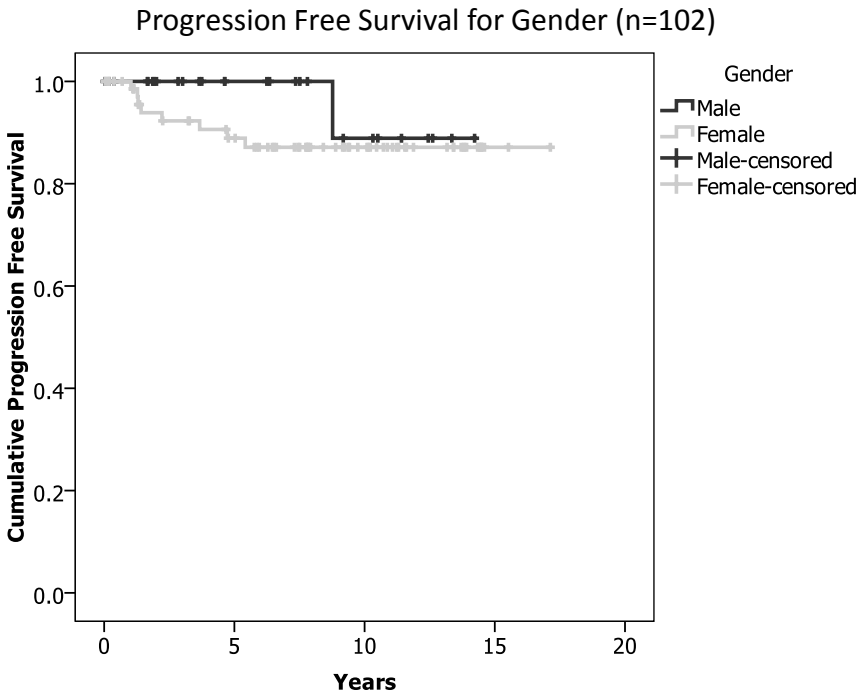


Figure 2.8 - Kaplan-Meier plot of progression free survival by gender. There was no difference in recurrence of tumour between males and females (Log rank = 0.938, df = 1, p = 0.333)

Overall there was a non-significant trend of association with reduction of surgical resection on the Simpson grade and recurrence (Figure 2.9).

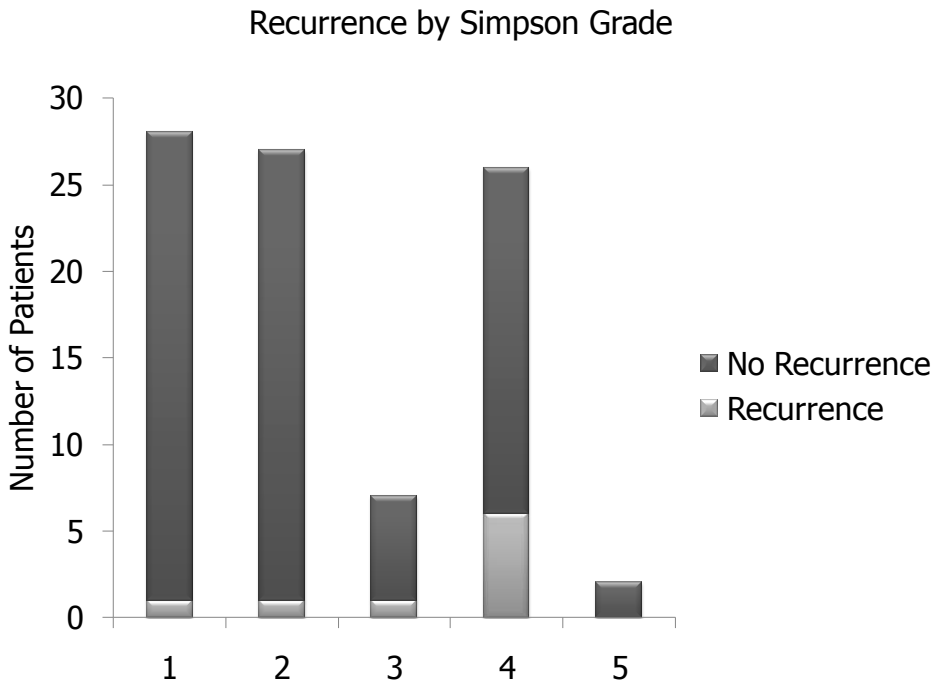


Figure 2.9 - Recurrence by individual Simpson meningioma resection grade. There was a non-significant trend of increase in recurrence with higher Simpson grades ie more residual tumour (χ^2 = 7.780, df 4, p = 0.100)

When the Simpson grade is dichotomised between grades 1 and 2 (no residual tumour with either excision or diathermy of dura) and 3,4 and 5 (dural origin unable to be diathermised, subtotal removal or biopsy) then there is a significant association with the grade 3,4 and 5 group and recurrence (Figures 2.10 & 2.11).

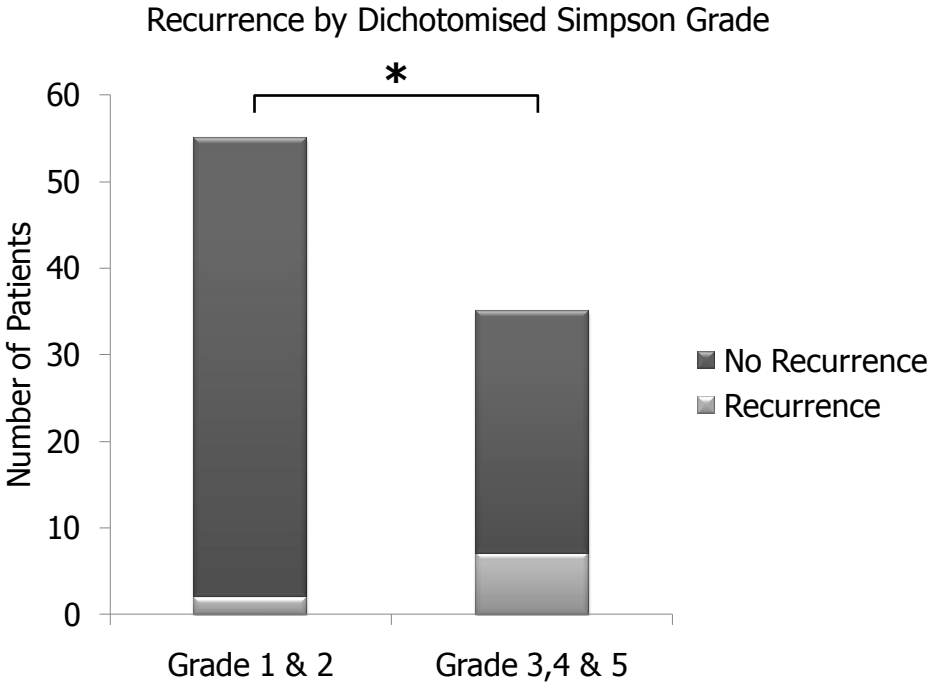


Figure 2.10 - Recurrence rate with dichotomised Simpson grade. There is a significant increase in recurrence with the grade 3,4 &5 group (χ^2 = 6.364, df = 1, Fisher's Exact 2-sided p = 0.025; Odds ratio = 6.623, 95% CI 1.289 to 34.483)

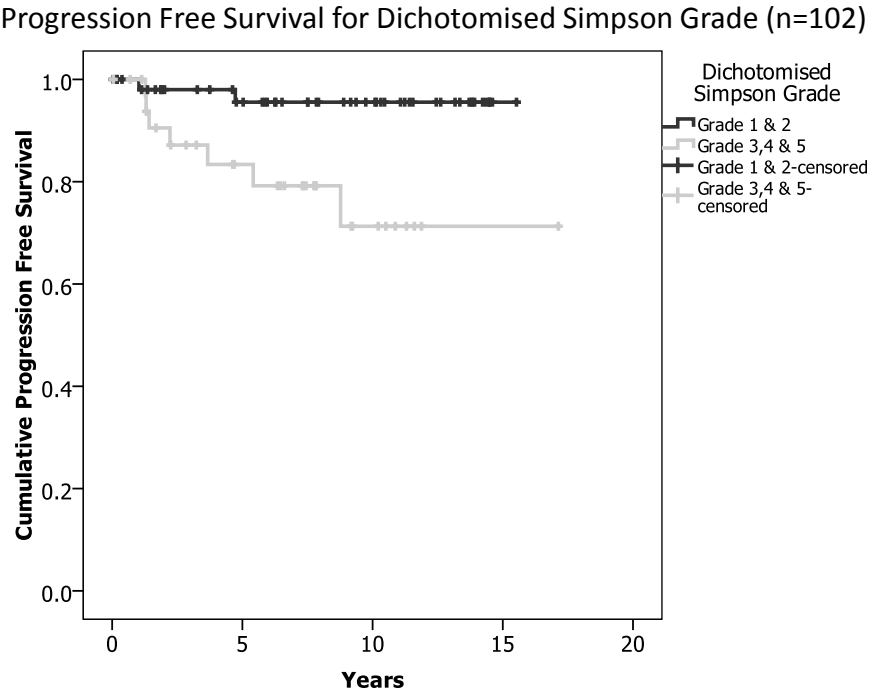


Figure 2.11 - Kaplan Meier plot of recurrence by dichotomised Simpson grade group. There is a significantly increased risk of recurrence with the grade 3,4 & 5 group (Log rank = 6.748, df = 1, p = 0.009)

There is an association with progesterone receptor negativity and meningioma recurrence (Figures 2.12 & 2.13).

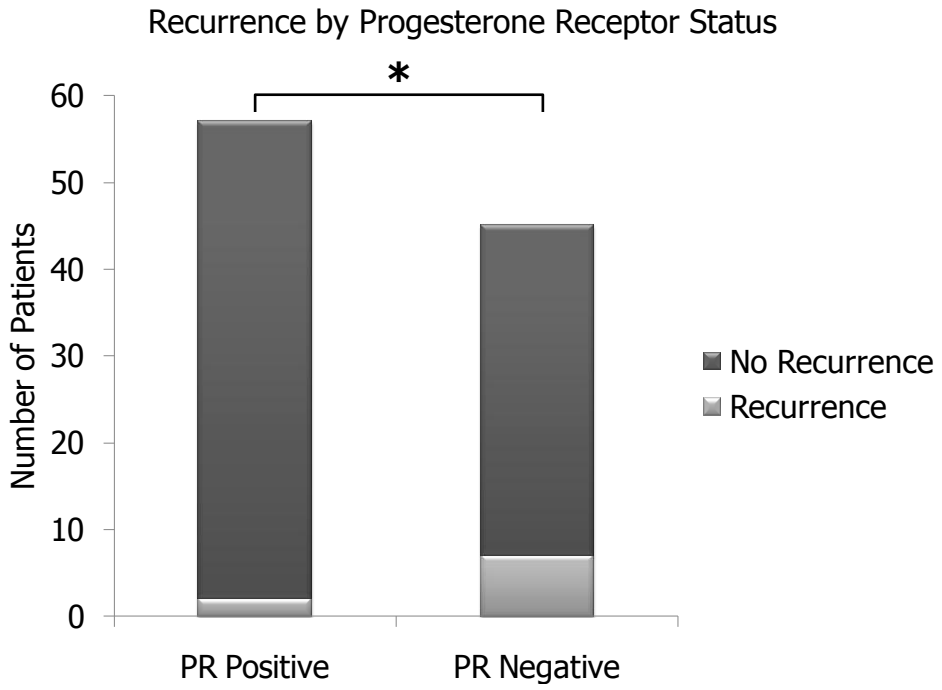


Figure 2.12 - Recurrence by progesterone receptor status. There is an increase in recurrence rate for meningiomas that are progesterone receptor negative (χ^2 = 4.536, df = 1, Fisher's Exact 2-sided p = 0.041; Odds ratio = 5.066, 95% CI 0.998 to 25.725)

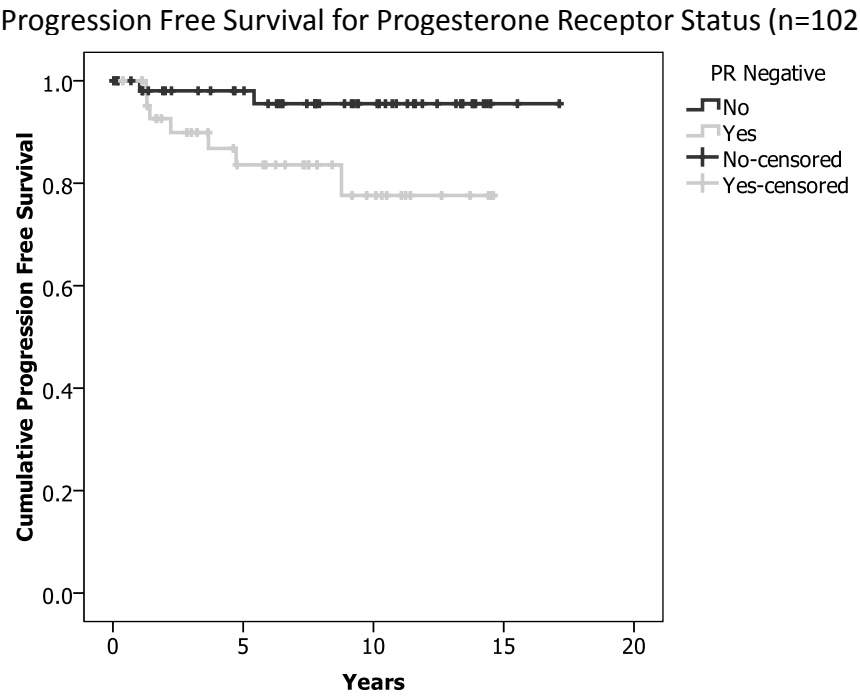


Figure 2.13 - Kaplan-Meier plot of recurrence by receptor status. There is a significantly increased risk of recurrence for meningiomas that are progesterone receptor negative (Log rank = 4.760, df = 1, p = 0.029)

2.2.4 Cox Proportional Hazards Model

The significantly associated variables of tumour grade, extent of surgical resection classified by Simpson grade and progesterone receptor status were entered into a proportional hazards model. The overall model was significant with only the dichotomised Simpson grade maintaining significance with atypical/anaplastic histology on the borderline of significance (Table 2.2). In this multivariable model progesterone receptor negativity was not an independent predictor of recurrence.

	P value	**Risk Ratio**	**95% CI for RR**	
			Lower	Upper
Atypical/Anaplastic (Tumour grade 2&3)	.070	4.657	.882	24.596
Progesterone Receptor Negative	.431	2.204	.308	15.788
Simpson Grade 3,4 & 5	.042	5.222	1.059	25.745

Table 2.2 - Cox regression analysis results for factors relating to meningioma progression free survival. Extent of resection as categorized by the dichotomized Simpson grade maintains significance with WHO tumour grade 2 & 3 borderline significant and progesterone receptor status not significant in this model.

Chapter 3 – The effect of neoadjuvant chemotherapy on operative blood loss and surgical resection for Choroid Plexus Carcinomas

3.1 Clinical Material and Methods

At the Hospital for Sick Children, Toronto, Canada 16 children with a diagnosis of Choroid Plexus Carcinoma were treated between 1982 and 2004. The charts, radiology and histology were reviewed for these patients.

The patients were split into two groups: group 1 consisted of patients who did not receive any chemotherapy prior to the attempt at surgical resection of their tumour (n=8) and group 2 who following an initial biopsy to confirm the diagnosis of their lesion were given neoadjuvant chemotherapy prior to an attempted surgical resection (n=8). The chemotherapy utilised was the ICE regimen (ifosfamide 3 g/m2 on days 1 and 2, etoposide 150 mg/m2 on days 1 and 2 and carboplatin 600 mg/m2 on day 3). The patients received a median of 4 cycles of the ICE chemotherapy prior to the operation (range 2 to 7).

Blood loss for each operation was calculated in terms of red blood cell volume using the previously described technique(16) and the following formula:

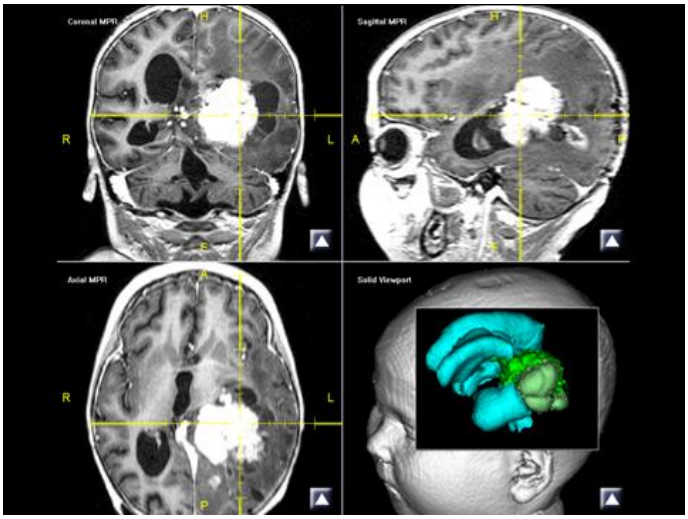
CBL = RBC Volume Transfused + EBV x (Hct Pre Op – Hct Post Op)

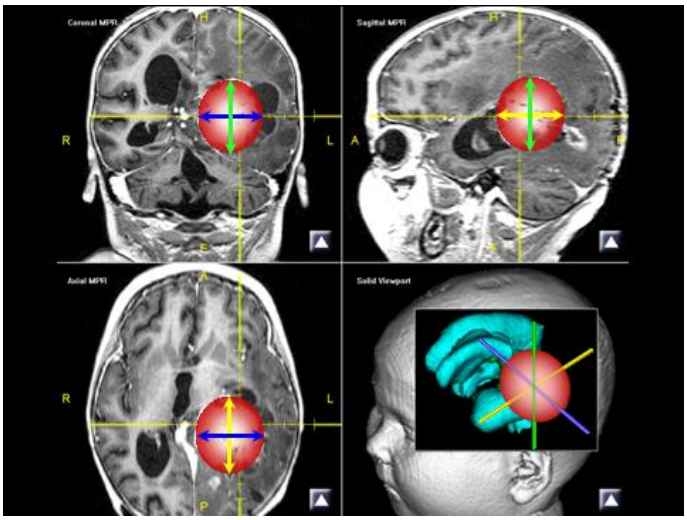
CBL, Calculated Blood Loss; RBC, Red Blood Cell; EBV, Estimated Blood Volume (80mls/Kg Weight); Hct, Hematocrit.

Tumour volume was calculated from either CT or MR scans using a 3 dimensional diameter method with the following formula:(17)

Volume = 1/6 x π x D1 x D2 x D3

D1 Anterior-Posterior Diameter; D2 Medial-Lateral Diameter; D3 Superior-Inferior Diameter (Figure 3.1)





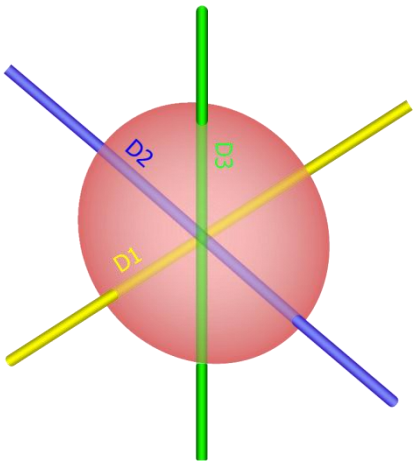


Figure 3.1 - Method for estimating tumour volume. These T1 weighted MR scans with contrast in 3 orthognal planes demonstrate a choroid plexus carcinoma in the left lateral ventricle. The estimation of tumour volume uses the formula for calculating the volume of a prolate ellipsoid by entering the three orthogonal diameters (D1-3) as measured on the scan.

3.1.1 Statistical Analysis

Comparison of means was undertaken using t tests with Levene's test for equality of variance. Multivariable linear regression analysis was used to identify the relationship between blood loss and potential predictive variables. Kaplan-Meier plots with the log rank test was used to analyse survival. Statistical analysis was performed using SPSS for Windows release 16.0.2 2008. Chicago: SPSS inc.

3.2 Results

3.2.1 General

The 16 patients consisted of 10 males and 6 females. The median age at presentation of all the patients was 21 months with a median duration of symptoms of 4 weeks. CSF diversion was required in 12 of 16 patients at some point during their treatment. There was no difference in the pre-operative characteristics of the two treatment groups in terms of tumour size, age or weight of patient (Table 3.1). There was one peri-operative death secondary to haemorrhage in a child who had not received pre-operative chemotherapy. This child was excluded from survival analysis.

By Operations	Group 1 No-Chemotherapy (n=13)		Group 2 Pre-Operation Chemotherapy (n=11)		
	Mean	**SE**	**Mean**	**SE**	**P Value**
Age	3 yr 5 mo	1 yr 3 mo	1 yr 9 mo	7 mo	0.25 NS
Patient Weight (kg)	15.5	4.3	12.9	1.8	0.60 NS
Size of Tumour (cm^3)	82.1	16.7	55.3	22.5	0.34 NS
By Patient	**Group 1 No-Chemotherapy (n=8)**		**Group 2 Pre-Operation Chemotherapy (n=8)**		
	Mean	**SE**	**Mean**	**SE**	**P Value**
Age	3 yr 7 mo	1 yr 6 mo	2 yr 0 mo	9 mo	0.38 NS
Patient Weight (kg)	15.0	5.0	13.0	2.4	0.72 NS
Size of Tumour (cm^3)	80.2	20.9	72.0	29.0	0.82 NS

Table 3.1 – Characteristics of the two treatment groups by operations undertaken and patient. There was no difference in the pre-operative age, weight or size of the tumour between groups.

One patient experienced marked cerebral oedema following her first cycle of chemotherapy which led to a hemiparesis that ultimately resolved and cortical blindness that was permanent. No other patients developed a permanent neurological deficit whilst on the chemotherapy regimen. The number of cycles of chemotherapy administered was dependent upon both response of the tumour and also the patients clinical condition. Symptoms from mass effect of the tumour with raised intracranial pressure were treated with steroids during the chemotherapy treatment, however

uncontrollable pressure symptoms were often the indication to discontinue chemotherapy and undertake surgical resection of the tumour.

The 16 patients underwent a total of 24 operations where the pre-operative surgical goal was gross total resection. For both groups, operations where only a biopsy was performed (less that 5% of total tumour volume removed) were not included in the analysis of blood loss and tumour volume reduction.

Figure 3.2 shows the year of presentation of each of the patients. The patients in the two treatment groups presented throughout the time period of the study with equal numbers from each group in the first and last 8 patients to present.

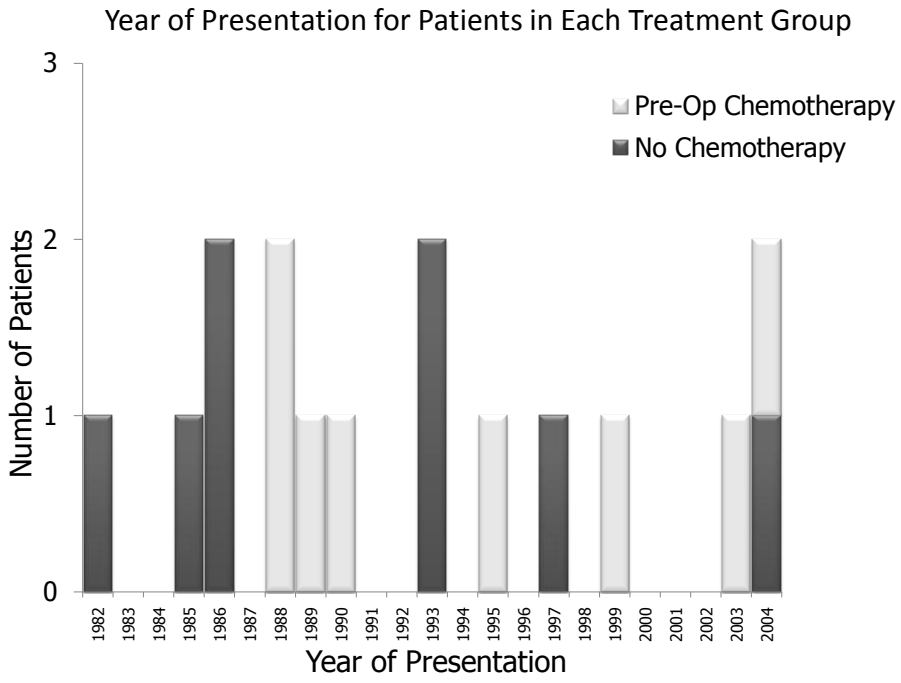


Figure 3.2 - Graph demonstrating the year of presentation of each of the patients in the two treatment groups.

3.2.2 Blood Loss

Operative blood loss was significantly reduced with pre-operative chemotherapy from a mean of 1.11 blood volumes in group 1 (n = 13, SE 0.33) to 0.22 blood volumes in group 2 (n = 11, SE 0.07) (t = -2.66, p = 0.02, two tailed)(Figure 3.3).

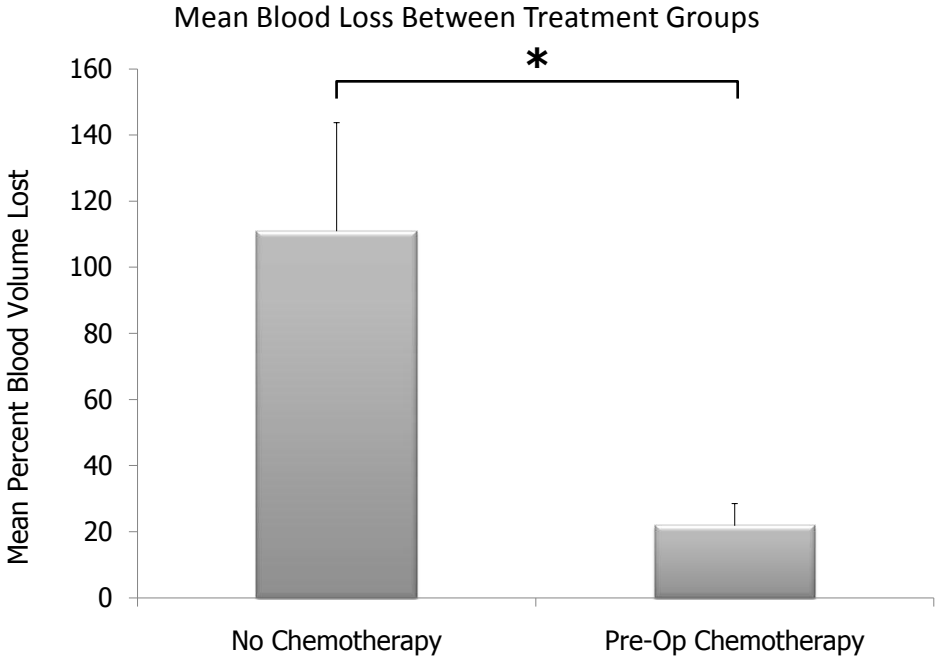


Figure 3.3 - Mean operative blood loss as a percentage of total patient blood volume in the two treatment groups. There was a significant reduction in blood loss in the neoadjuvant chemotherapy group (t = -2.66, p = 0.02, two tailed).

A linear regression model was set up using blood loss/volume as the dependent variable and post-chemotherapy, age at operation and volume of tumour removed as independent variables. Using the enter method, a significant model emerged (F = 4.125, p = 0.02. Adjusted R^2 = 0.29). P values for predictor variables are shown in table 2 with pre operative chemotherapy being the most significant variable and age at operation also

found to be significant. The Beta value in table 2 indicates the relative contribution of the predictor variables to blood loss i.e. being in the post-chemotherapy group contributes 52.9% of the total reduction (given the negative value) of blood loss seen. Volume of tumour removed was not a significant variable in this model.

Predictor variable	Beta	p
Post-chemotherapy	-0.529	p = 0.008**
Age at operation	-0.436	p = 0.042*
Volume of tumour removed	0.343	p = 0.098 NS

Table 3.2 – The predictors of blood loss in a linear regression model with blood loss/volume the dependent factor. Neoadjuvant was the most significant factor with age at operation also significant but extent of surgical resection becoming non-significant in this model.

3.2.3 Extent of Surgical Resection

Mean percentage of tumour removed was higher in group 2 (97.3%, n = 11) compared with group 1 (64.3%, n = 13) (t = 2.969, p = 0.01)(Figure 3.4).

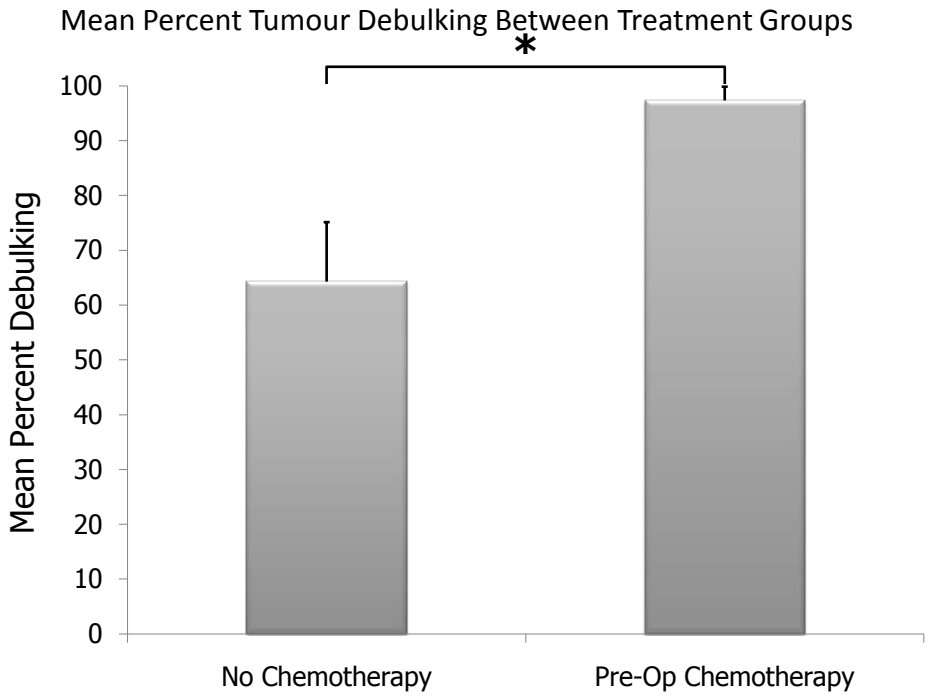


Figure 3.4 - Mean percent debulking between the two treatment groups. There was a significantly higher mean percent debulking in the neoadjuvant chemotherapy group (t = 2.969, p = 0.01).

Gross total resection was ultimately achieved in seven out of eight patients in group 2 compared with only 4 out of eight in group 1.

3.2.4 Survival

Kaplan-Meier survival analysis showed that gross total resection (Figure 3.5; Log rank = 6.107, df = 1, p=0.013) and age at presentation of 3 years or greater (Figure 3.6; Log rank = 4.860, df = 1, p=0.027) were associated with improved survival.

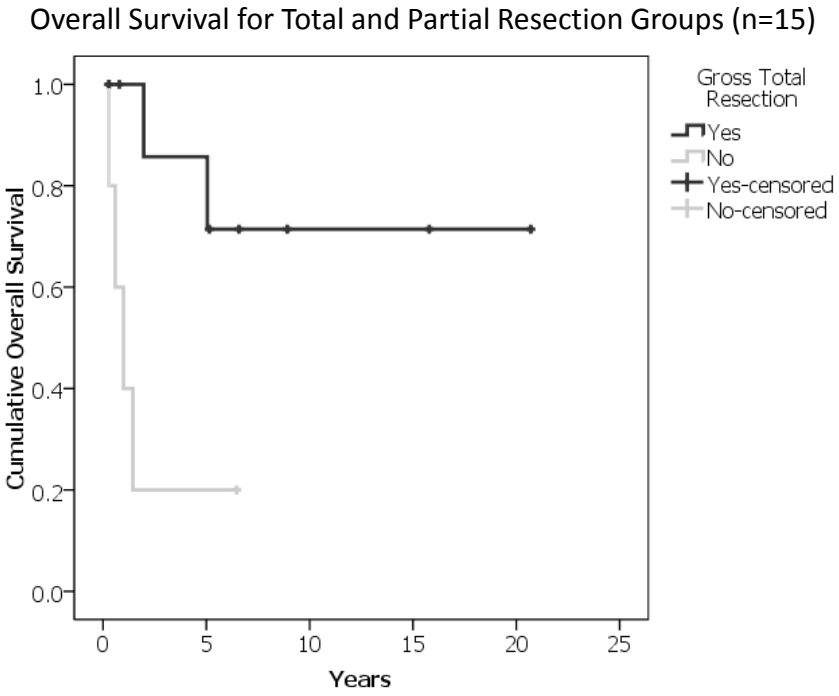


Figure 3.5 - Kaplan-Meier plot for survival between total and partial resection groups. There is a significant survival advantage for those patients that underwent a gross total resection (Log rank = 6.107, df = 1, p=0.013).

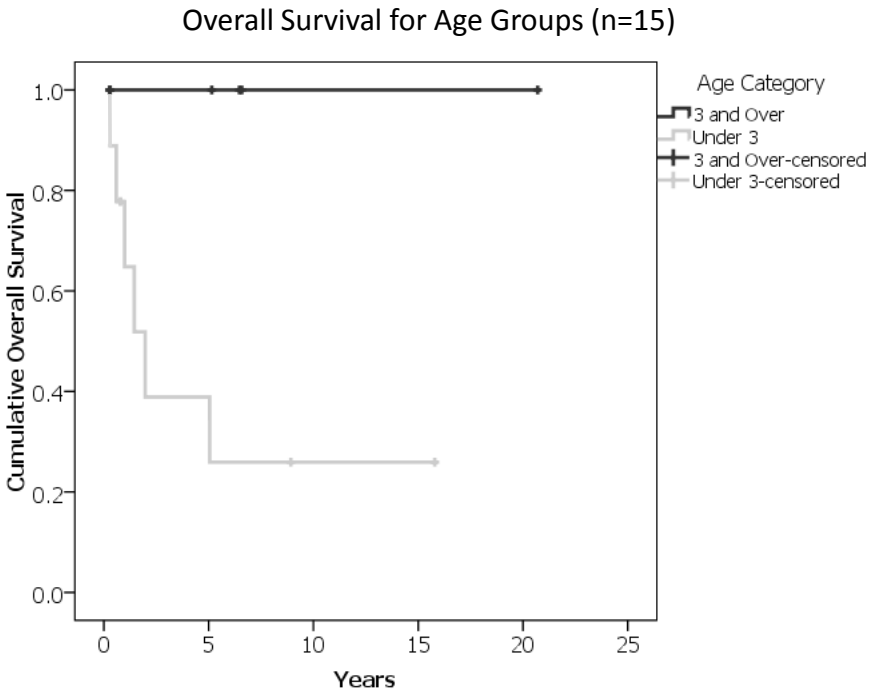


Figure 3.6 - Kaplan-Meier plot for survival between age groups. There is a significant survival advantage for patients age 3 and over (Log rank = 4.860, df = 1, p=0.027).

Mean survival was 15 years 9 months (SE 2 years 11 months) in the gross total resection group compared with 1 year 11 months (SE 1 year 0 months) in patients with a partial resection (Figure 3.7).

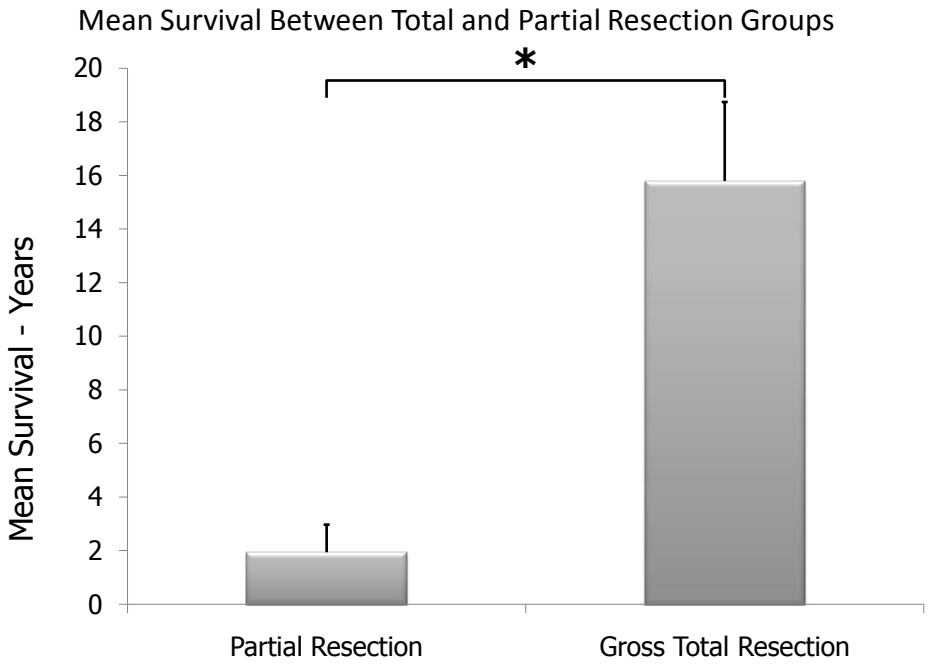


Figure 3.7 - Mean survival between total and partial resection groups. There was a significant mean survial advantage for those patients in which a gross total resection was achieved (Log rank = 6.107, df = 1, p=0.013).

Looking at all the patients there was no survival advantage in the group receiving pre-operative chemotherapy. However, in the under 3 age group (n = 9) there was a significant advantage in following this treatment paradigm (Figure 3.8).

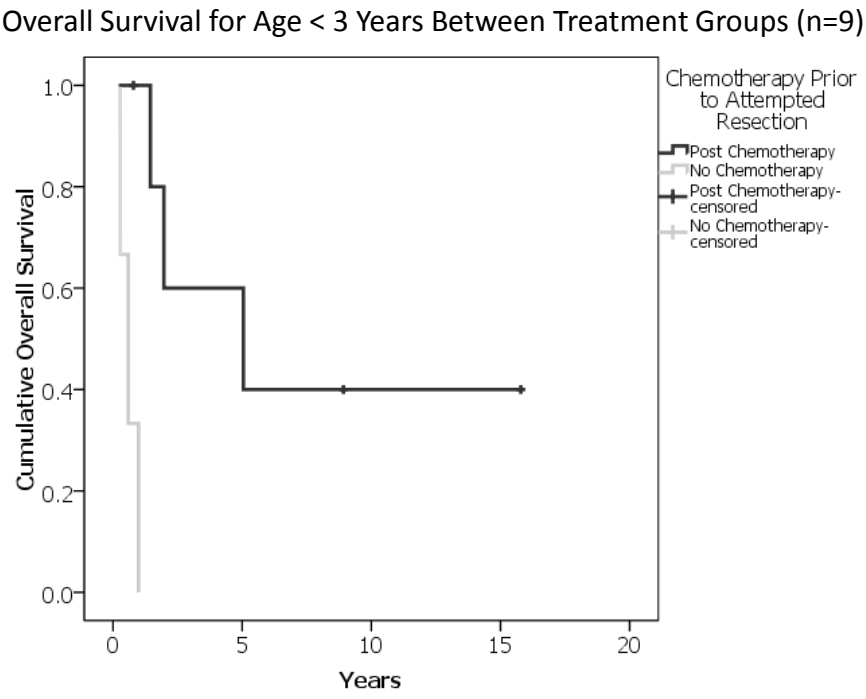


Figure 3.8 - Kaplan-Meier plot of overall survival between treatment groups in patients under 3 years old. There was a significant survival advantage in the group receiving neoadjuvant chemotherapy (Log rank = 9.228, df = 1, p=0.002).

For the under 3 year olds, mean survival was 7 months (SE 3 months) in the group not receiving chemotherapy (n = 3) compared to 8 years 0 months (SE 11 months) in those who did (n = 6) (Figure 3.9; Log rank = 9.228, df = 1, p=0.002).

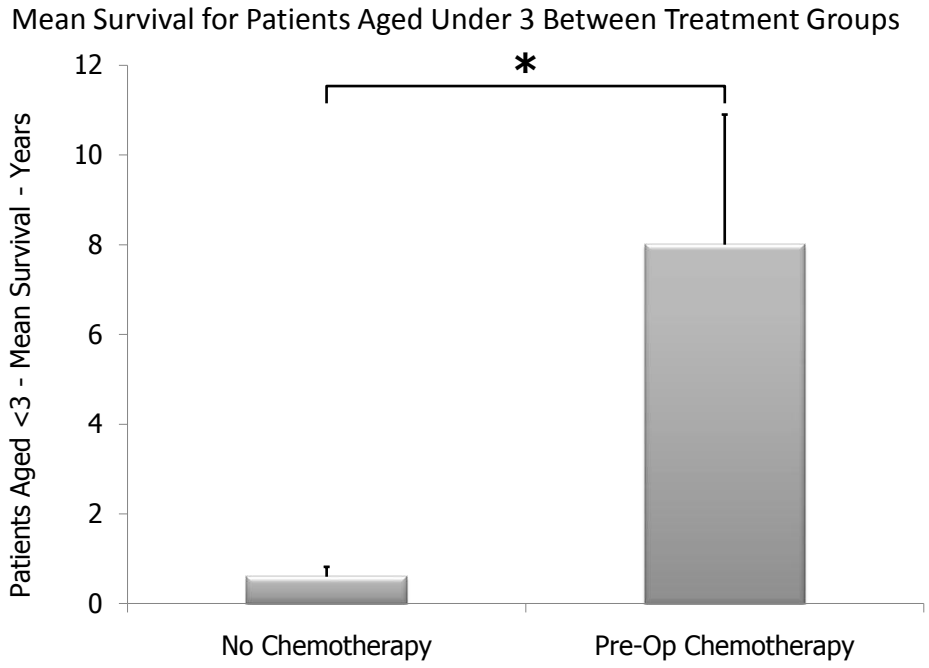


Figure 3.9 - Mean survival between treatment groups in patients under 3 years old. There is a significant increase in mean survival in those patients receiving neoadjuvant chemotherapy (Log rank = 9.228, df = 1, p=0.002).

Chapter 4 – Single nucleotide polymorphisms of the vascular endothelial growth factor gene and their association with the development and survival of adult cerebral gliomas

4.1 Clinical Material and Methods

Samples from 129 patients with a confirmed diagnosis of glioma from 1980 to 1997 in the Brigham and Women's/Boston Children's Hospital tumour bank were analysed. Patient samples were either tumour or whole blood and were collected with prospective clinical information under Institutional Review Board approved protocols. In addition 101 healthy adult controls were recruited for the study to donate whole blood. The study adhered to the Declaration of Helsinki Guidelines and required all subjects to give written, informed consent.

Clinical information was retrieved from the prospectively collected database of patients within the tumour bank.

4.1.1 DNA Extraction

DNA was extracted from tumour or whole blood using Qiagen DNA extraction kits. Genomic DNA was isolated from whole blood and red cell pellets using Qiagen QIAamp DNA blood midi kit (Qiagen Ltd UK, Crawley, West Sussex, UK). Samples of whole blood or red cell pellets were mixed with protease and incubated at 70^{o}C for 10 minutes. After further mixing with absolute ethanol the samples were loaded into QIAamp Midi columns and centrifuge

filtered, allowing DNA to remain within the filter but permitting residual waste to pass through. After two washing steps the entrapped DNA was eluted from the filter with distilled water. Quantification of DNA concentration and purity was performed using a spectrophotometer.

4.1.2 VEGF SNP Genotyping

Genotyping of the -460 and +405 VEGF SNPs (Genebank numbers rs833061 and rs2010963) was performed using the SNP genotyping assay (Applied Biosystems, Foster City, CA). A 10μl rather than a 25μl reaction volume was used but otherwise the assay was performed according to manufacturer's instructions. The primers and probes were designed using the assays-by-design™ service for the -460 SNP (Applied Biosystems) and were available from the assays-on-demand™ service for the +405 SNP (Applied Biosystems). Each PCR reaction contained 15ng genomic DNA, 5μl TaqMan Universal PCR Master Mix, No AmpErase® UNG, 0.5μl 20X SNP Genotyping Mix, and 4.5μl DNase-free water. PCR and genotyping analysis was performed using 96-well plates on an ABI PRISM® 7000 Sequence Detection System (Applied Biosystems). Thermal cycling conditions consisted of an initial denaturation step at 95^{o}C for 10 minutes followed by 40 cycles at 92^{o}C for 15 seconds (denature) and 60^{o}C for one minute (anneal/extension).

Allelic discrimination using the TaqMan assay is possible because the fluorescence signal generated by PCR amplification is indicative of which alleles are present within the sample. The technique is advantageous over

conventional techniques such as PCR-RFLP in that it requires no post-PCR manipulation or agarose gel electrophoresis.

Two allele-specific probes are used which contain reporter and quencher dyes bound to a minor groove binder. After strong probe binding, which is allele-specific, PCR causes probe disintegration (Figure 4.1).

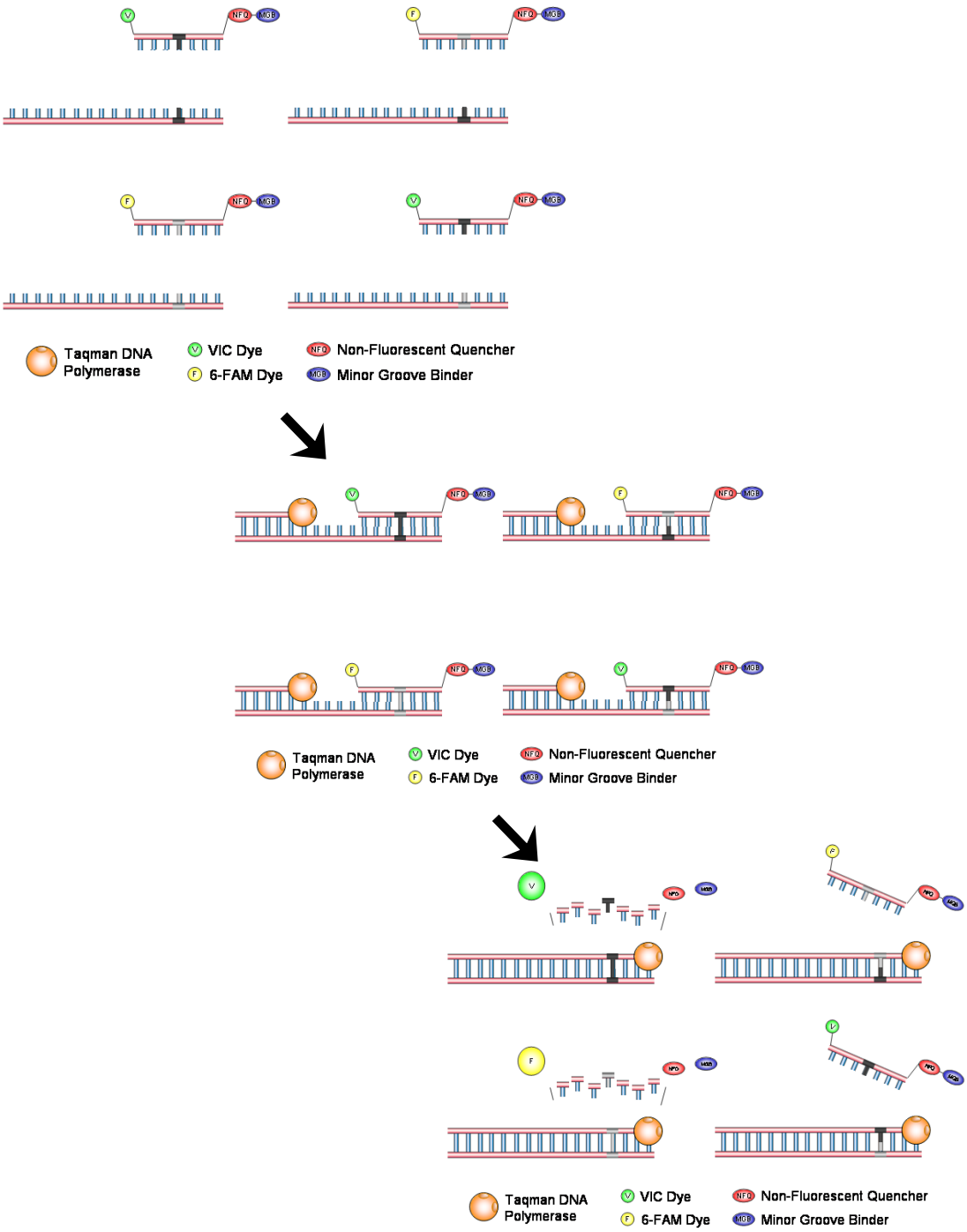


Figure 4.1 - TaqMan allelic discrimination assay. This utilizes two allele specific probes that when bound strongly with an allelic match, will result in disintegration at the next PCR cycle thus releasing the fluorescent dye from its quencher. If there is no match then the probe will dislodge intact and not cause fluorescence.

Dissociation between reporter dye and quencher results in a fluorescent emissions which permits allelic discrimination. The TaqMan genotyping method results in allele-specific fluorescent emissions which permits allelic discrimination using a computer software package (Figure 4.2).

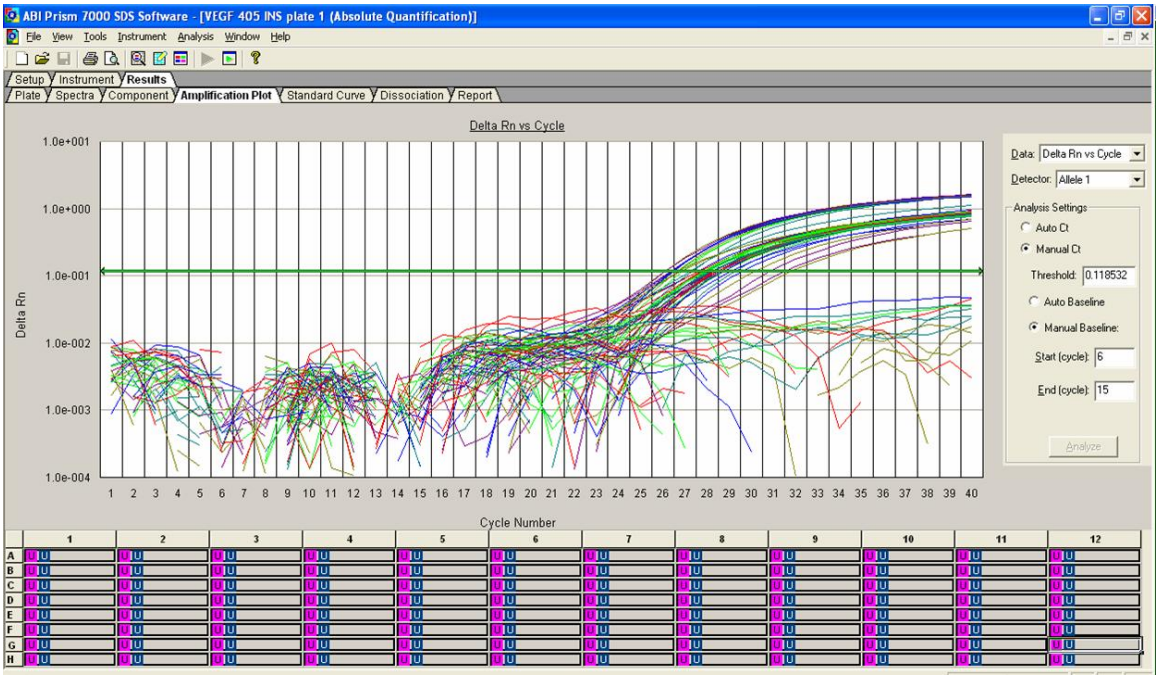


Figure 4.2 - Allelic discrimination software. This program detects the fluorescence giving a read out of the genotype of the DNA in each well.

Linkage disequilibrium for the -460 and +405 SNPs was investigated using the HelixTree programme (Golden Helix Inc, Montana, USA – www.goldenhelix.com) and the D' and r^2 normalised co-efficients of disequilibrium were estimated. Haplotypes were determined from the unphased genotype data using the Expectation/Maximization (EM) algorithm and assigned to individuals using SNPHAP software (http://www-

gene.cimr.cam.ac.uk/clayton/software/snphap.txt). This required confirmation that the populations were in Hardy-Weinberg equilibrium.[30]

4.1.3 Statistical Analysis

Hardy-Weinberg equilibrium for cases and controls at both the -460 and +405 SNPs was tested using Chi squared. The Chi squared test with the Mantel-Haenszel estimate of common odds ratio was undertaken to compare differences in the observed genotype and haplotype frequencies. Kaplan-Meier survival analysis with log rank testing and the Cox proportional hazards model were used to estimate differences in survival.

4.2 Results

4.2.1 General

Ethnicity was similar in the patient and control groups (χ^2 = 5.146, df = 3, p = 0.161). There was a male predominance in the glioma group compared to the controls; however, there was no gender difference in -460 or +405 genotype frequency for either group (-460 χ^2 = 0.545, df = 2, p = 0.761; +405 χ^2 = 3.236, df = 2, p = 0.198) (Table 4.1).

			VEGF -460 Genotype			χ^2 p value	VEGF +405 Genotype			χ^2 p value
		Total	**CC (%)**	**CT (%)**	**TT (%)**		**CC (%)**	**CG (%)**	**GG (%)**	
Controls	All Controls	101	21 (21)	60 (59)	20 (20)		7 (7)	48 (47.5)	46 (45.5)	
	Male Controls	26	8 (31)	16 (61)	2 (8)	0.117	0 (0)	12 (46)	14 (54)	0.226
	Female Controls	75	13 (17)	44 (59)	18 (24)		7 (9)	36 (48)	32 (43)	
Patients	All Patients	129	29 (22)	54 (42)	46 (36)	0.014* vs. control	19 (15)	53 (41)	57 (44)	0.165 vs. control
Gender	Male	77	19 (25)	31 (40)	27 (35)	0.761	10 (13)	28 (36)	39 (51)	0.198
	Female	52	10 (19)	23 (44)	19 (37)		9 (17)	25 (48)	18 (35)	
Histology	Astrocytoma	51	13 (26)	22 (43)	16 (31)	0.055	6 (12)	21 (41)	24 (47)	0.219
	Oligodendroglioma	21	1 (5)	9 (43)	11 (52)		6 (29)	10 (48)	5 (24)	
	Oligoastrocytoma	19	6 (31.5)	7 (37)	6 (31.5)		4 (21)	6 (32)	9 (47)	
	Neuroepithelial	4	1 (25)	3 (75)	0 (0)		0 (0)	2 (50)	2 (50)	
	Glioblastoma	34	8 (24)	13 (38)	13 (38)		3 (9)	14 (41)	17 (50)	
WHO Tumour Grade	Grade 1		1 (12.5)	4 (50)	3 (37.5)	0.723	2 (25)	0 (0)	6 (75)	0.200
	Grade 2		9 (35)	9 (35)	8 (30)		3 (12)	11 (42)	12 (46)	
	Grade 3		11 (18)	28 (46)	22 (36)		11 (18)	28 (46)	22 (36)	
	Grade 4		8 (24)	13 (38)	13 (38)		3 (9)	14 (41)	17 (50)	

Table 4.1 – Distribution of genotypes for adult gliomas. For overall genotype there was a significant difference between patients and controls in distribution of frequencies at the VEGF -460 locus. There was no difference in frequencies within the groups of gender, histology or tumour grade at either locus.

Histological subtypes of the gliomas were 51 (40%) Astrocytomas, 21 (16%) Oligodendrogliomas, 19 (15%) Oligoastrocytomas, 34 (26%) Glioblastomas and 4 (3%) Neuroepithelial tumours. The tumour grades for the study population were 8 (6%) grade one, 26 (20%) grade two, 61 (47%) grade three and 34 (27%) grade four.

4.2.2 VEGF -460 Locus

At the VEGF -460 locus the overall distribution of genotype frequencies was significantly different to controls (Figure 4.3; $\chi^2 = 8.556$, df = 2, p = 0.014). The main factor for this was a significantly increased frequency of the TT genotype compared to controls (Figure 4.4; TT vs. non-TT $\chi^2 = 6.961$, df = 1, p = 0.008; Odds ratio = 2.245, 95% CI 1.222 - 4.121).

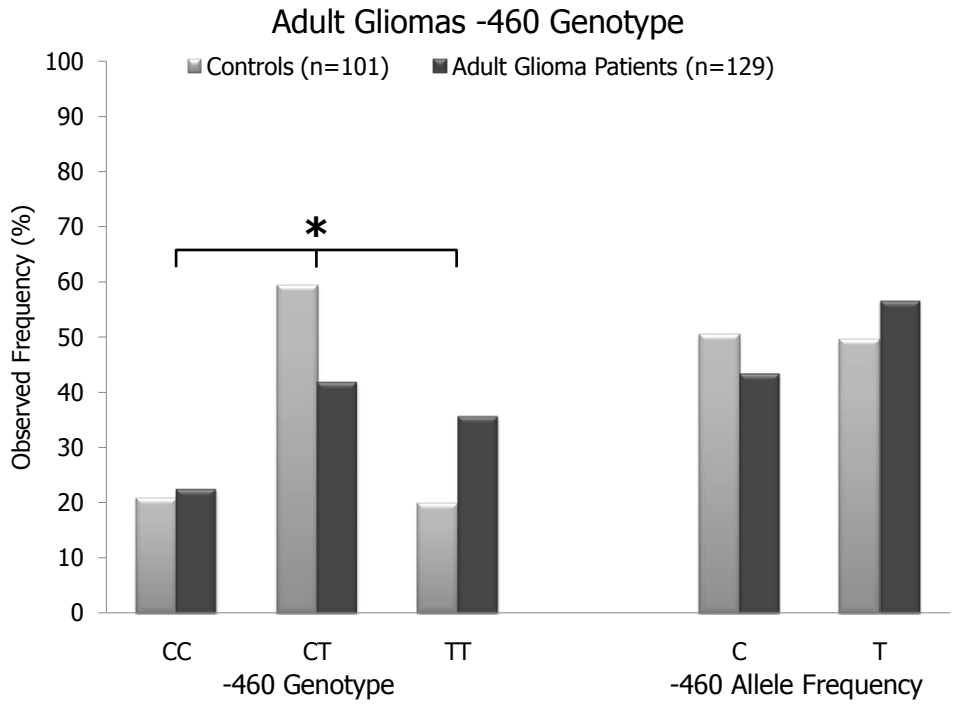


Figure 4.3 - Distribution of genotypes at the VEGF -460 locus. There was a significant difference in frequency of genotypes between glioma patients and controls ($\chi^2 = 8.556$, df = 2, p = 0.014) but no difference in allele frequency ($\chi^2 = 2.285$, df = 1, p = 0.131).

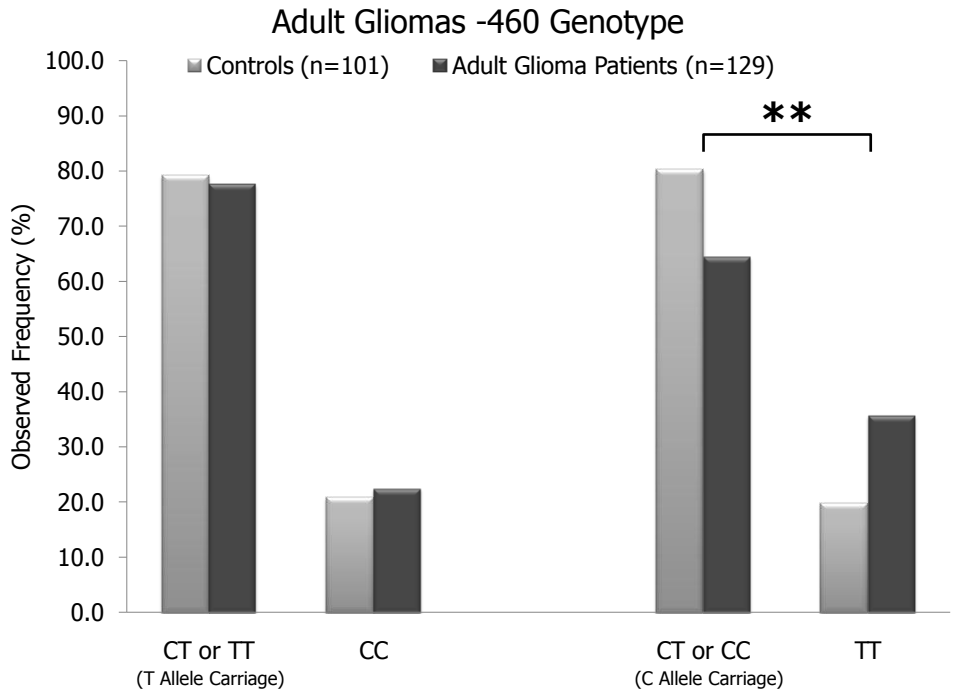


Figure 4.4 - Distribution of homozygous genotypes at the VEGF -460 locus in adult gliomas. There was a significant increase in the TT homozygous genotype in glioma patients compared to controls (TT vs. non-TT χ^2 = 6.961, df = 1, p = 0.008; Odds ratio = 2.245, 95% CI 1.222 - 4.121).

4.2.3 VEGF +405 Locus

At the +405 locus there was no overall difference in genotype frequencies (Figure 4.5; χ^2 = 3.605, df = 1, p = 0.165) but there was a trend of increased CC genotype that did not quite reach statistical significance (Figure 4.6; CC vs. non-CC χ^2 = 3.436, p = 0.06; Odds ratio = 2.319, 95% CI 0.934 – 5.758).

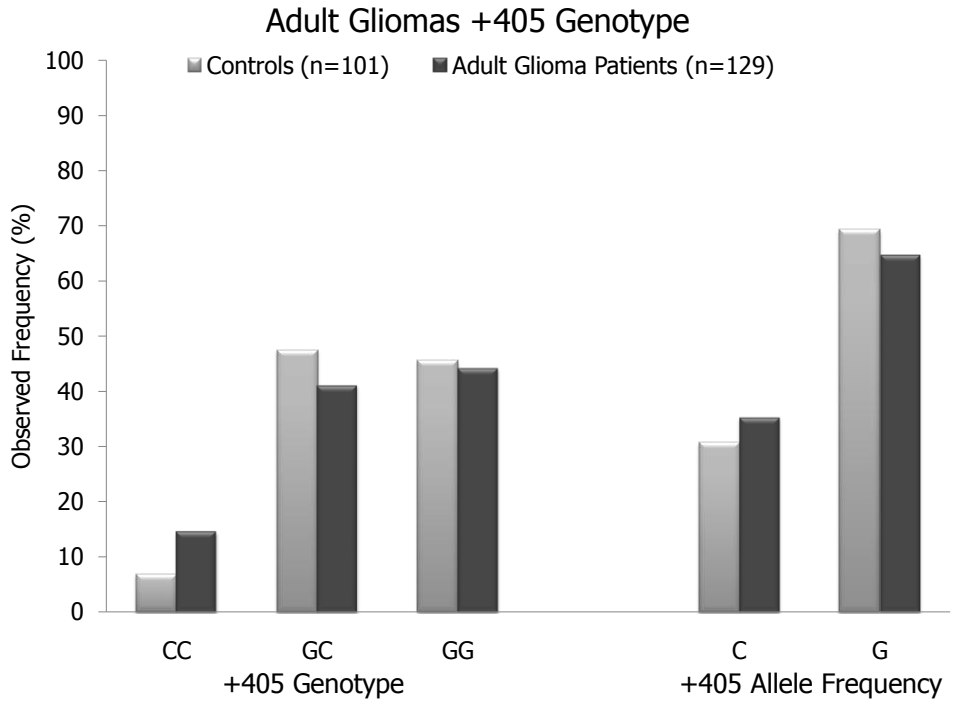


Figure 4.5 - Distribution of genotype and allele frequencies at the VEGF +405 locus. There was no difference between in either genotype or allele frequency between glioma patients and controls (Genotype χ^2 = 3.605, df = 2, p = 0.165; Allele χ^2 = 1.070, df = 1, p = 0.300).

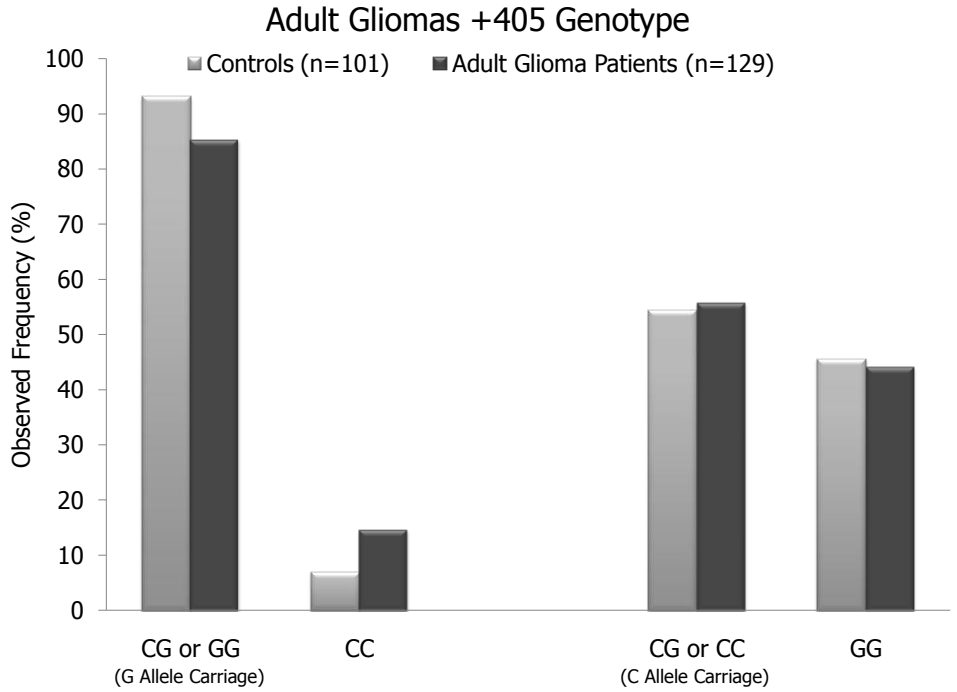


Figure 4.6 - Distribution of homozygous genotypes at the VEGF +405 locus in adult gliomas. There was a trend towards increase in the CC homozygous genotype in glioma patients compared to controls (CC vs. non-CC χ^2 = 3.436, df = 1, p = 0.064; Odds ratio = 2.319, 95% CI 0.934 – 5.758).

4.2.4 Haplotype Analysis

There was no significant difference in overall distribution of haplotype pairs comparing adult gliomas to controls (Figure 4.7). There was however, in the glioma group a significant reduction in the frequency of subjects that carried a copy of the CG haplotype (Figure 4.8).

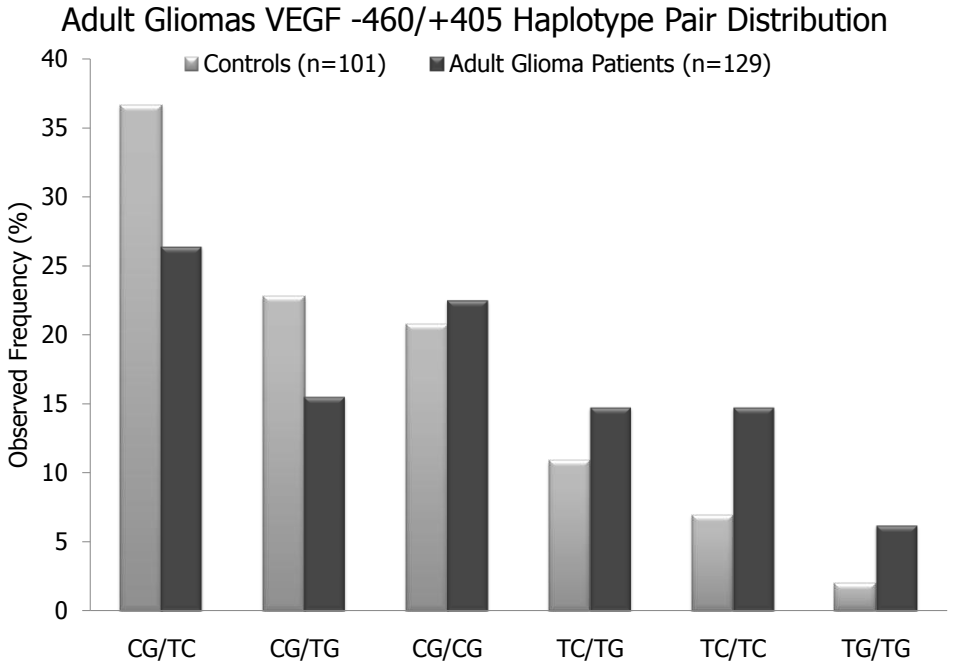


Figure 4.7 - Distribution of haplotype pairs for the VEGF -460/+405 haplotype in adult gliomas. There was no significant difference in haplotype pair frequencies (χ^2 = 9.622, df = 5, p = 0.087).

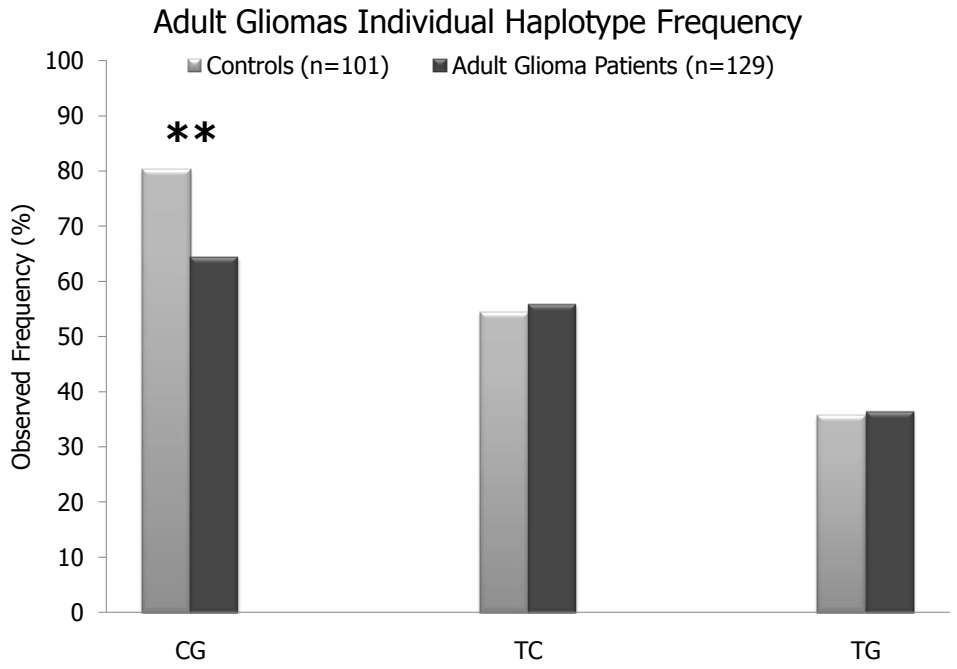


Figure 4.8 - Individual haplotype frequencies for the VEGF -460/+405 haplotype. There was a significantly reduced frequency of the CG haplotype in adult glioma patients compared to controls (χ^2 = 6.961, df = 1, p = 0.008; Odds ratio = 0.446, 95% CI 0.243 to 0.818).

4.2.5 Tumour Grade

There was no difference in tumour grade for any genotype of either the -460 (χ^2 = 3.656, df = 6, p = 0.723) or +405 SNPs (χ^2 = 8.563, df = 6, p = 0.200) (Table 4.1).

4.2.6 Survival Analysis

Kaplan-Meier analysis of the grade 2 tumours (n=26) shows a significant survival advantage for the CC genotype (i.e. absence of the T allele) of the -460 SNP, with mean survival of 10.9 years compared to 5.3 years for non-CC genotypes (Figure 4.9; Log rank = 5.679, df = 1, p = 0.017). Five year survival was 89% for the -460 CC genotype group compared to 45% for the non-CC group (χ^2 = 4.683, df = 1, p = 0.027).

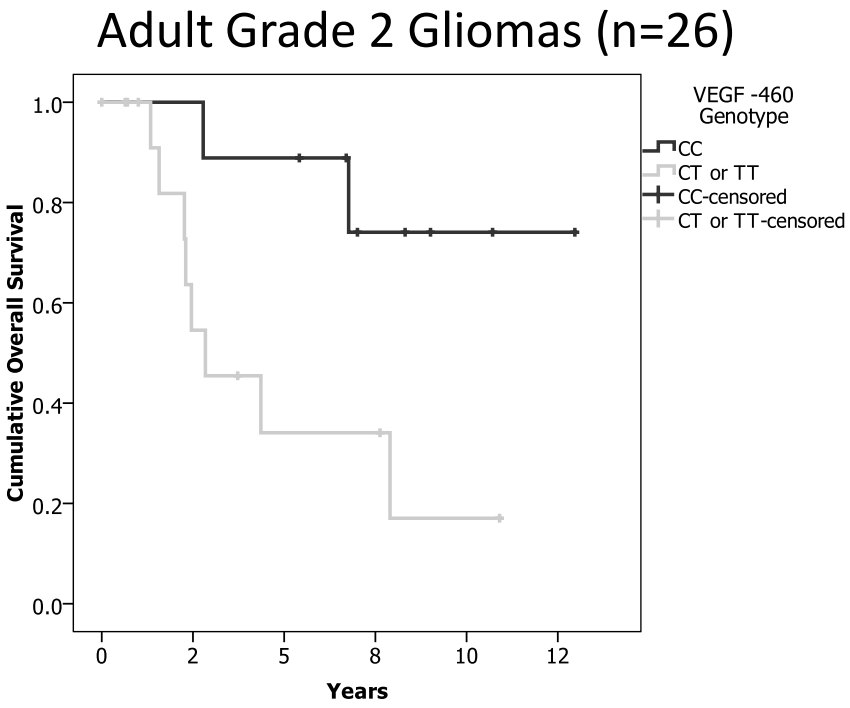


Figure 4.9 - Kaplan-Meier plot of overall survival in the grade 2 glioma patients between CC and non-CC genotypes at the VEGF -460 locus. There was a significant survival advantage for the CC genotype patients (Log rank = 5.679, df = 1, p = 0.017)

For the grade 2 tumours at the +405 locus there appeared to be spreading of the Kaplan-Meier survival lines but this was not statistically significant (Figure 4.10; Log rank = 1.915, df = 1, p = 0.166).

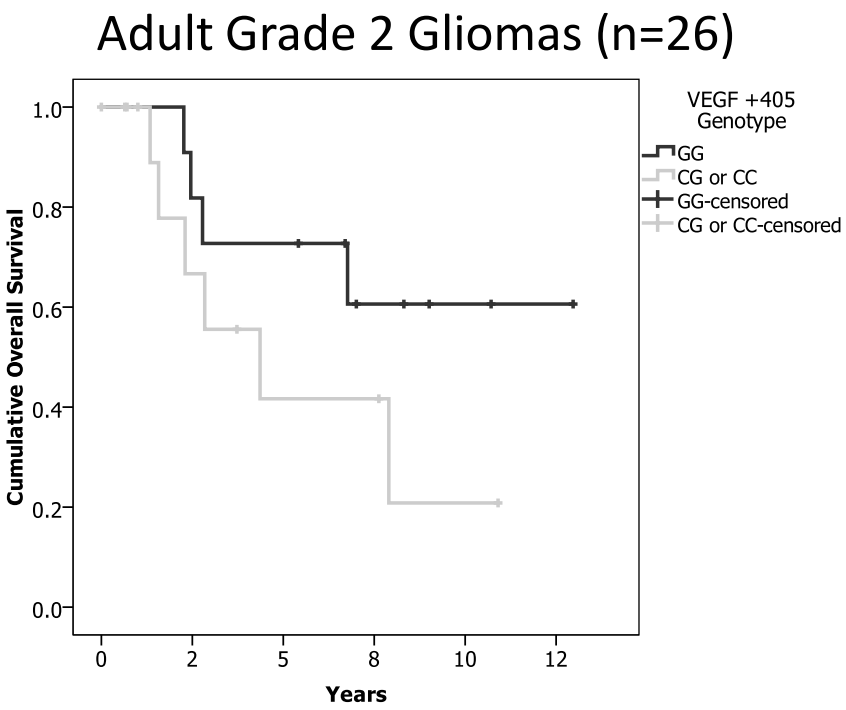


Figure 4.10 - Kaplan-Meier plot of survival in adult grade 2 gliomas between VEGF +405 genotypes. There was spreading of the lines but no significant difference in survival comparing GG to non-GG genotypes (Log rank = 1.915, df = 1, p = 0.166).

Kaplan-Meier analysis of the grade 2 tumour patient's -460/+405 haplotype assignments showed a significantly worse survival for patients who have the TG haplotype on either chromosome with mean survival of 3.2 years compared to 8.7 years for patients without the TG haplotype (Figure 4.11; Log rank = 7.01, df = 1, p = 0.008). Five year survival figures were 20% for those patients with TG haplotype on either chromosome compared to 76% for the Non-TG haplotypes (χ^2 = 4.877, df = 1, p = 0.027).

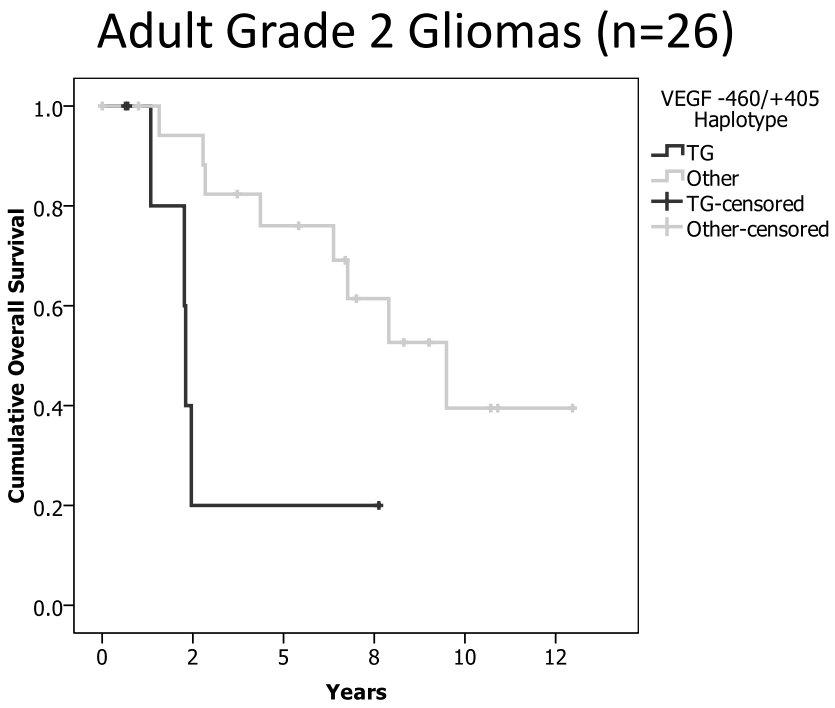


Figure 4.11 - Kaplan-Meier plot of survival in adult grade 2 gliomas between presence and absence of the VEGF -460/+405 TG haplotype. There was a significant worsening in survival if the TG haplotype was present on either chromosome (Log rank = 7.01, df = 1, p = 0.008)

For our group of grade 2 glioma patients Kaplan-Meier survival analysis for both age and Oligodendroglial histological subtype did not show any survival difference (Log rank = 0.46 - 1.34, p = 0.795 - 0.247) and these factors

were therefore not entered into further proportional hazards assessment for this population.

4.2.7 Cox proportional hazard model

Cox proportional hazards analysis was performed with -460 CC vs. Non-CC genotype and -460/+405 TG vs. Non-TG Haplotype as factors (Table 1). Significance was maintained in the model for both factors with risk ratios of 5.3 for CC genotype (improved survival) and 5.0 for TG haplotype (worsened survival).

	P value	**Risk Ratio**	**95% CI for RR**	
			Lower	Upper
CC Genotype (Improved Survival)	0.032	5.304	1.155	24.355
TG Haplotype (Worsened Survival)	0.016	5.010	1.351	18.575

Table 4.2 – Cox regression analysis results for VEGF -460 CC genotype and -460 /+405 TG Haplotype. Significance is maintained for both factors in the model.

Chapter 5 – Single nucleotide polymorphisms of the vascular endothelial growth factor gene and their association with the development and survival of paediatric brain tumours

5.1 Clinical Material and Methods

This study utilised the Ontario molecular-epidemiological, case-control database of childhood brain tumours. This was a Government backed, Province wide initiative in Ontario, Canada, that recruited all children with brain tumours along with age and gender matched healthy controls identified through Ontario Government records between 1997 and 2005. Controls were recruited on a two per case basis. Cases and controls donated whole blood for the purpose of DNA extraction. The study was subject to ethical committee approval and written, informed consent from all parents was obtained.

Clinical information was retrieved from the prospectively collected database of patients within the tumour bank which is held at the Hospital for Sick Children in Toronto. Extent of resection was assessed using both operative reports and post operative radiological reports.

5.1.1 DNA Extraction

See Chapter 3.1.1

5.1.2 VEGF SNP Genotyping

See Chapter 3.1.2

5.1.3 Statistical Analysis

Hardy-Weinberg equilibrium for cases and controls at both the -460 and +405 SNPs was tested using Chi squared. The Chi squared test with the Mantel-Haenszel estimate of common odds ratio was undertaken to compare differences in the observed genotype and haplotype frequencies. Kaplan-Meier survival analysis with log rank testing and the Cox proportional hazards model were used to estimate differences in survival.

5.2 Results

There were 193 cases representing the spectrum of paediatric brain tumours (Figure 5.1).

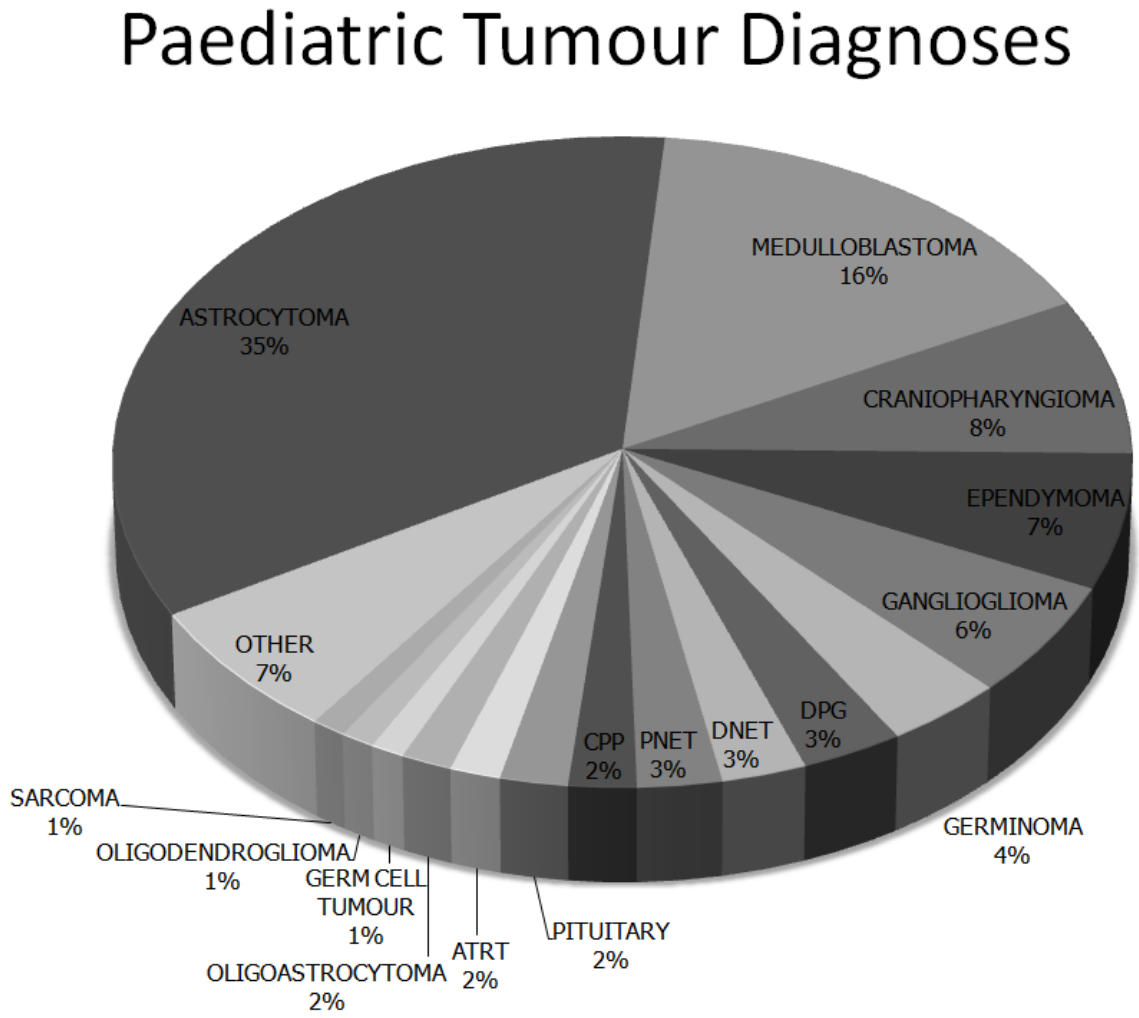


Figure 5.1 – Frequency of histological paediatric tumour types in the study (n = 193).

5.2.1 VEGF -460 Locus

Looking at the overall group of paediatric brain tumours there was no difference in the distribution of genotypes at the -460 locus (Table 5.1). For all patients with Astrocytomas (n=89), there was no difference in either the genotype distribution or allele frequency at -460 (Figure 5.2).

			VEGF -460 Genotype			χ^2 p value	VEGF +405 Genotype			χ^2 p value
		Total	**CC (%)**	**CT (%)**	**TT (%)**		**CC (%)**	**CG (%)**	**GG (%)**	
Controls	All Controls	474	100 (21)	224 (47)	150 (32)		42 (9)	208 (44)	224 (47)	
Patients	All Patients	193	42 (22)	94 (49)	57 (29)	0.867[a]	24 (12)	87 (45)	82 (43)	0.287[a]
Histology	Astrocytoma	89	12 (13)	53 (60)	24 (27)	0.081[a]	12 (14)	44 (49)	33 (37)	0.143[a]
	Low Gr Astrocytoma	79	8 (10)	49 (62)	22 (28)	0.024[a]*	12 (15)	39 (50)	28 (35)	0.070[a]
	Medulloblastoma	31	8 (26)	12 (39)	11 (35)	0.640[a]	5 (16)	13 (42)	13 (42)	0.396[a]
	Ependymoma	13	3 (23)	7 (54)	3 (23)	0.805[a]	1 (8)	5 (38)	7 (54)	0.896[a]
Gender	Male	112	25 (22)	53 (47)	34 (31)	0.959[b]	18 (16)	45 (40)	49 (44)	0.265[b]
	Female	78	16 (21)	40 (51)	22 (28)		6 (8)	41 (52)	31 (40)	
WHO Tumour Grade	Grade 1	108	18 (17)	59 (54)	31 (29)	0.374[b]	13 (12)	50 (46)	45 (42)	0.999[b]
	Grade 2	11	3 (27)	5 (46)	3 (27)		1 (9)	5 (45.5)	5 (45.5)	
	Grade 3	22	7 (32)	11 (50)	4 (18)		3 (14)	10 (45)	9 (41)	
	Grade 4	38	11 (29)	14 (37)	13 (34)		4 (10)	17 (45)	17 (45)	

Table 5.1 – Distribution of genotypes for paediatric tumours at the VEGF -460 and +405 Loci. Looking at overall genotype distribution there was a significant difference between low grade astrocytomas and controls at the VEGF -460 locus. [a] χ^2 p value comparing genotype distribution of factor with control. [b] χ^2 p value comparing genotype distribution within the group.

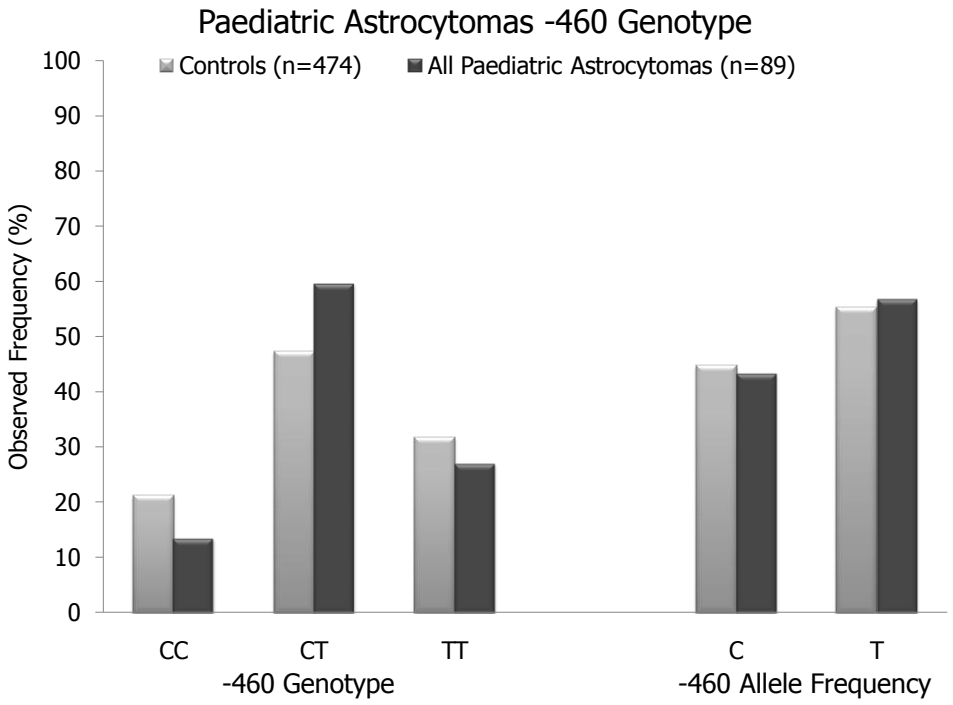


Figure 5.2 - Genotype and allele frequencies for all paediatric astrocytomas at the VEGF -460 locus. There were no differences in either genotype or allele frequencies between cases and controls (Genotype $\chi^2 = 3.887$, df = 2, p = 0.143; Allele $\chi^2 = 0.013$, df = 1, p = 0.908).

However looking at the low grade (grades 1 & 2) glioma group (n=79), there was an overall difference in genotype distribution with an increased frequency of the heterozygous CT genotype and a decrease in the homozygous CC genotype (Figures 5.3 & 5.4).

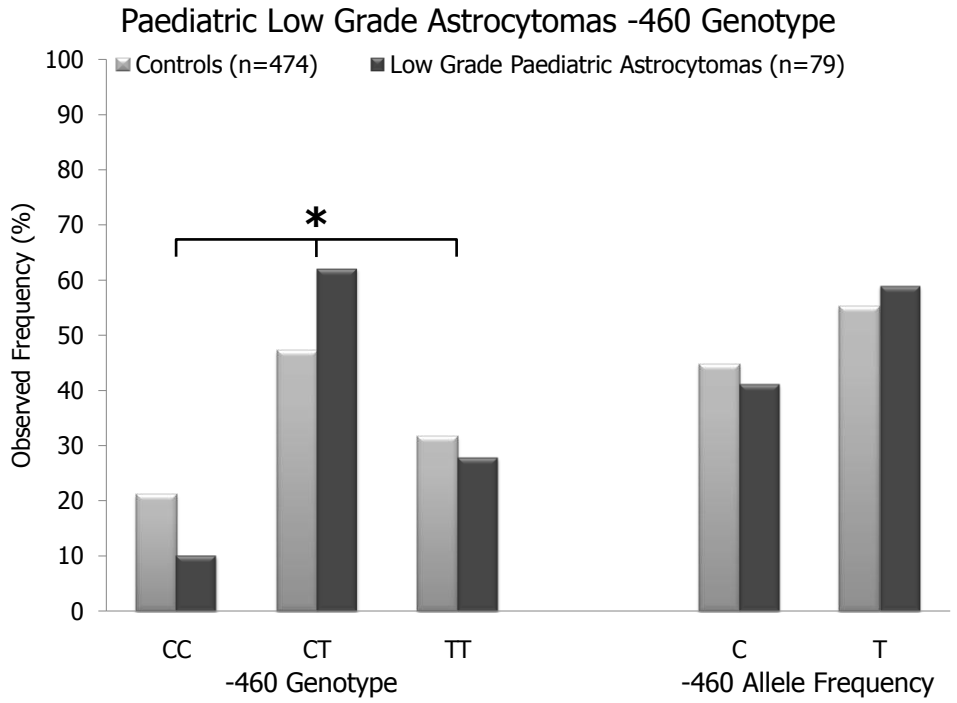


Figure 5.3 - Distribution of genotype and allele frequency for paediatric low grade astrocytomas at the VEGF -460 locus. There was a significant difference in genotype frequency but not allele frequency between cases and controls (Genotype $\chi^2 = 7.478$, df = 2, p = 0.024; Allele $\chi^2 = 0.706$, df = 1, p = 0.401).

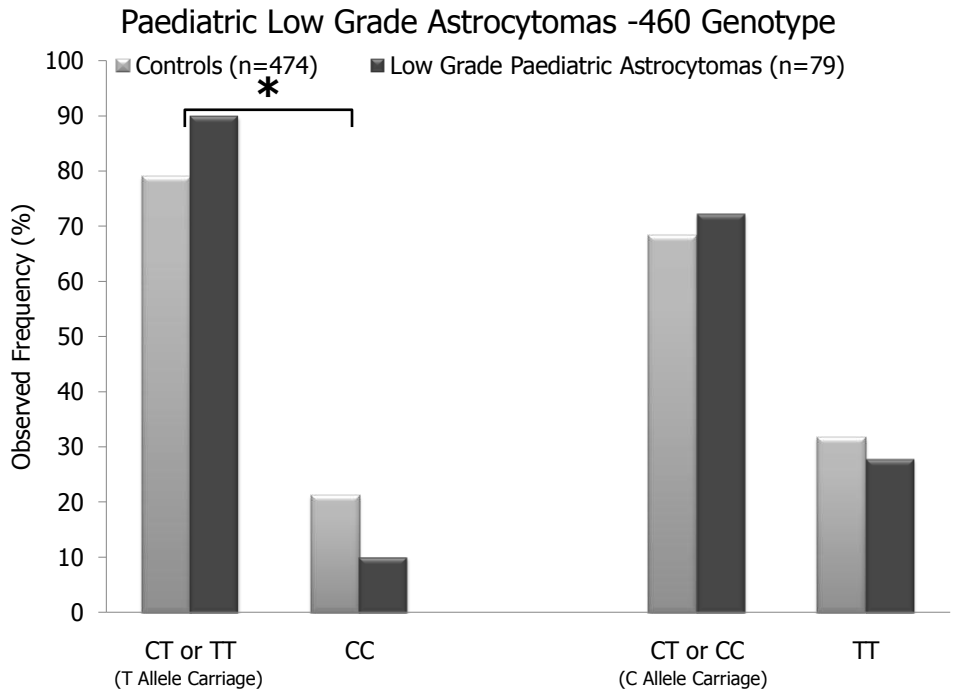


Figure 5.4 - Distribution of homozygous genotypes at the VEGF -460 locus for low grade paediatric astrocytomas. There was a significant decrease in the CC homozygous genotype in low grade astrocytoma patients compared to controls (CC vs. non-CC χ^2 = 5.186, df = 1, p = 0.023; Odds ratio = 0.421, 95% CI 0.196 to 0.904).

5.2.2 VEGF +405 Locus

Again, looking at all tumour types combined, there was no difference in genotype or allele frequency at the +405 locus. For the group of Astrocytomas, there was a borderline significant increase in C allele frequency at +405 (Figure 5.5).

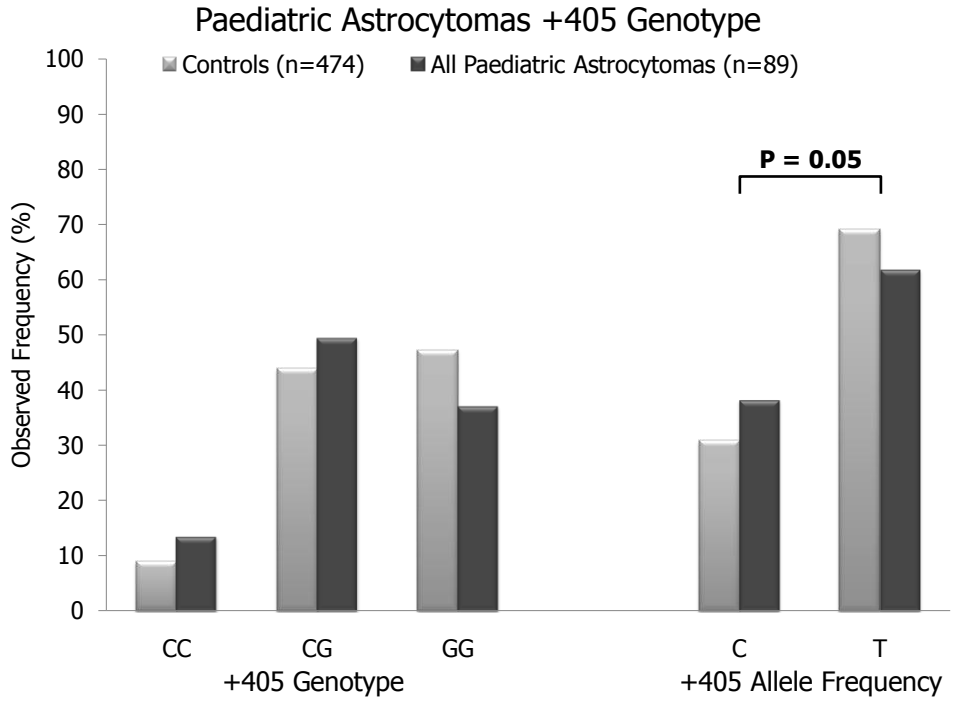


Figure 5.5 - Distribution of genotype and allele frequency for all paediatric astrocytomas at the VEGF +405 locus. There was a significant difference in allele frequency but not genotype frequency between cases and controls (Genotype $\chi^2 = 3.887$, df = 2, p = 0.143; Allele $\chi^2 = 3.774$, df = 1, p = 0.052).

For the low grade gliomas there was a significant increase in C allele frequency with a decrease in homozygous GG frequency at +405 (Figures 5.6 & 5.7).

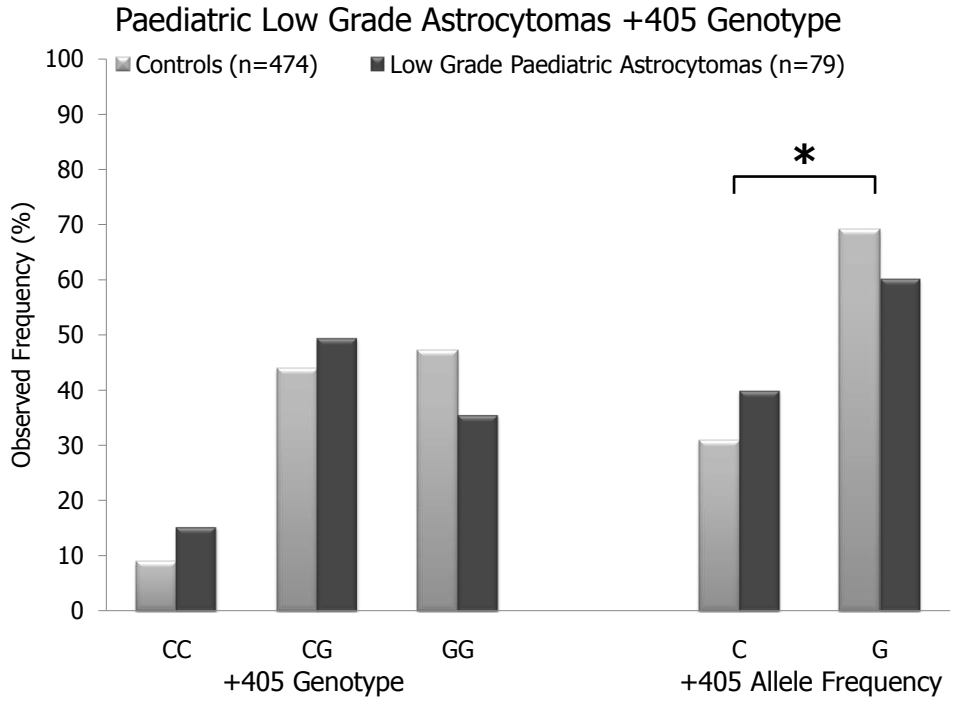


Figure 5.6 - Distribution of genotype and allele frequency for paediatric low grade astrocytomas at the VEGF +405 locus. There was a significant increase in C allele frequency but not genotype frequency between cases and controls (Genotype $\chi^2 = 5.308$, df = 2, p = 0.070; Allele $\chi^2 = 5.114$, df = 1, p = 0.024).

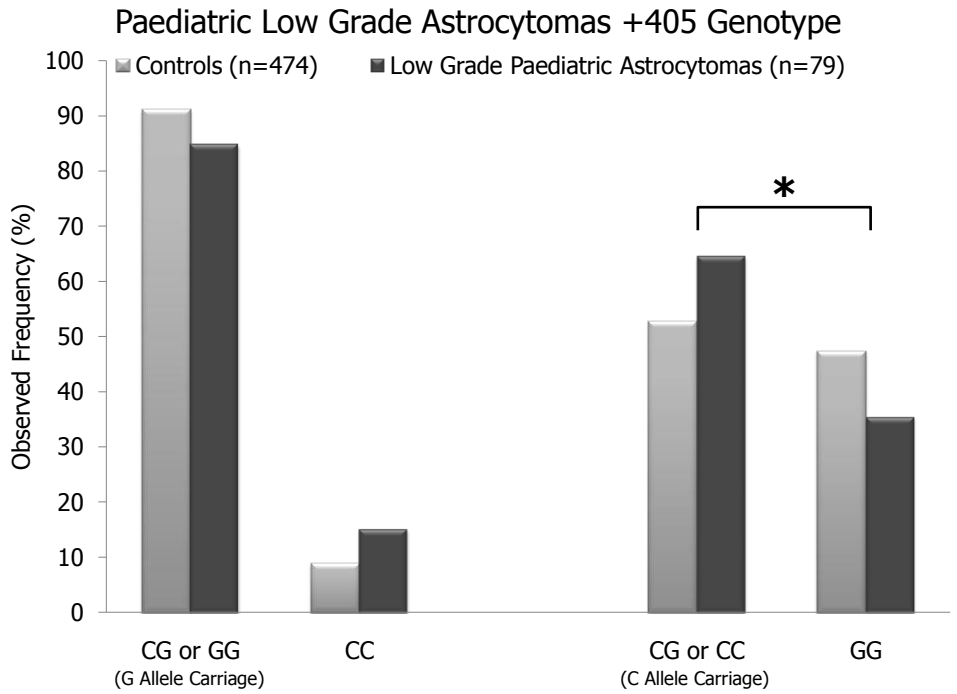


Figure 5.7 - Distribution of homozygous genotypes at the VEGF +405 locus for low grade paediatric astrocytomas. There was a significant decrease in the GG homozygous genotype in low grade astrocytoma patients compared to controls (GG vs. non-GG χ^2 = 3.873, df = 1, p = 0.049; Odds ratio = 0.610, 95% CI 0.372 to 1.001).

5.2.3 Haplotype Analysis

For the collective group of all paediatric tumours, there was no difference in haplotype frequency when compared to controls. For all grades of Astrocytomas there was an overall difference in distribution of haplotype pairs compared to controls with a decreased frequency of TG haplotype on either chromosome (Figures 5.8 & 5.9).

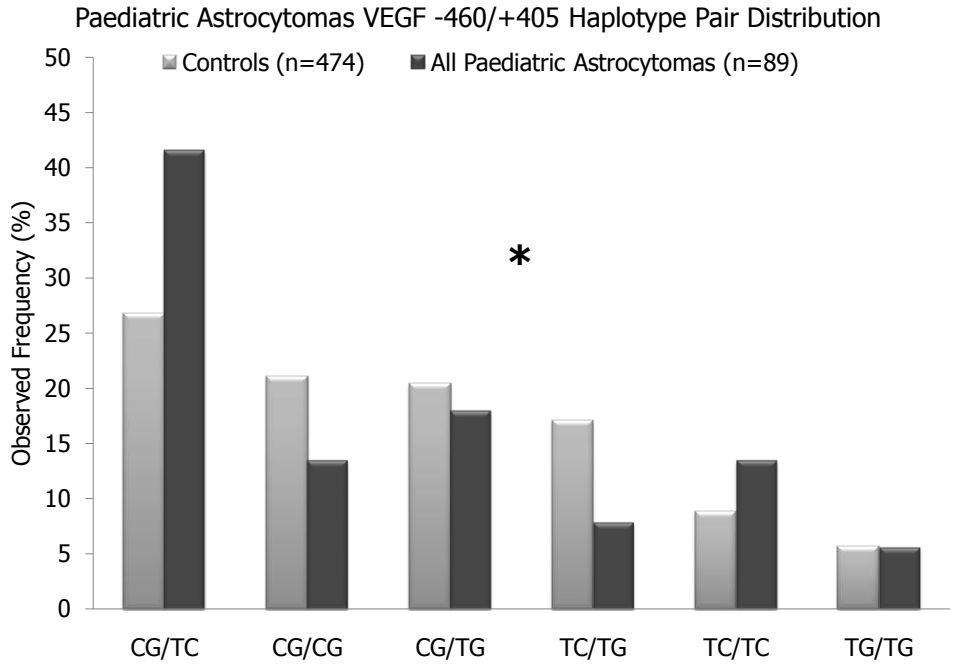


Figure 5.8 - Distribution of haplotype pairs for the VEGF -460/+405 haplotype in all paediatric astrocytomas. There was significant difference in haplotype pair frequencies comparing cases with controls (χ^2 = 13.782, df = 5, p = 0.017).

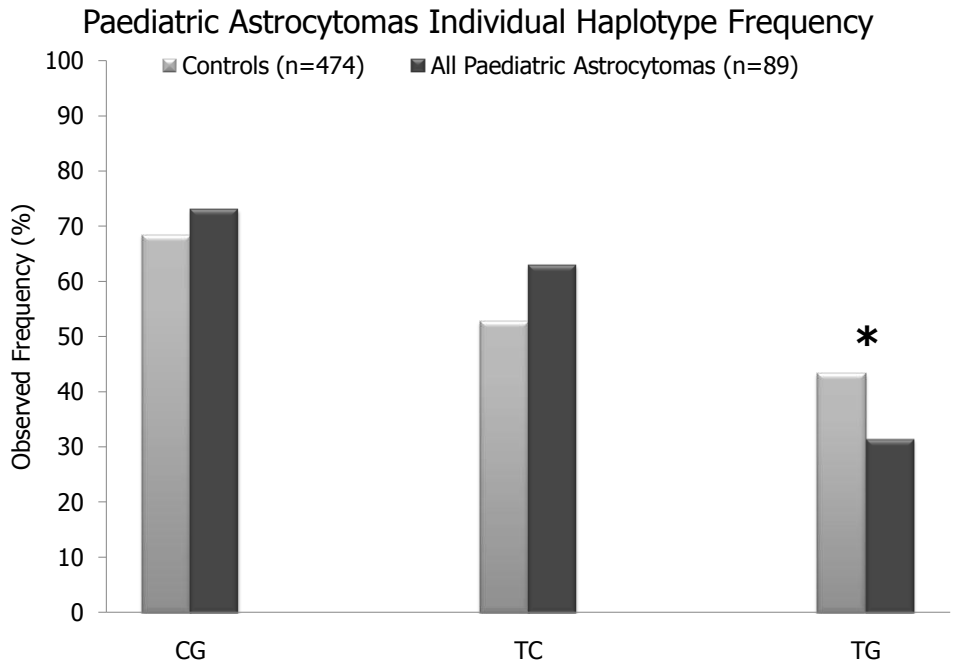


Figure 5.9 - Individual haplotype frequencies for the VEGF -460/+405 haplotype. There was a significantly reduced frequency of the TG haplotype in paediatric astrocytoma patients compared to controls (χ^2 = 4.292, df = 1, p = 0.038; Odds ratio = 0.602, 95% CI 0.372 to 0.976).

For low grade Astrocytomas there was again a difference in distribution of haplotype pairs together with borderline increase of TC haplotype and borderline decrease of TG haplotype when compared to controls (Figures 5.10 & 5.11).

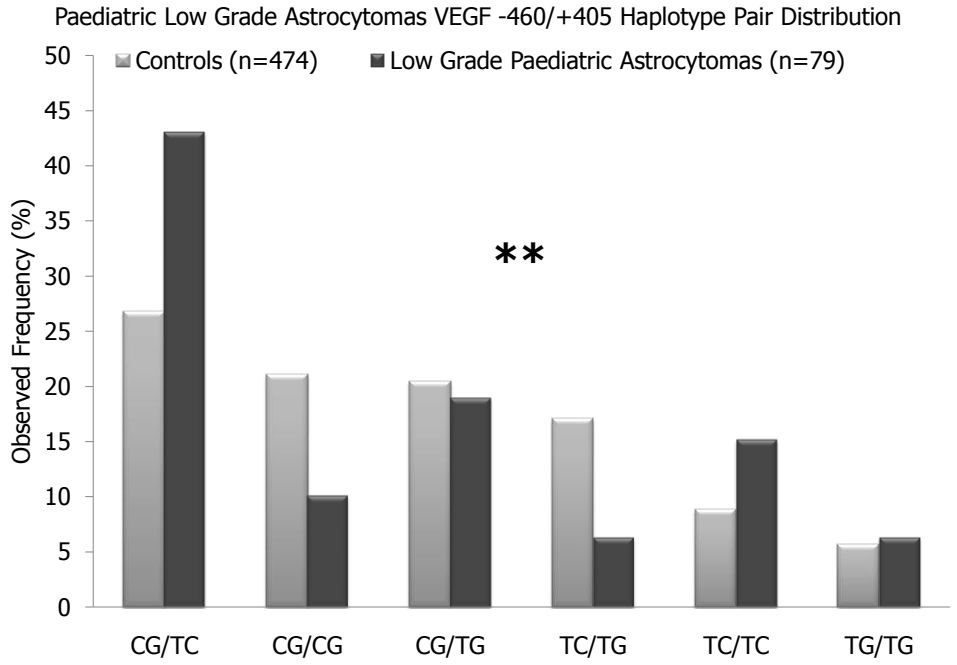


Figure 5.10 - Distribution of haplotype pairs for the VEGF -460/+405 haplotype in paediatric low grade astrocytomas. There was a significant difference in haplotype pair frequencies between cases and controls (χ^2 = 18.249, df = 5, p = 0.003).

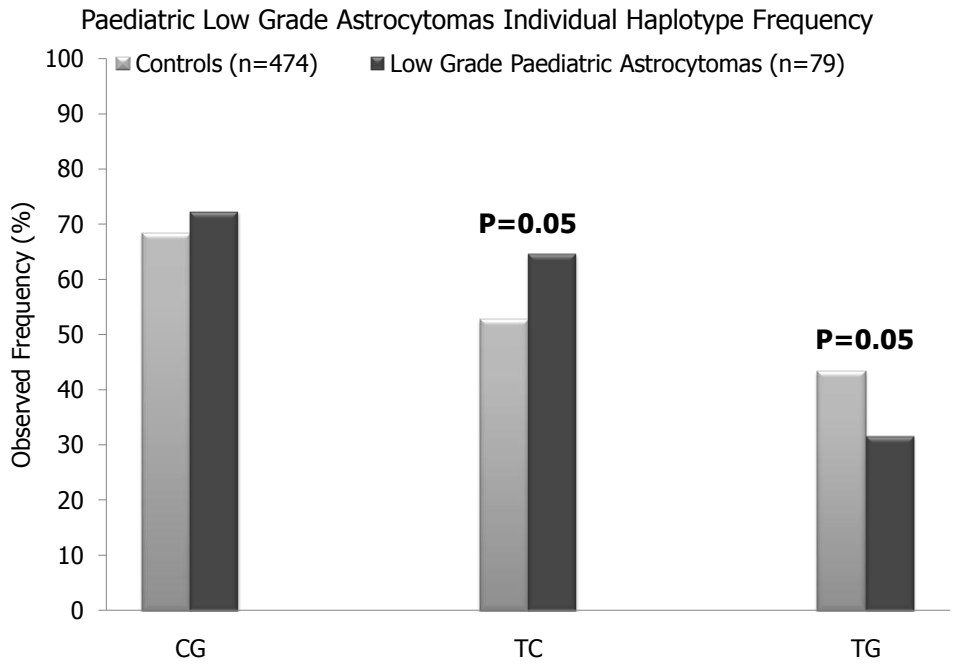


Figure 5.11 - Individual haplotype frequencies for the VEGF -460/+405 haplotype. There was a borderline increased frequency of the TC haplotype and borderline decrease in the TG haplotype in paediatric low grade astrocytoma patients compared to controls (TC χ^2 = 3.811, df = 1, p = 0.051; Odds ratio = 1.632, 95% CI 0.995 to 2.677; TG χ^2 = 3.753, df = 1, p = 0.053; Odds ratio = 0.607, 95% CI 0.366 to 1.009).

For Medulloblastomas (n=31), there was an overall significant difference in distribution of haplotype pairs compared to controls (Figure 5.12) but otherwise no difference in genotype at either locus.

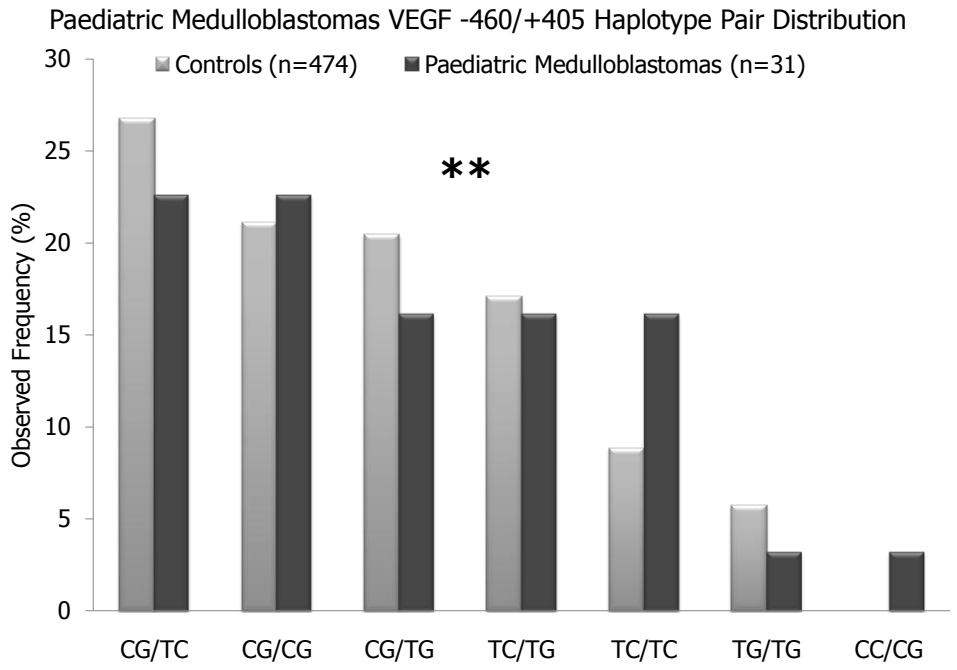


Figure 5.12 - Distribution of haplotype pairs for the VEGF -460/+405 haplotype in paediatric medulloblastomas. There was a significant difference in haplotype pair frequencies between cases and controls (χ^2 = 17.773, df = 5, p = 0.007).

5.2.4 Survival Analysis

5.2.4.a Low Grade Astrocytoma – Progression Free Survival

For the group of low grade Astrocytomas there was 100% progression free survival in the VEGF -460 CC genotype patients (n=4) compared to 15% of cases recurring in the other genotype groups (n=53). Although the Kaplan-Meier plots appear to separate, this was not a statistically significant difference (Figure 5.13).

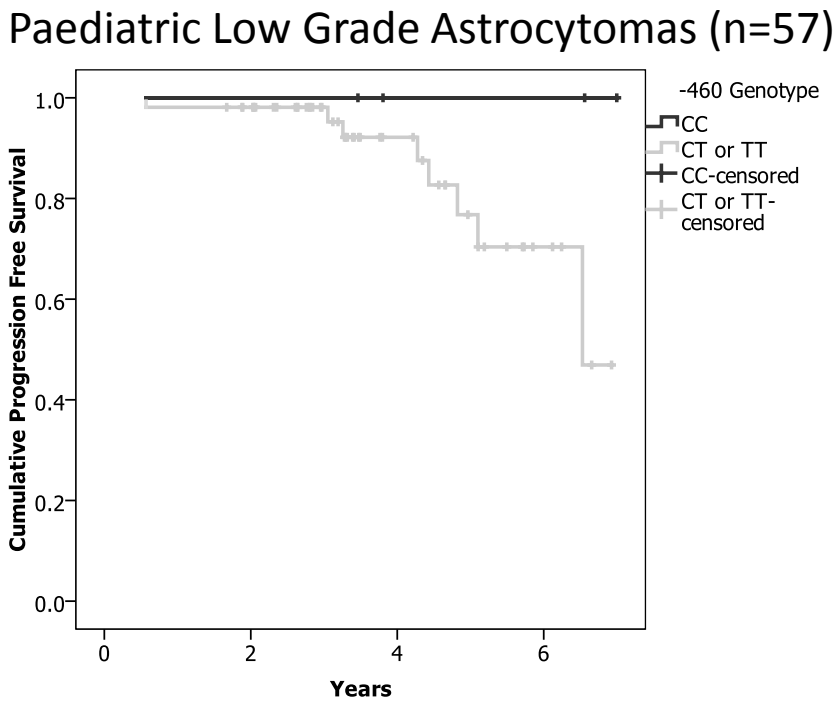


Figure 5.13 - Kaplan-Meier plot of progression free survival in paediatric low grade astrocytomas between CC and non-CC genotype at the VEGF -460 locus. Although the lines separate this was not statistically significant (Log rank = 1.457, df = 1, p = 0.227).

At the +405 locus there was again a trend in progression free survival differences with patients having the CC genotype appearing to have a tendency to recur (Figure 5.14).

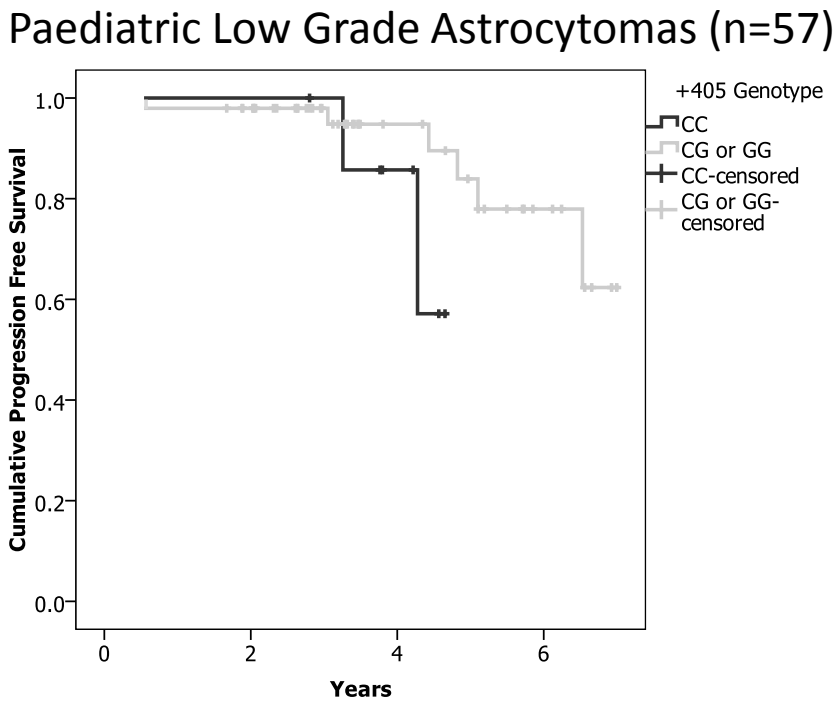


Figure 5.14 - Kaplan-Meier plot of progression free survival in paediatric low grade astrocytomas between CC and non-CC genotype at the VEGF +405 locus. Although the lines separate indicating a tendency to recur with CC genotype this was not statistically significant (Log rank = 2.404, df = 1, p = 0.121).

5.2.4.b Medulloblastomas and Ependymomas – Overall Survival

5.2.4.b.i VEGF Genotype and Haplotype

For the combined group of Medulloblastomas and Ependymomas there appeared to be several trends in overall survival between the different genotypes and haplotypes. Survival of the VEGF -460 CC genotype in this group was 100% with no deaths in the 11 patients with CC compared to 10 deaths in the 34 patients (29.6%) with other (CT or TT) genotypes (Figure 5.15).

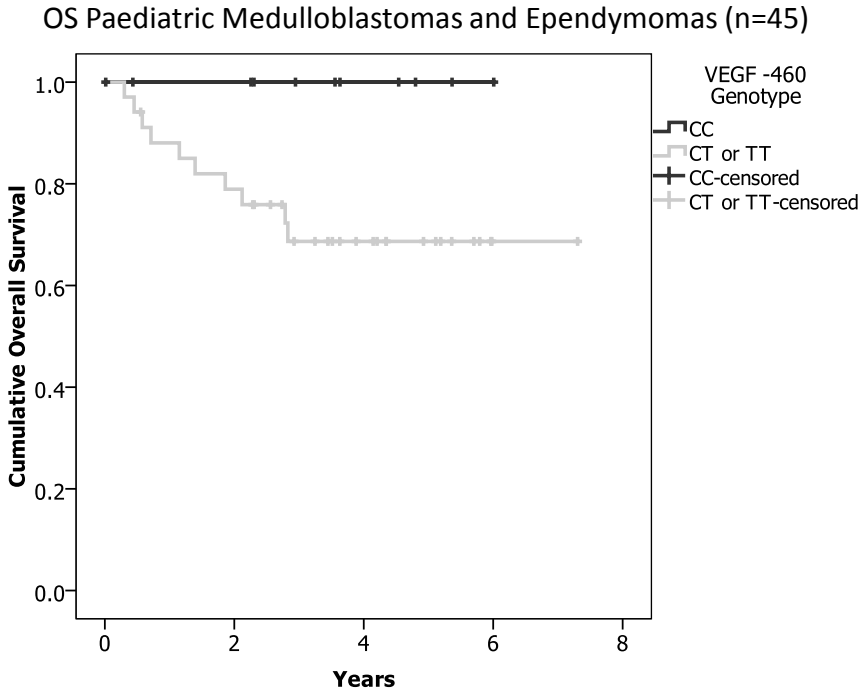


Figure 5.15 - Kaplan-Meier plot of overall survival in paediatric medulloblastomas and ependymomas between CC and non-CC genotype at the VEGF -460 locus. Although the lines separate with a trend to improved survival with the CC genotype, this was not statistically significant (Log rank = 3.151, df = 1, p = 0.076).

At the +405 locus, there was a trend for the CC genotype to have a better overall survival compared to other (CG and GG) genotypes. There were no deaths in the 6 CC genotype patients with 10 deaths out of 39 (25.6%) in the patients with other genotypes (Figure 5.16).

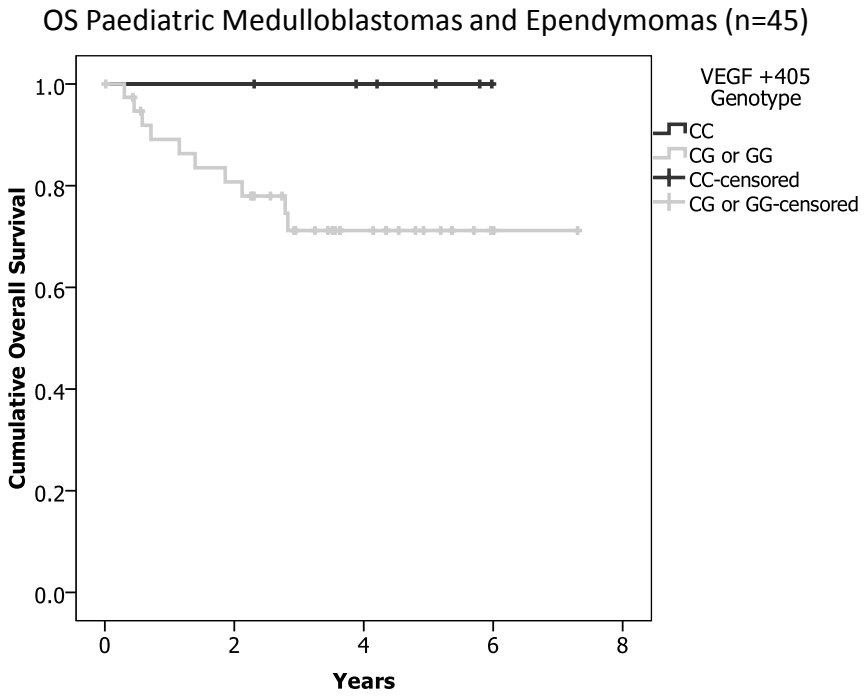


Figure 5.16 - Kaplan-Meier plot of overall survival in paediatric medulloblastomas and ependymomas between CC and non-CC genotype at the VEGF +405 locus. Although the lines separate with a trend to improved survival with the CC genotype, this was not statistically significant (Log rank = 1.915, df = 1, p = 0.166).

For the haplotype analysis there was a trend of worse survival with the presence of the TG haplotype on either chromosome (Figure 5.17).

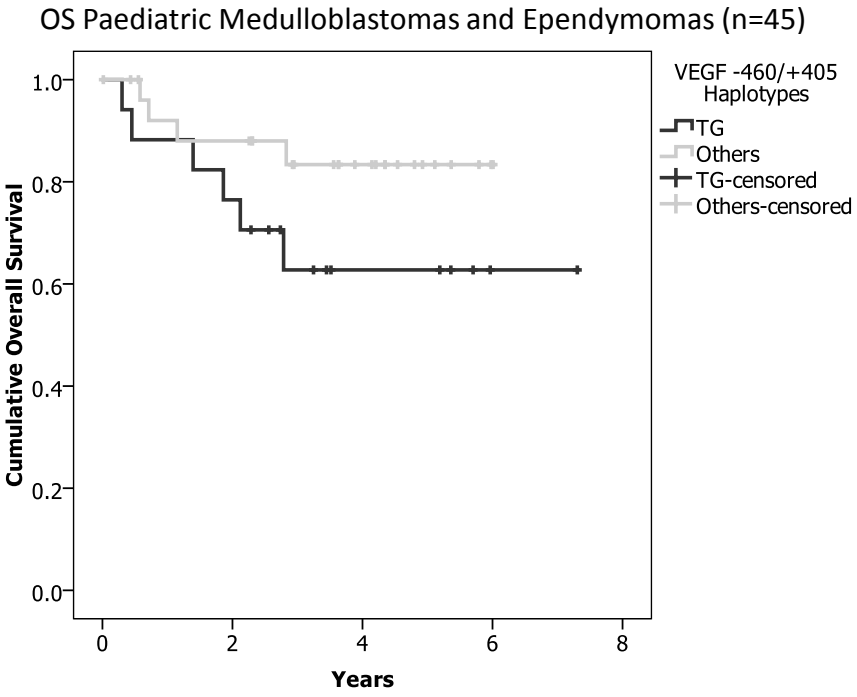


Figure 5.17 - Kaplan-Meier plot of overall survival in paediatric medulloblastomas and ependymomas between presence and absence of VEGF -460/+405 TG haplotype. Although the lines separate with a trend to worse survival with presence of the TG haplotype, this was not statistically significant (Log rank = 2.268, df = 1, p = 0.132).

5.2.4.b.ii Gender

For this combined group of patients there was a significant survival advantage in male patients (Figure 5.18). Looking at the Medulloblastomas and Ependymomas separately there was a survival advantage in the males but this was not independently significant in either group (Medulloblastoma Log rank = 1.195, df = 1, p = 0.274 and Ependymoma Log rank = 2.718, df = 1, p = 0.099).

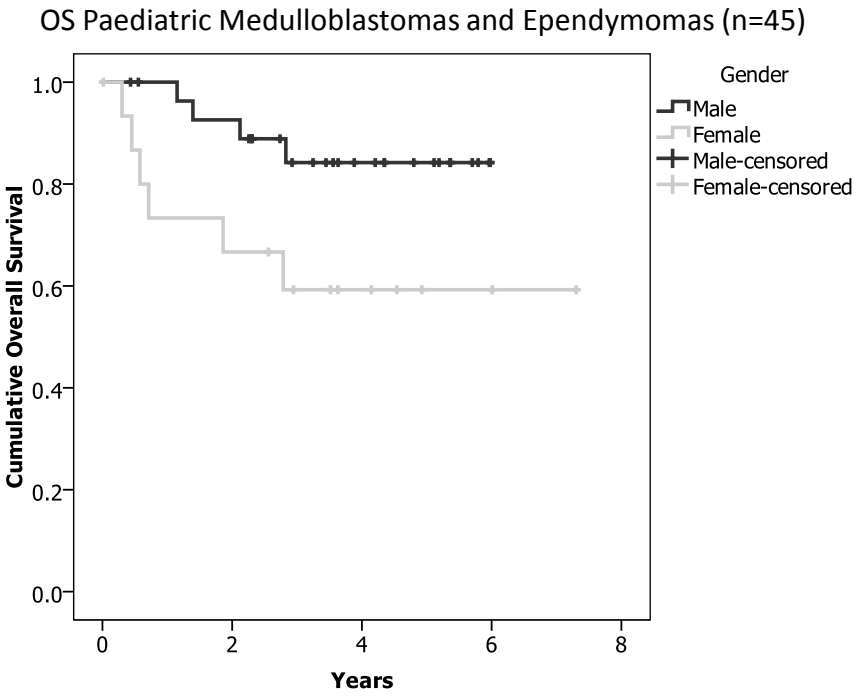


Figure 5.18 - Kaplan-Meier plot of overall survival in paediatric medulloblastomas and ependymomas between genders. There was a significantly better survival in males in this combined group (Log rank = 3.928, df = 1, p = 0.047).

5.2.4.b.iii Extent of Resection

The amount of surgical resection had a significant effect on survival with patients where a gross total resection (GTR) or almost total resection (ATR), i.e. >95% resection, was achieved having a significant survival advantage over subtotal resection or biopsy (Figure 5.19).

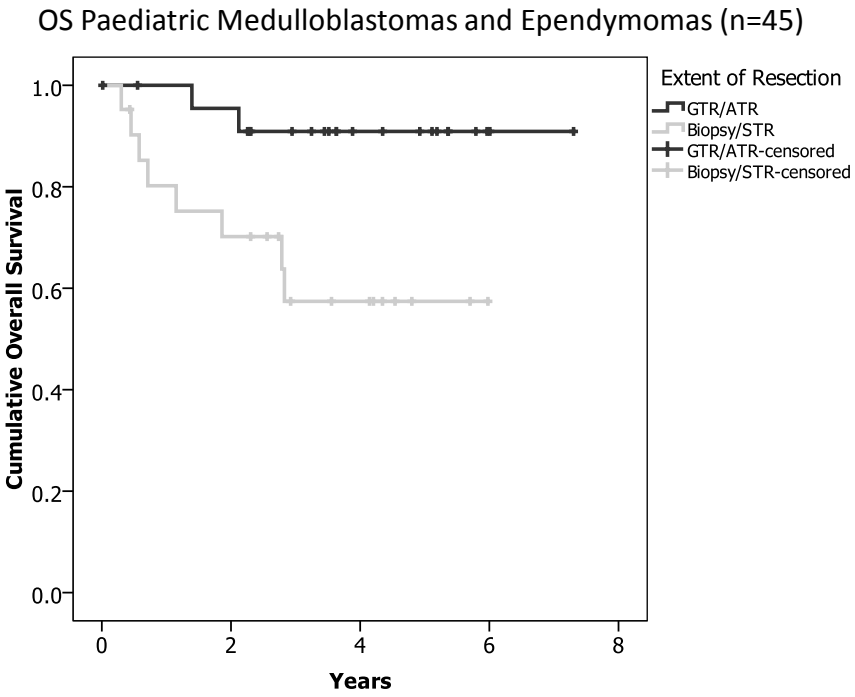


Figure 5.19 - Kaplan-Meier plot of overall survival in paediatric medulloblastomas and ependymomas between gross or almost total resection and subtotal resection or biopsy. There was a significant survival advantage with gross or almost total resection (Log rank = 5.874, df = 1, p = 0.015).

5.2.4.b.iv Age at Presentation

There was a strong influence of age at presentation on survival with patients under 3 years of age at presentation having a significantly worse prognosis compared to older patients (Figure 5.20).

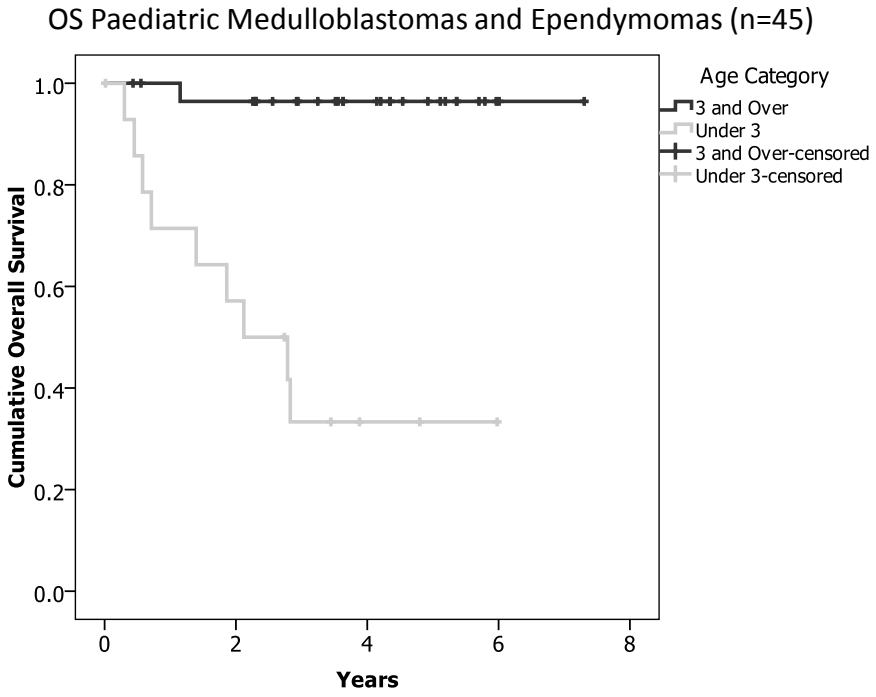


Figure 5.20 - Kaplan-Meier plot of overall survival in paediatric medulloblastomas and ependymomas between age groups. There was a strongly significant survival advantage for patients age 3 and over (Log rank = 21.221, df = 1, p = 0.000004).

5.2.5 Cox proportional hazards model

The statistically significant variables on univariable analysis were entered into a Cox proportional hazards model. Significance was maintained in the model for age category and gender but not extent of resection (table).

	P value	Risk Ratio	95% CI for RR	
			Lower	Upper
Age under 3 (Worsened Survival)	0.004	30.693	3.038	310.110
Gender (Female Worsened Survival)	0.039	4.860	1.081	21.848
Extent of Resection	0.730	1.381	0.220	8.654

Table 5.2 – Cox regression analysis results for age under 3, gender and extent of resection. Significance is maintained for age under 3 and gender but not extent of resection in the model.

Chapter 6 Discussion

6.1 Prognostic factors relating to sex steroid receptors in meningiomas

This study like many before has identified sex steroid receptors in meningiomas. Our findings of the distribution of tumour grades and subtypes would be consistent with the published literature.[35] Our overall recurrence rate for such a long period of follow up is somewhat low at 9%.[71] This should be an accurate figure as the patients are from an area with relatively low levels of migration and they are followed up both clinically and radiologically in a specific meningioma clinic.

6.1.1 Sex steroid receptors

For progesterone receptors our proportion of patients who are receptor positive (55%) was again consistent with the published literature.[58, 65]

Oestrogen receptor levels is more variable and controversial in the literature. The findings of low levels of nuclear, positive oestrogen receptors (1%) has been the finding of many studies but others have described higher results, up to 94%.[574] This is partly a manifestation of assay type with most of the studies showing higher levels of oestrogen receptors having performed cytosol assays of receptor levels. This study demonstrated that oestrogen receptors are present in the cytoplasm in 53% of tumours and obviously this would have given a positive result on cytosol assay, but the receptor is only biologically active in the nucleus.[575]

In this study there is a correlation with progesterone receptors and gender. Tumours were progesterone receptor negative in 60.7% of males and 37.8% of females. This has been found in other studies,[58, 65] but there is no known mechanism that explains this finding.

This study also showed a strong association with tumour grade and progesterone receptor status. All of the grade 2 and 3 tumours were progesterone receptor negative. Many other studies have shown a similar correlation with tumour grade and progesterone receptor status, but they have not found an absolute relationship, describing some grade 2 and 3 tumours as being progesterone receptor positive.[58, 65]

6.1.2 Recurrence

In univariable analysis tumour grade was associated with recurrence with an odds ratio of 11.667. This is a consistent finding with other published studies.[58, 65] The numbers were too small in this study to separate out the magnitude of effect between grade 2 and 3 tumours.

The absolute Simpson grade was not statistically significant in relation to recurrence in this cohort but this was presumably due to small numbers of recurrences. When the Simpson grade was dichotomised to grades 1 and 2 vs. grades 3-5 (where there is some amount of residual nodular tumour left at the time of resection) then there was a clear association with recurrence and less extent of resection.

For this study, overall gender was not a prognostic factor for tumour recurrence. This is, as with other studies, despite their being an increased incidence of higher grade tumours in males and the implication is that males must have other protective factors to counteract the larger proportion of higher risk tumours.

In univariable analysis on this study, progesterone receptor status is predictive of tumour recurrence, with an odds ratio of five times higher in PR negative tumours. This has been a finding in other studies, looking at both grade 1 tumours and all tumour types.[58, 63-65]

6.1.3 Multivariable analysis

Of the papers who have found an association with progesterone receptor status and recurrence, only one undertook multivariable analysis to control for other known predictive factors.[58] In this study a Cox regression model was constructed with a combination of progesterone receptor status, number of mitoses and grade 3 histology as co-variables. They found that the models were significant overall but there was no indication as to whether or not each of the individual co-variables maintained their significance in the model.

In the two studies that found progesterone receptor status to be predictive of recurrence in grade 1 patients,[63, 64] both had high rates of recurrence for grade 1 patients of 28% and 50% and it may be that some of these tumours were actually grade 2.

In this study, the Cox regression analysis with progesterone receptor status, grade 2 and 3 tumours and extent of resection using a dichotomised Simpson grade was a significant model overall, but progesterone receptor status clearly lost significance. Extent of resection was borderline significant and tumour grade 2 and 3 was borderline non-significant. This finding is due to the very strong association with progesterone receptor status and tumour grade and implies that decreased nuclear progesterone receptor expression is a secondary phenomenon to increasing tumour grade.

6.1.4 Biological influence of progesterone receptors

The evidence that progesterone receptors are biologically important in meningiomas is reasonably strong with evidence of increased growth of tumours at the time of high circulating progestogens,[40, 41] increased frequency in females,[36] and an association with breast cancer.[42] In addition, a study in nude mice showed a reduction of growth of a human meningioma xenograft with the administration of an antiprogesterone.[576] Finally there have been a variety of studies looking at hormonal manipulation of meningiomas with mixed success but no proven therapy shown in a phase III randomised controlled trial.[577-581]

With the advent of high throughput chromosomal and genetic expression analysis recent studies have shown an increase in chromosomal abnormalities[65] and changes in gene expression[66] in meningiomas with loss of progesterone receptor expression. The chromosomal losses are most

notable in chromosomes 22 and 14, particularly in the progesterone receptor negative tumours. The location of the progesterone receptor gene on chromosome 11q22 did not appear to be involved in the chromosomal changes seen in more progressive tumours.[65] However, there is altered gene expression in progesterone negative meningiomas, including the mitogen activated protein kinase 4 (*MAP4K4*) gene.[66] MAP kinases have been shown to be important in the regulation of progesterone receptor activity and localisation within the nucleus.[582, 583] Also, increased cell turnover in itself has a down regulating effect on progesterone receptor expression.[62]

6.1.5 Limitations

This study involved the retrospective analysis of the clinical findings of the patient cohort. Although this is one of the biggest studies in the published literature, the relatively small number of recurrences meant a limited number of events in the survival analysis.

6.1.6 Summary

It is reasonable from this evidence to assume that progesterone receptors are important in the growth of meningiomas. The recurrent finding in many studies of worse prognosis with loss of nuclear progesterone receptors would initially seem counter intuitive to this argument. The findings of this study would imply that atypical and malignant grade is a more important factor in terms of recurrence than progesterone receptor status. Findings from

genetic studies have shown evidence of altered expression of genes important in the biological localisation and activity of progesterone receptors. This study demonstrated that there were progesterone receptors present in the cytoplasm of 35 out of the 46 nuclear progesterone receptor negative patients. It may be that alteration of MAP kinases or other co-factors localising the receptors to the cytoplasm with increasing atypia in meningiomas is the reason why there is an association with negative nuclear progesterone receptors and worse outcome.

6.2 The effect of neoadjuvant chemotherapy on operative blood loss and surgical resection for Choroid Plexus Carcinomas

Complete surgical resection has consistently been shown to be the most important factor in the survival of patients with choroid plexus carcinomas[81] and this finding is evident in this study. In addition we also demonstrated the previously described finding that young age at presentation was associated with a poor outcome.

6.2.1 Neoadjuvant chemotherapy

In this patient group, giving neoadjuvant chemotherapy as a pre-operative intervention does appear to facilitate surgical resection by reducing the vascularity of the tumours rather than their size. It is certainly my experience that the tumours operatively handle more favourably following chemotherapy and these quantitative findings of a reduction of mean blood loss from 1.11 blood volumes to 0.22 of a blood volume would appear to support that perception.

The process of administering the chemotherapy in the per-operative setting can be very difficult and requires a lot of input from a multidisciplinary team to maintain control of raised intracranial pressures secondary to the tumour mass and associated hydrocephalus.

6.2.2 Limitations

Given that choroid plexus carcinomas are rare tumours the study is understandably limited in its size. In addition the choice of whether a child received neoadjuvant chemotherapy was multifactorial and based upon surgeon/oncologist preference.

6.2.3 Summary

The striking benefits seen within the limitations of this study would support neoadjuvant chemotherapy for choroid plexus carcinomas as a valid treatment option and warrant further studies of its use in this often devastating condition.

6.3 Single nucleotide polymorphisms of the vascular endothelial growth factor gene and their association with the development and survival of adult cerebral gliomas

This study involved the analysis of VEGF genotype and haplotype in a cohort of adult glioma patients

6.3.1 VEGF -460 locus

At the -460 locus there was an overall difference in the distribution of genotypes between gliomas patients and controls and this was most directly associated with an increase in the presence of the homozygous TT genotype in gliomas patients. There was a non-significant trend towards an overall increase in the T allele frequency.

6.3.2 VEGF +405 locus

Overall at the +405 locus there was no difference in genotype frequencies between cases and controls but there was a trend towards the presence of the homozygous CC genotype in gliomas patients.

6.3.3 VEGF -460/+405 haplotype analysis

Linkage disequilibrium allows a highly predictive allocation of haplotypes for the locus. Overall there was no difference in haplotype frequency but there was a significant decrease seen in the presence of the CG haplotype in gliomas patients.

6.3.4 Survival analysis

For the group as a whole the VEGF polymorphisms were not predictive of survival. When the subgroup of grade 2 astrocytomas was analysed a significant influence was found with the patients with the -460 CC genotype having a better outcome. At the +405 locus there was a trend to better survival with the GG genotype and on haplotype analysis the patients with the TG haplotype on either chromosome having a significantly worse survival.

Both -460 CC genotype and TG haplotype maintained their significance in a Cox regression analysis model.

6.3.5 Limitations

Although the cases and control group have a similar ethnic distribution, they are taken from geographically different areas and this may have an influence on genotype frequencies. This would not impact on the survival analysis.

The whole group is made up of a heterogeneous cohort of gliomas and therefore the subgroup sample size is small.

For the group of grade 2 astrocytomas, accurate information on initial tumour size and extent of resection was not available. These are factors that have been shown to influence survival for these tumours although this is not a universal finding in all studies.[35]

6.3.6 Summary

This is the first study to show an association with a single nucleotide polymorphism of the *VEGF* gene and cerebral gliomas. In addition, genotype and haplotype of the *VEGF* gene has been shown to have an association with outcome for grade 2 astrocytomas.

6.4 Single nucleotide polymorphisms of the vascular endothelial growth factor gene and their association with the development and survival of paediatric brain tumours

This study utilised the Ontario molecular-epidemiological, case-control database of childhood brain tumours to investigate the association of *VEGF* gene polymorphisms and paediatric brain tumours.

6.4.1 VEGF -460 locus

Looking at the variety of tumour types in total there was no significant difference in genotype frequency compared with controls.

For patients with low grade astrocytomas there was an increase in the frequency of the CT genotype and a decrease in the homozygous CC genotype at the -460 locus compared to controls.

6.4.2 VEGF +405 locus

There was no difference in genotype frequency between all the combined tumour types and controls.

For all grades of astrocytoma there was a significant increase in the overall C allele frequency. This was also seen in the low grade astrocytoma subgroup along with a decrease in the homozygous GG genotype.

6.4.3 VEGF -460/+405 haplotype analysis

For the subgroup of all grades of astrocytoma there was an overall difference in the frequency of haplotype pairs compared to controls. This was most evident with the TG haplotype which was decreased compared to controls.

For low grade astrocytomas there was again an overall difference in the haplotype pairs compared to controls. For individual haplotypes there was a significant increase in the TC haplotype and a decrease in the TG haplotype.

For medulloblastomas there was an overall difference in distribution of haplotype pairs compared to controls.

6.4.4 Survival analysis

For low grade astrocytomas there was a non-significant progression free survival advantage for patients with the VEGF -460 CC genotype with no recurrence of tumours in the patients with this genotype. At the VEGF +405 locus there was also a non-significant progression free survival advantage for patients with the CC genotype.

The group of medulloblastomas and ependymomas were combined for this analysis. These tumour types have a similar outcome and progression profile overall and it is generally different to the other common paediatric brain tumours.[35] At the VEGF -460 locus the CC genotype patients showed 100% overall survival which was just not significant compared to other genotypes. At the VEGF +405 locus the CC genotype again showed a non-

significant overall survival advantage with 100% survival in this group. On haplotype analysis the TG haplotype of VEGF -460/+405 showed a trend to worse overall survival compared to patients with non-TG haplotypes.

In this combined group of medulloblastomas and ependymomas there was an overall survival advantage for males compared to females. There have been conflicting studies as to the effect of gender in medulloblastomas and ependymomas. The largest series from the Surveillance, Epidemiology, and End Results (SEER) database have shown for medulloblastomas, no overall survival difference but in the over 3 years patients a female advantage and in the under 3 years a trend towards a male survival advantage.[9] For ependymomas there was an overall survival advantage for females in all age groups including adults.[584]

Overall in the group there was a significant survival advantage for a greater than 95% surgical resection and age 3 or greater at presentation. These associations have been shown previously in various studies for both tumour types.[35]

A Cox proportional hazards model of the significant variables on univariable analysis showed an overall significant model with age greater than 3 at presentation and gender maintaining their significance but extent of resection becoming non-significant.

6.4.5 Limitations

Given the rare nature of these tumours the sample sizes, particularly in the subgroups were small with a limited number of events in the survival analysis. This is, however, one of the largest cohorts of its nature looking at paediatric brain tumours, collected over several years from large catchment population.

6.5 Possible influence of VEGF SNPs on development and progression of CNS tumours

6.5.1 Tumour type

For both the adults and paediatric CNS tumours presented in these studies there have been associations demonstrated with the development of certain tumour types. In addition, differences in survival have also been shown depending upon genotype and haplotype within the gene.

The tumours that have been shown to be most influenced are adult low grade astrocytomas and paediatric medulloblastomas and ependymomas. The other large groups of tumours; adult high grade astrocytomas/glioblastomas and paediatric high grade astrocytomas tend to be more aggressive tumours with much shorter median survivals.

The angiogenic profile of high grade astrocytomas is different to that of low grade astrocytomas.[585] A study from 2003 showed that angiogenic gene profiling, particularly *VEGF* was different in low grade astrocytomas compared to glioblastomas. This was most marked with primary glioblastomas where secondary glioblastomas shared some similarities with the other two types.[585] Another paper from 2006 confirmed this finding in primary and secondary glioblastomas.[586]

It has been postulated for many years that for a tumour to grow and progress to a more malignant lesion, there is a need for the switch to an angiogenic phenotype.[290] To have a constitutional angiogenic genotype would make this process more straightforward. This is a disease modulating

process as angiogenic factors such as VEGF will in themselves not promote malignant transformation. A study on anaplastic astrocytoma cells that were genetically modified to secrete four times the normal levels of VEGF found that the mutant cells grew more quickly but did not transform in glioblastomas.[587]

VEGF genotype did not appear to have an influence on survival of high grade astrocytomas/glioblastomas in this study. This is probably a consequence of loss of multiple controls of cell division and apoptosis in these malignant tumours together with marked autocrine expression of angiogenic factors that would render a background constitutional elevation of VEGF a non-significant factor. There is some evidence for this in the levels of VEGFR-1 (a physiological inhibitor of VEGF) in gliomas of different grades. Levels of VEGFR-1 rise from grade 2 to grade 3 tumours but then fall off again for grade 4 tumours.[588] The authors of this study postulate that this is a sign that the constitutional antiangiogenic pathways have given up the battle by the time a lesion is grade 4.

6.5.2 Mechanism of variable action of VEGF SNPs

Within the literature there have been a variety of studies that have demonstrated functional differences between VEGF SNPs and haplotypes.[342, 511, 525, 528, 568] Often these studies appear to directly contradict each other. Lambrechts found that the -2578/-1154/-634 AAG haplotype reduced VEGF expression and Stevens found that the same AAG genotype increased VEGF

expression.[342, 528] These studies both looked at this in cell cultures with Lambrechts using gliosarcoma and Stevens using breast cancer cell lines. Other studies have shown that within the same patient the VEGF -460 T allele and the +405 C allele were associated with lower levels of VEGF in normal colorectal tissue but this was not the case in adjacent colorectal cancer tissue.[568]

Awata found an association with the VEGF +405 C allele and diabetic proliferative retinopathy.[511] In the same study they found an increase in serum levels of VEGF in 11 healthy volunteers with the VEGF +405 CC genotype. The measurement of serum VEGF levels can be questioned as it will increase from normal background plasma levels because of release of VEGF from platelets during clotting.[571] Watson found the opposite to Awata with the VEGF +405 GG showing the highest levels of VEGF protein production in lipopolysaccharide stimulated peripheral blood mononuclear cells.[13] Koukourakis showed a borderline decrease in vascular density with the +405 GG genotype, but in this study there was no correlation to outcome.[541]

Gender has been shown to have a variable influence on VEGF SNPs with Lambrechts paper where subgroup analysis by gender revealed that the VEGF -2578 AA genotype, which lowers VEGF expression, increased the risk of ALS in males.

Race can also have an influence on the association of VEGF SNPs and disease. A large meta analysis of general cancer risk for the VEGF -460 T allele found an association with Asian people but not with Europeans.[589]

Environmental factors have also been shown to play a part in the association of VEGF SNPs and cancer. In oesophageal cancer patients with the C allele at VEGF -460 locus had an increased risk of developing cancer if they were smokers and a decreased risk compared to other genotypes if they were non-smokers.[590]

Finally angiogenesis involves a complex genetic interaction and polymorphisms at a variety of associated genes such as *VEGFR-2* and *EGFR* have also been shown to have an influence on survival in cancer patients.[591, 592]

6.5.3 What are the significant VEGF genotypes and haplotypes?

As can be seen in table 1.3, there is much variation and no consistency in the literature regarding the VEGF SNPs and their associations, functional significance and prognostic value. One example of this is two studies in breast cancer. One showed the CC genotype was associated with large tumours and high grade[544] and the other with small tumours.[554]

Overall the literature is fairly evenly split between VEGF -460 T and C alleles associations and VEGF +405 C and G allele associations (Table 1.3). There may be many reason for these discrepancies. As mentioned above gene-gene and gene-environmental interactions are evident but not usually

identified in a large proportion of genetic association studies. The SNPs that are chosen are not consistent and often not functionally (haplotypes) assessed. Multiple SNPs often on multiple genes are assessed without taking into account corrections for multiple testing. Finally the phenotypes are often not clearly defined and other proven associated factors not taken into account in multivariable testing.

With this study we found a variety of associations with different tumour types in adults and children. At the VEGF -460 locus there was a theme of increased T allele and decreased C allele. At VEGF +405 there was a general trend towards increased C allele and decreased G allele. In terms of survival there was significant improvement and trends towards better survival with VEGF -460 CC and VEGF +405 CC genotypes in both adult and paediatric tumours. With regards to haplotype a significant worsening in survival is seen with adult low grade astrocytomas and VEGF -460/+405 TG haplotype and this is mirrored with a trend to worse survival with TG haplotype in paediatric medulloblastomas and ependymomas.

6.5.4 Antiangiogenic therapy

With the advent of multiple antiangiogenic therapies becoming available to treat CNS malignancies,[12] the best use of VEGF SNPs would be as factors predictive of response to treatment. Young was the first to describe VEGF SNPs as being predictive of treatment response with retinoids in patients with psoriasis.[593] This study found an improved response to retinoids in the

VEGF -460 TC patients compared to other genotypes. Subsequent to this a phase III study comparing paclitaxel versus paclitaxel plus bevacizumab as initial chemotherapy for metastatic breast cancer showed improved response to the antiangiogenic treatment arm for patients with VEGF -1540 AA and VEGF -116 AA genotypes.[594] In addition the VEGF -460 TT and the VEGF +405 CC genotypes were associated with less grade 3 and 4 hypertension compared to the other genotypes. Overall survival in the study was improved for those patients who did experience grade 3-4 hypertension. This phenomenon has been seen in other studies.[595, 596] It is also felt that a low background level of plasma VEGF production predicts a better outcome with antiangiogenic therapy.[597]

6.6 Conclusions

Studies examining prognostic factors have the potential to be incredibly helpful in the management of patients. There are, however many ways that such studies can become confusing and misleading. This is particularly true in the field of neuro-oncology where sample size is often necessarily small making the affirmation of correct conclusions often very difficult.

A good understanding of all the various influences that have an impact on the prognostic factors discussed in this work is vital so that the information that the studies provide is utilised to bring the most benefit to patients, both young and old.

6.7 Future work from this thesis

The findings of my research throughout all the chapters lead to many further questions that I wish to pursue in the future.

For the progesterone receptors in meningiomas, I wish to further study the altered expression of genes associated with progesterone receptor function, notably the mitogen activated protein kinase 4 as postulated earlier in this chapter.

In my practice I have adopted the treatment paradigm of neo-adjuvant chemotherapy for choroid plexus carcinomas. I have also used this with great anecdotal effect in a patient with a large and vascular choroid plexus papilloma and further study of this patient group would be warranted. I am attempting with these patients to more accurately assess the reduction of vascularity by undertaking serial CT perfusion scans.

For the VEGF polymorphisms, there is a need for ongoing prospective validation of the findings from this thesis. In Manchester we are enrolling patients into trials using the anti-VEGF Bevacizumab (Avastin) and I would aim to undertake a pilot study to determine the VEGF genotype of these patients and their response to treatment. The long term goal would be to be able to predict response to anti-angiogenic therapy, based upon VEGF genotype and therefore provide a tailored pharmacogenomic based therapy to patients with brain tumours.

References

1. McShane LM, Altman DG, Sauerbrei W, Taube SE, Gion M, Clark GM. Reporting recommendations for tumor marker prognostic studies (REMARK). J Natl Cancer Inst 2005;97:1180-4.
2. Gospodarowicz M, O'Sullivan B. Prognostic factors in cancer. Semin Surg Oncol 2003;21:13-8.
3. Adams FS. The Genuine Works of Hippocrates. Translated ... by Francis Adams, etc: pp. viii. 384. Baillie\0300re & Co.: London; printed in America; 1939.
4. Riley RD, Sauerbrei W, Altman DG. Prognostic markers in cancer: the evolution of evidence from single studies to meta-analysis, and beyond. Br J Cancer 2009;100:1219-29.
5. Moons KG, Royston P, Vergouwe Y, Grobbee DE, Altman DG. Prognosis and prognostic research: what, why, and how? BMJ 2009;338:b375.
6. Gospodarowicz M, Benedet L, Hutter RV, Fleming I, Henson DE, Sobin LH. History and international developments in cancer staging. Cancer Prev Control 1998;2:262-8.
7. Burger PC, Green SB. Patient age, histologic features, and length of survival in patients with glioblastoma multiforme. Cancer 1987;59:1617-25.
8. Chen P, Aldape K, Wiencke JK, et al. Ethnicity delineates different genetic pathways in malignant glioma. Cancer Res 2001;61:3949-54.
9. Curran EK, Sainani KL, Le GM, Propp JM, Fisher PG. Gender affects survival for medulloblastoma only in older children and adults: a study from the Surveillance Epidemiology and End Results Registry. Pediatr Blood Cancer 2009;52:60-4.
10. Smith JS, Perry A, Borell TJ, et al. Alterations of chromosome arms 1p and 19q as predictors of survival in oligodendrogliomas, astrocytomas, and mixed oligoastrocytomas. JClinOncol 2000;18:636-45.
11. Tow SL, Chandela S, Miller NR, Avellino AM. Long-term outcome in children with gliomas of the anterior visual pathway. Pediatr Neurol 2003;28:262-70.
12. Jain RK, di Tomaso E, Duda DG, Loeffler JS, Sorensen AG, Batchelor TT. Angiogenesis in brain tumours. Nat Rev Neurosci 2007;8:610-22.
13. Watson CJ, Webb NJ, Bottomley MJ, Brenchley PE. Identification of polymorphisms within the vascular endothelial growth factor (VEGF) gene: correlation with variation in VEGF protein production. Cytokine 2000;12:1232-5.
14. Gillis CR, Hole DJ. Survival outcome of care by specialist surgeons in breast cancer: a study of 3786 patients in the west of Scotland. BMJ 1996;312:145-8.
15. Mackillop WJ, Groome PA, Zhang-Solomons J, et al. Does a centralized radiotherapy system provide adequate access to care? J Clin Oncol 1997;15:1261-71.
16. Selby P, Gillis C, Haward R. Benefits from specialised cancer care. Lancet 1996;348:313-8.

17. Newton RU, Galvao DA. Exercise in prevention and management of cancer. Curr Treat Options Oncol 2008;9:135-46.
18. Shackley DC, Clarke NW. Impact of socioeconomic status on bladder cancer outcome. Curr Opin Urol 2005;15:328-31.
19. Smith JJ, Tilney HS, Heriot AG, et al. Social deprivation and outcomes in colorectal cancer. Br J Surg 2006;93:1123-31.
20. Altman DG, Lausen B, Sauerbrei W, Schumacher M. Dangers of using "optimal" cutpoints in the evaluation of prognostic factors. J Natl Cancer Inst 1994;86:829-35.
21. Altman DG, Royston P. What do we mean by validating a prognostic model? Stat Med 2000;19:453-73.
22. Altman DG, Vergouwe Y, Royston P, Moons KG. Prognosis and prognostic research: validating a prognostic model. BMJ 2009;338:b605.
23. Halabi S, Owzar K. The importance of identifying and validating prognostic factors in oncology. Semin Oncol 2010;37:e9-18.
24. Kyzas PA, Denaxa-Kyza D, Ioannidis JP. Almost all articles on cancer prognostic markers report statistically significant results. Eur J Cancer 2007;43:2559-79.
25. Kyzas PA, Denaxa-Kyza D, Ioannidis JP. Quality of reporting of cancer prognostic marker studies: association with reported prognostic effect. J Natl Cancer Inst 2007;99:236-43.
26. Kyzas PA, Loizou KT, Ioannidis JP. Selective reporting biases in cancer prognostic factor studies. J Natl Cancer Inst 2005;97:1043-55.
27. Mallett S, Royston P, Dutton S, Waters R, Altman DG. Reporting methods in studies developing prognostic models in cancer: a review. BMC Med 2010;8:20.
28. McShane LM, Altman DG, Sauerbrei W. Identification of clinically useful cancer prognostic factors: what are we missing? J Natl Cancer Inst 2005;97:1023-5.
29. Vaquero J, Zurita M, Coca S, Oya S, Morales C. Prognostic significance of clinical and angiogenesis-related factors in low-grade oligodendrogliomas. SurgNeurol 2000;54:229-34.
30. Narum S. Beyond Bonferroni: Less conservative analyses for conservation genetics. Conservation Genetics 2006;7:783-7.
31. Vittinghoff E, McCulloch CE. Relaxing the rule of ten events per variable in logistic and Cox regression. Am J Epidemiol 2007;165:710-8.
32. Altman DG. Systematic reviews of evaluations of prognostic variables. BMJ 2001;323:224-8.
33. Stewart LA, Clarke MJ. Practical methodology of meta-analyses (overviews) using updated individual patient data. Cochrane Working Group. Stat Med 1995;14:2057-79.
34. Trivella M, Pezzella F, Pastorino U, Harris AL, Altman DG. Microvessel density as a prognostic factor in non-small-cell lung carcinoma: a meta-analysis of individual patient data. Lancet Oncol 2007;8:488-99.
35. Tonn JC, Westphal, M., Rutka, J.T. Neuro-oncology of CNS tumors 2nd Ed. Berlin ; London: Springer; 2010.

36. CBTRUS. Statistical Report: Primary Brain Tumours in the United States, 2000-2004. Published by the Central Brain Tumour Registry of the United States 2008.
37. Sadetzki S, Modan B, Chetrit A, Freedman L. An iatrogenic epidemic of benign meningioma. Am J Epidemiol 2000;151:266-72.
38. Preston DL, Ron E, Yonehara S, et al. Tumors of the nervous system and pituitary gland associated with atomic bomb radiation exposure. J Natl Cancer Inst 2002;94:1555-63.
39. Strojan P, Popovic M, Jereb B. Secondary intracranial meningiomas after high-dose cranial irradiation: report of five cases and review of the literature. Int J Radiat Oncol Biol Phys 2000;48:65-73.
40. Cushing H, Eisenhardt L. Meningiomas : their classification, regional behaviour, life history, and surgical end results: New York : Hafner, 1929; 1929.
41. Bickerstaff ER, Small JM, Guest IA. The relapsing course of certain meningiomas in relation to pregnancy and menstruation. J Neurol Neurosurg Psychiatry 1958;21:89-91.
42. Schoenberg BS, Christine BW, Whisnant JP. Nervous system neoplasms and primary malignancies of other sites. The unique association between meningiomas and breast cancer. Neurology 1975;25:705-12.
43. Schnegg JF, Gomez F, LeMarchand-Beraud T, de Tribolet N. Presence of sex steroid hormone receptors in meningioma tissue. Surg Neurol 1981;15:415-8.
44. Magdelenat H, Pertuiset BF, Poisson M, et al. Progestin and oestrogen receptors in meningiomas. Biochemical characterization, clinical and pathological correlations in 42 cases. Acta Neurochir (Wien) 1982;64:199-213.
45. Markwalder TM, Zava DT, Goldhirsch A, Markwalder RV. Estrogen and progesterone receptors in meningiomas in relation to clinical and pathologic features. Surg Neurol 1983;20:42-7.
46. Blankenstein MA, Blaauw G, Lamberts SW. Progestin and estrogen receptors in human meningioma. Clin Neuropharmacol 1984;7:363-7.
47. Cahill DW, Bashirelahi N, Solomon LW, Dalton T, Salcman M, Ducker TB. Estrogen and progesterone receptors in meningiomas. J Neurosurg 1984;60:985-93.
48. Hayward E, Whitwell H, Paul KS, Barnes DM. Steroid receptors in human meningioma. Clin Neuropharmacol 1984;7:351-6.
49. Markwalder TM, Markwalder RV, Zava DT. Estrogen and progestin receptors in meningiomas: clinicopathological correlations. Clin Neuropharmacol 1984;7:368-74.
50. Moguilewsky M, Pertuiset BF, Verzat C, Philibert D, Philippon J, Poisson M. Cytosolic and nuclear sex steroid receptors in meningioma. Clin Neuropharmacol 1984;7:375-81.
51. Blaauw G, Blankenstein MA, Lamberts SW. Sex steroid receptors in human meningiomas. Acta Neurochir (Wien) 1986;79:42-7.
52. Goffin J. Estrogen- and progesterone-receptors in meningiomas. Review article. Clin Neurol Neurosurg 1986;88:169-75.

53. Ironside JW, Battersby RD, Dangerfield VJ, Parsons MA, Timperley WR, Underwood JC. Cryostat section assay of oestrogen and progesterone receptors in meningiomas: a clinicopathological study. J Clin Pathol 1986;39:44-50.
54. Ironside JW, Battersby RD, Dangerfield VJ, Timperley WR, Underwood JC. Progesterone receptors in meningiomas: morphometric assessment of vascularity and cellularity on near facsimile cryostat sections. J Clin Pathol 1986;39:810-1.
55. Maiuri F, Montagnani S, Gallicchio B. Estrogen and progesterone receptors in meningiomas. Surg Neurol 1986;26:435-40.
56. Carroll RS, Glowacka D, Dashner K, Black PM. Progesterone receptor expression in meningiomas. Cancer Res 1993;53:1312-6.
57. Brandis A, Mirzai S, Tatagiba M, Walter GF, Samii M, Ostertag H. Immunohistochemical detection of female sex hormone receptors in meningiomas: correlation with clinical and histological features. Neurosurgery 1993;33:212-7; discussion 7-8.
58. Hsu DW, Efird JT, Hedley-Whyte ET. Progesterone and estrogen receptors in meningiomas: prognostic considerations. J Neurosurg 1997;86:113-20.
59. Roser F, Nakamura M, Ritz R, et al. Proliferation and progesterone receptor status in benign meningiomas are not age dependent. Cancer 2005;104:598-601.
60. Lesch KP, Gross S. Estrogen receptor immunoreactivity in meningiomas. Comparison with the binding activity of estrogen, progesterone, and androgen receptors. J Neurosurg 1987;67:237-43.
61. Nagashima G, Aoyagi M, Wakimoto H, Tamaki M, Ohno K, Hirakawa K. Immunohistochemical detection of progesterone receptors and the correlation with Ki-67 labeling indices in paraffin-embedded sections of meningiomas. Neurosurgery 1995;37:478-82; discussion 83.
62. Tonn JC, Ott MM, Bouterfa H, et al. Inverse correlation of cell proliferation and expression of progesterone receptors in tumor spheroids and monolayer cultures of human meningiomas. Neurosurgery 1997;41:1152-9.
63. Fewings PE, Battersby RD, Timperley WR. Long-term follow up of progesterone receptor status in benign meningioma: a prognostic indicator of recurrence? J Neurosurg 2000;92:401-5.
64. Maiuri F, De Caro M, Esposito F, et al. Recurrences of meningiomas: predictive value of pathological features and hormonal and growth factors. J Neurooncol 2007;82:63-8.
65. Pravdenkova S, Al-Mefty O, Sawyer J, Husain M. Progesterone and estrogen receptors: opposing prognostic indicators in meningiomas. J Neurosurg 2006;105:163-73.
66. Claus EB, Park PJ, Carroll R, Chan J, Black PM. Specific genes expressed in association with progesterone receptors in meningioma. Cancer Res 2008;68:314-22.
67. Lamszus K, Vahldiek F, Mautner VF, et al. Allelic losses in neurofibromatosis 2-associated meningiomas. J Neuropathol Exp Neurol 2000;59:504-12.

68. Riemenschneider MJ, Perry A, Reifenberger G. Histological classification and molecular genetics of meningiomas. Lancet Neurol 2006;5:1045-54.
69. Louis DN. WHO classification of tumours of the central nervous system. 4th ed. ed. Lyon: International Agency for Research on Cancer; 2007.
70. PathConsult Website. 2010. (Accessed September, 2010, at http://www.pathconsultddx.com.)
71. Simpson D. The recurrence of intracranial meningiomas after surgical treatment. J Neurol Neurosurg Psychiatry 1957;20:22-39.
72. Minniti G, Amichetti M, Enrici RM. Radiotherapy and radiosurgery for benign skull base meningiomas. Radiat Oncol 2009;4:42.
73. Bendszus M, Rao G, Burger R, et al. Is there a benefit of preoperative meningioma embolization? Neurosurgery 2000;47:1306-11; discussion 11-2.
74. Probst EN, Grzyska U, Westphal M, Zeumer H. Preoperative embolization of intracranial meningiomas with a fibrin glue preparation. AJNR Am J Neuroradiol 1999;20:1695-702.
75. Chen TC, Chamberlain MC. Adjuvant therapy for unresectable meningiomas: benign and malignant. Neurosurg Focus 2007;23:1.
76. Chamberlain MC. Adjuvant combined modality therapy for malignant meningiomas. J Neurosurg 1996;84:733-6.
77. Norden AD, Drappatz J, Wen PY. Targeted drug therapy for meningiomas. Neurosurg Focus 2007;23:E12.
78. Loven D, Hardoff R, Sever ZB, et al. Non-resectable slow-growing meningiomas treated by hydroxyurea. J Neurooncol 2004;67:221-6.
79. Mason WP, Gentili F, Macdonald DR, Hariharan S, Cruz CR, Abrey LE. Stabilization of disease progression by hydroxyurea in patients with recurrent or unresectable meningioma. J Neurosurg 2002;97:341-6.
80. Gupta N. Choroid plexus tumors in children. NeurosurgClinNAm 2003;14:621-31.
81. Wolff JE, Sajedi M, Brant R, Coppes MJ, Egeler RM. Choroid plexus tumours. BrJCancer 2002;87:1086-91.
82. Ellenbogen RG, Winston KR, Kupsky WJ. Tumors of the choroid plexus in children. Neurosurgery 1989;25:327-35.
83. Asai A, Hoffman HJ, Hendrick EB, Humphreys RP, Becker LE. Primary intracranial neoplasms in the first year of life. Childs NervSyst 1989;5:230-3.
84. Sherr CJ. Principles of tumor suppression. Cell 2004;116:235-46.
85. Pollack IF, Finkelstein SD, Woods J, et al. Expression of p53 and prognosis in children with malignant gliomas. NEnglJMed 2002;346:420-7.
86. Krutilkova V, Trkova M, Fleitz J, et al. Identification of five new families strengthens the link between childhood choroid plexus carcinoma and germline TP53 mutations. EurJCancer 2005;41:1597-603.
87. Malkin D, Chilton-MacNeill S, Meister LA, Sexsmith E, Diller L, Garcea RL. Tissue-specific expression of SV40 in tumors associated with the Li-Fraumeni syndrome. Oncogene 2001;20:4441-9.
88. Varley JM, Evans DG, Birch JM. Li-Fraumeni syndrome--a molecular and clinical review. BrJCancer 1997;76:1-14.

89. Vital A, Bringuier PP, Huang H, et al. Astrocytomas and choroid plexus tumors in two families with identical p53 germline mutations. JNeuropatholExpNeurol 1998;57:1061-9.
90. Zakrzewska M, Wojcik I, Zakrzewski K, et al. Mutational analysis of hSNF5/INI1 and TP53 genes in choroid plexus carcinomas. Cancer GenetCytogenet 2005;156:179-82.
91. Carlotti CG, Jr., Salhia B, Weitzman S, et al. Evaluation of proliferative index and cell cycle protein expression in choroid plexus tumors in children. Acta Neuropathol(Berl) 2002;103:1-10.
92. Mueller W, Eum JH, Lass U, et al. No evidence of hSNF5/INI1 point mutations in choroid plexus papilloma. NeuropatholApplNeurobiol 2004;30:304-7.
93. Rutherford J, Chu CE, Duddy PM, et al. Investigations on a clinically and functionally unusual and novel germline p53 mutation. BrJCancer 2002;86:1592-6.
94. Sevenet N, Sheridan E, Amram D, Schneider P, Handgretinger R, Delattre O. Constitutional mutations of the hSNF5/INI1 gene predispose to a variety of cancers. AmJHumGenet 1999;65:1342-8.
95. Taylor MD, Gokgoz N, Andrulis IL, Mainprize TG, Drake JM, Rutka JT. Familial posterior fossa brain tumors of infancy secondary to germline mutation of the hSNF5 gene. AmJHumGenet 2000;66:1403-6.
96. Gessi M, Giangaspero F, Pietsch T. Atypical teratoid/rhabdoid tumors and choroid plexus tumors: when genetics "surprise" pathology. Brain Pathol 2003;13:409-14.
97. Wyatt-Ashmead J, Kleinschmidt-DeMasters B, Mierau GW, et al. Choroid plexus carcinomas and rhabdoid tumors: phenotypic and genotypic overlap. PediatrDevPathol 2001;4:545-9.
98. Judkins AR, Burger PC, Hamilton RL, et al. INI1 protein expression distinguishes atypical teratoid/rhabdoid tumor from choroid plexus carcinoma. JNeuropatholExpNeurol 2005;64:391-7.
99. Judkins AR, Mauger J, Ht A, Rorke LB, Biegel JA. Immunohistochemical analysis of hSNF5/INI1 in pediatric CNS neoplasms. AmJSurgPathol 2004;28:644-50.
100. Vajtai I, Varga Z, Aguzzi A. MIB-1 immunoreactivity reveals different labelling in low-grade and in malignant epithelial neoplasms of the choroid plexus. Histopathology 1996;29:147-51.
101. Brat DJ, Parisi JE, Kleinschmidt-DeMasters BK, et al. Surgical neuropathology update: a review of changes introduced by the WHO classification of tumours of the central nervous system, 4th edition. Arch Pathol Lab Med 2008;132:993-1007.
102. Chow E, Reardon DA, Shah AB, et al. Pediatric choroid plexus neoplasms. IntJRadiatOncolBiolPhys 1999;44:249-54.
103. St Clair SK, Humphreys RP, Pillay PK, Hoffman HJ, Blaser SI, Becker LE. Current management of choroid plexus carcinoma in children. PediatrNeurosurg 1991;17:225-33.
104. Levy ML, Goldfarb A, Hyder DJ, et al. Choroid plexus tumors in children: significance of stromal invasion. Neurosurgery 2001;48:303-9.

105. Chow E, Jenkins JJ, Burger PC, et al. Malignant evolution of choroid plexus papilloma. PediatrNeurosurg 1999;31:127-30.
106. Wrede B, Liu P, Ater J, Wolff JE. Second surgery and the prognosis of choroid plexus carcinoma--results of a meta-analysis of individual cases. Anticancer Res 2005;25:4429-33.
107. Berger C, Thiesse P, Lellouch-Tubiana A, Kalifa C, Pierre-Kahn A, Bouffet E. Choroid plexus carcinomas in childhood: clinical features and prognostic factors. Neurosurgery 1998;42:470-5.
108. McEvoy AW, Harding BN, Phipps KP, et al. Management of choroid plexus tumours in children: 20 years experience at a single neurosurgical centre. PediatrNeurosurg 2000;32:192-9.
109. Wolff JE, Sajedi M, Coppes MJ, Anderson RA, Egeler RM. Radiation therapy and survival in choroid plexus carcinoma. Lancet 1999;353:2126.
110. Wrede B, Liu P, Wolff JE. Chemotherapy improves the survival of patients with choroid plexus carcinoma: a meta-analysis of individual cases with choroid plexus tumors. JNeurooncol 2007.
111. Fitzpatrick LK, Aronson LJ, Cohen KJ. Is there a requirement for adjuvant therapy for choroid plexus carcinoma that has been completely resected? JNeurooncol 2002;57:123-6.
112. Greenberg ML. Chemotherapy of choroid plexus carcinoma. Childs NervSyst 1999;15:571-7.
113. Packer RJ, Perilongo G, Johnson D, et al. Choroid plexus carcinoma of childhood. Cancer 1992;69:580-5.
114. Razzaq AA, Cohen AR. Neoadjuvant chemotherapy for hypervascular malignant brain tumors of childhood. PediatrNeurosurg 1997;27:296-303.
115. Souweidane MM, Johnson JH, Jr., Lis E. Volumetric reduction of a choroid plexus carcinoma using preoperative chemotherapy. JNeurooncol 1999;43:167-71.
116. Inskip PD, Linet MS, Heineman EF. Etiology of brain tumors in adults. Epidemiol Rev 1995;17:382-414.
117. Guillamo JS, Creange A, Kalifa C, et al. Prognostic factors of CNS tumours in Neurofibromatosis 1 (NF1): a retrospective study of 104 patients. Brain 2003;126:152-60.
118. James CD, Carlbom E, Nordenskjold M, Collins VP, Cavenee WK. Mitotic recombination of chromosome 17 in astrocytomas. ProcNatlAcadSciUSA 1989;86:2858-62.
119. Hermanson M, Funa K, Koopmann J, et al. Association of loss of heterozygosity on chromosome 17p with high platelet-derived growth factor alpha receptor expression in human malignant gliomas. Cancer Res 1996;56:164-71.
120. Balss J, Meyer J, Mueller W, Korshunov A, Hartmann C, von Deimling A. Analysis of the IDH1 codon 132 mutation in brain tumors. Acta Neuropathol 2008;116:597-602.
121. Reifenberger J, Reifenberger G, Liu L, James CD, Wechsler W, Collins VP. Molecular genetic analysis of oligodendroglial tumors shows preferential allelic deletions on 19q and 1p. AmJPathol 1994;145:1175-90.

122. Pfister S, Janzarik WG, Remke M, et al. BRAF gene duplication constitutes a mechanism of MAPK pathway activation in low-grade astrocytomas. J Clin Invest 2008;118:1739-49.
123. Kluwe L, Hagel C, Tatagiba M, et al. Loss of NF1 alleles distinguish sporadic from NF1-associated pilocytic astrocytomas. J Neuropathol Exp Neurol 2001;60:917-20.
124. Tihan T, Vohra P, Berger MS, Keles GE. Definition and diagnostic implications of gemistocytic astrocytomas: a pathological perspective. J Neurooncol 2006;76:175-83.
125. Prayson RA, Estes ML. Protoplasmic astrocytoma. A clinicopathologic study of 16 tumors. AmJClinPathol 1995;103:705-9.
126. Tihan T, Fisher PG, Kepner JL, et al. Pediatric astrocytomas with monomorphous pilomyxoid features and a less favorable outcome. J Neuropathol Exp Neurol 1999;58:1061-8.
127. Giannini C, Scheithauer BW, Burger PC, et al. Pleomorphic xanthoastrocytoma: what do we really know about it? Cancer 1999;85:2033-45.
128. Ichikawa T, Wakisaka A, Daido S, et al. A case of solitary subependymal giant cell astrocytoma: two somatic hits of TSC2 in the tumor, without evidence of somatic mosaicism. J Mol Diagn 2005;7:544-9.
129. Lam C, Bouffet E, Tabori U, Mabbott D, Taylor M, Bartels U. Rapamycin (sirolimus) in tuberous sclerosis associated pediatric central nervous system tumors. Pediatr Blood Cancer;54:476-9.
130. Recht LD, Lew R, Smith TW. Suspected low-grade glioma: is deferring treatment safe? AnnNeurol 1992;31:431-6.
131. van Veelen ML, Avezaat CJ, Kros JM, van Putten W, Vecht C. Supratentorial low grade astrocytoma: prognostic factors, dedifferentiation, and the issue of early versus late surgery. JNeurolNeurosurgPsychiatry 1998;64:581-7.
132. Lunsford LD, Somaza S, Kondziolka D, Flickinger JC. Survival after stereotactic biopsy and irradiation of cerebral nonanaplastic, nonpilocytic astrocytoma. JNeurosurg 1995;82:523-9.
133. Woodworth GF, McGirt MJ, Samdani A, Garonzik I, Olivi A, Weingart JD. Frameless image-guided stereotactic brain biopsy procedure: diagnostic yield, surgical morbidity, and comparison with the frame-based technique. J Neurosurg 2006;104:233-7.
134. Sanai N, Berger MS. Glioma extent of resection and its impact on patient outcome. Neurosurgery 2008;62:753-64; discussion 264-6.
135. Smith JS, Chang EF, Lamborn KR, et al. Role of extent of resection in the long-term outcome of low-grade hemispheric gliomas. J Clin Oncol 2008;26:1338-45.
136. Claus EB, Horlacher A, Hsu L, et al. Survival rates in patients with low-grade glioma after intraoperative magnetic resonance image guidance. Cancer 2005;103:1227-33.
137. Hammoud MA, Ligon BL, elSouki R, Shi WM, Schomer DF, Sawaya R. Use of intraoperative ultrasound for localizing tumors and determining the extent of resection: a comparative study with magnetic resonance imaging. J Neurosurg 1996;84:737-41.

138. Sanai N, Mirzadeh Z, Berger MS. Functional outcome after language mapping for glioma resection. N Engl J Med 2008;358:18-27.
139. Karim AB, Maat B, Hatlevoll R, et al. A randomized trial on dose-response in radiation therapy of low-grade cerebral glioma: European Organization for Research and Treatment of Cancer (EORTC) Study 22844. IntJRadiatOncolBiolPhys 1996;36:549-56.
140. Shaw E, Arusell R, Scheithauer B, et al. Prospective randomized trial of low- versus high-dose radiation therapy in adults with supratentorial low-grade glioma: initial report of a North Central Cancer Treatment Group/Radiation Therapy Oncology Group/Eastern Cooperative Oncology Group study. JClinOncol 2002;20:2267-76.
141. Karim AB, Afra D, Cornu P, et al. Randomized trial on the efficacy of radiotherapy for cerebral low-grade glioma in the adult: European Organization for Research and Treatment of Cancer Study 22845 with the Medical Research Council study BRO4: an interim analysis. IntJRadiatOncolBiolPhys 2002;52:316-24.
142. van den Bent MJ, Afra D, de Witte O, et al. Long-term efficacy of early versus delayed radiotherapy for low-grade astrocytoma and oligodendroglioma in adults: the EORTC 22845 randomised trial. Lancet 2005;366:985-90.
143. Burzynski SR. Treatments for Astrocytic Tumors in Children: Current and Emerging Strategies. Pediatric Drugs 2006;8:167-78.
144. Brada M, Viviers L, Abson C, et al. Phase II study of primary temozolomide chemotherapy in patients with WHO grade II gliomas. Ann Oncol 2003;14:1715-21.
145. van den Bent MJ. Can chemotherapy replace radiotherapy in low-grade gliomas? Time for randomized studies. SeminOncol 2003;30:39-44.
146. Mueller S, Chang S. Pediatric brain tumors: current treatment strategies and future therapeutic approaches. Neurotherapeutics 2009;6:570-86.
147. Packer RJ, Lange B, Ater J, et al. Carboplatin and vincristine for recurrent and newly diagnosed low-grade gliomas of childhood. JClinOncol 1993;11:850-6.
148. Perilongo G. Considerations on the role of chemotherapy and modern radiotherapy in the treatment of childhood low grade glioma. J Neurooncol 2005;75:301-7.
149. Bauman G, Lote K, Larson D, et al. Pretreatment factors predict overall survival for patients with low-grade glioma: a recursive partitioning analysis. IntJRadiatOncolBiolPhys 1999;45:923-9.
150. Lote K, Egeland T, Hager B, et al. Survival, prognostic factors, and therapeutic efficacy in low-grade glioma: a retrospective study in 379 patients. JClinOncol 1997;15:3129-40.
151. McCormack BM, Miller DC, Budzilovich GN, Voorhees GJ, Ransohoff J. Treatment and survival of low-grade astrocytoma in adults--1977-1988. Neurosurgery 1992;31:636-42.
152. Shaw EG, Scheithauer BW, O'Fallon JR. Supratentorial gliomas: a comparative study by grade and histologic type. JNeurooncol 1997;31:273-8.

153. Vertosick FT, Jr., Selker RG, Arena VC. Survival of patients with well-differentiated astrocytomas diagnosed in the era of computed tomography. Neurosurgery 1991;28:496-501.
154. Brown PD, Buckner JC, O'Fallon JR, et al. Importance of baseline mini-mental state examination as a prognostic factor for patients with low-grade glioma. IntJRadiatOncolBiolPhys 2004;59:117-25.
155. Franzini A, Leocata F, Cajola L, Servello D, Allegranza A, Broggi G. Low-grade glial tumors in basal ganglia and thalamus: natural history and biological reappraisal. Neurosurgery 1994;35:817-20.
156. Janny P, Cure H, Mohr M, et al. Low grade supratentorial astrocytomas. Management and prognostic factors. Cancer 1994;73:1937-45.
157. Leighton C, Fisher B, Bauman G, et al. Supratentorial low-grade glioma in adults: an analysis of prognostic factors and timing of radiation. JClinOncol 1997;15:1294-301.
158. Pignatti F, van den BM, Curran D, et al. Prognostic factors for survival in adult patients with cerebral low-grade glioma. JClinOncol 2002;20:2076-84.
159. Soffietti R, Chio A, Giordana MT, Vasario E, Schiffer D. Prognostic factors in well-differentiated cerebral astrocytomas in the adult. Neurosurgery 1989;24:686-92.
160. Desai KI, Nadkarni TD, Muzumdar DP, Goel A. Prognostic factors for cerebellar astrocytomas in children: a study of 102 cases. PediatrNeurosurg 2001;35:311-7.
161. Morreale VM, Ebersold MJ, Quast LM, Parisi JE. Cerebellar astrocytoma: experience with 54 cases surgically treated at the Mayo Clinic, Rochester, Minnesota, from 1978 to 1990. J Neurosurg 1997;87:257-61.
162. Due-Tonnessen BJ, Helseth E, Scheie D, Skullerud K, Aamodt G, Lundar T. Long-term outcome after resection of benign cerebellar astrocytomas in children and young adults (0-19 years): report of 110 consecutive cases. Pediatr Neurosurg 2002;37:71-80.
163. Gunny RS, Hayward RD, Phipps KP, Harding BN, Saunders DE. Spontaneous regression of residual low-grade cerebellar pilocytic astrocytomas in children. Pediatr Radiol 2005;35:1086-91.
164. Palma L, Celli P, Mariottini A. Long-term follow-up of childhood cerebellar astrocytomas after incomplete resection with particular reference to arrested growth or spontaneous tumour regression. Acta Neurochir (Wien) 2004;146:581-8; discussion 8.
165. Jenkins RB, Blair H, Ballman KV, et al. A t(1;19)(q10;p10) mediates the combined deletions of 1p and 19q and predicts a better prognosis of patients with oligodendroglioma. Cancer Res 2006;66:9852-61.
166. Mollemann M, Wolter M, Felsberg J, Collins VP, Reifenberger G. Frequent promoter hypermethylation and low expression of the MGMT gene in oligodendroglial tumors. Int J Cancer 2005;113:379-85.
167. Cairncross JG, Ueki K, Zlatescu MC, et al. Specific genetic predictors of chemotherapeutic response and survival in patients with anaplastic oligodendrogliomas. J Natl Cancer Inst 1998;90:1473-9.

168. Cairncross G, Berkey B, Shaw E, et al. Phase III trial of chemotherapy plus radiotherapy compared with radiotherapy alone for pure and mixed anaplastic oligodendroglioma: Intergroup Radiation Therapy Oncology Group Trial 9402. J Clin Oncol 2006;24:2707-14.
169. Cairncross G, MacDonald D, Ludwin S, et al. Chemotherapy for anaplastic oligodendroglioma. National Cancer Institute of Canada Clinical Trials Group. JClinOncol 1994;12:2013-21.
170. Mikkelsen T, Doyle T, Anderson J, et al. Temozolomide single-agent chemotherapy for newly diagnosed anaplastic oligodendroglioma. J Neurooncol 2009;92:57-63.
171. van den Bent MJ, Taphoorn MJ, Brandes AA, et al. Phase II study of first-line chemotherapy with temozolomide in recurrent oligodendroglial tumors: the European Organization for Research and Treatment of Cancer Brain Tumor Group Study 26971. J Clin Oncol 2003;21:2525-8.
172. Weller M, Berger H, Hartmann C, et al. Combined 1p/19q loss in oligodendroglial tumors: predictive or prognostic biomarker? Clin Cancer Res 2007;13:6933-7.
173. Ohgaki H, Kleihues P. Epidemiology and etiology of gliomas. Acta Neuropathol 2005;109:93-108.
174. Todd DW, Christoferson LA, Leech RW, Rudolf L. A family affected with intestinal polyposis and gliomas. Ann Neurol 1981;10:390-2.
175. Ohgaki H, Kleihues P. Genetic pathways to primary and secondary glioblastoma. Am J Pathol 2007;170:1445-53.
176. Reifenberger G, Collins VP. Pathology and molecular genetics of astrocytic gliomas. J Mol Med 2004;82:656-70.
177. The Cancer Genome Atlas Research Network. Comprehensive genomic characterization defines human glioblastoma genes and core pathways. Nature 2008;455:1061-8.
178. Kozak KR, Mahadevan A, Moody JS. Adult gliosarcoma: epidemiology, natural history, and factors associated with outcome. Neuro Oncol 2009;11:183-91.
179. Gorlia T, van den Bent MJ, Hegi ME, et al. Nomograms for predicting survival of patients with newly diagnosed glioblastoma: prognostic factor analysis of EORTC and NCIC trial 26981-22981/CE.3. Lancet Oncol 2008;9:29-38.
180. McGirt MJ, Chaichana KL, Gathinji M, et al. Independent association of extent of resection with survival in patients with malignant brain astrocytoma. J Neurosurg 2009;110:156-62.
181. Westphal M, Hilt DC, Bortey E, et al. A phase 3 trial of local chemotherapy with biodegradable carmustine (BCNU) wafers (Gliadel wafers) in patients with primary malignant glioma. Neuro Oncol 2003;5:79-88.
182. Nicholas MK. Glioblastoma multiforme: evidence-based approach to therapy. Expert Rev Anticancer Ther 2007;7:S23-7.
183. Chang JE, Khuntia D, Robins HI, Mehta MP. Radiotherapy and radiosensitizers in the treatment of glioblastoma multiforme. Clin Adv Hematol Oncol 2007;5:894-902, 7-15.

184. Randomized trial of procarbazine, lomustine, and vincristine in the adjuvant treatment of high-grade astrocytoma: a Medical Research Council trial. J Clin Oncol 2001;19:509-18.
185. Stupp R, Mason WP, van den Bent MJ, et al. Radiotherapy plus concomitant and adjuvant temozolomide for glioblastoma. N Engl J Med 2005;352:987-96.
186. Hegi ME, Diserens AC, Gorlia T, et al. MGMT gene silencing and benefit from temozolomide in glioblastoma. N Engl J Med 2005;352:997-1003.
187. Rutka JT. Medulloblastoma. ClinNeurosurg 1997;44:571-85.
188. Polkinghorn WR, Tarbell NJ. Medulloblastoma: tumorigenesis, current clinical paradigm, and efforts to improve risk stratification. Nat Clin Pract Oncol 2007;4:295-304.
189. Thompson MC, Fuller C, Hogg TL, et al. Genomics identifies medulloblastoma subgroups that are enriched for specific genetic alterations. J Clin Oncol 2006;24:1924-31.
190. de Haas T, Hasselt N, Troost D, et al. Molecular risk stratification of medulloblastoma patients based on immunohistochemical analysis of MYC, LDHB, and CCNB1 expression. Clin Cancer Res 2008;14:4154-60.
191. Kool M, Koster J, Bunt J, et al. Integrated genomics identifies five medulloblastoma subtypes with distinct genetic profiles, pathway signatures and clinicopathological features. PLoS One 2008;3:e3088.
192. Northcott PA, Korshunov A, Witt H, et al. Medulloblastoma Comprises Four Distinct Molecular Variants. J Clin Oncol.
193. Di Marcotullio L, Ferretti E, De Smaele E, et al. REN(KCTD11) is a suppressor of Hedgehog signaling and is deleted in human medulloblastoma. Proc Natl Acad Sci U S A 2004;101:10833-8.
194. Waha A, Koch A, Meyer-Puttlitz B, et al. Epigenetic silencing of the HIC-1 gene in human medulloblastomas. JNeuropatholExpNeurol 2003;62:1192-201.
195. Ehrbrecht A, Muller U, Wolter M, et al. Comprehensive genomic analysis of desmoplastic medulloblastomas: identification of novel amplified genes and separate evaluation of the different histological components. J Pathol 2006;208:554-63.
196. Taylor MD, Liu L, Raffel C, et al. Mutations in SUFU predispose to medulloblastoma. Nat Genet 2002;31:306-10.
197. Taylor MD, Mainprize TG, Rutka JT. Molecular insight into medulloblastoma and central nervous system primitive neuroectodermal tumor biology from hereditary syndromes: a review. Neurosurgery 2000;47:888-901.
198. Bruggers CS, Tai KF, Murdock T, et al. Expression of the c-Myc protein in childhood medulloblastoma. J Pediatr Hematol Oncol 1998;20:18-25.
199. Herms J, Neidt I, Luscher B, et al. C-MYC expression in medulloblastoma and its prognostic value. Int J Cancer 2000;89:395-402.
200. Moriuchi S, Shimizu K, Miyao Y, Hayakawa T. An immunohistochemical analysis of medulloblastoma and PNET with emphasis on N-myc protein expression. Anticancer Res 1996;16:2687-92.

201. Eberhart CG, Kepner JL, Goldthwaite PT, et al. Histopathologic grading of medulloblastomas: a Pediatric Oncology Group study. Cancer 2002;94:552-60.
202. Rieken S, Gaiser T, Mohr A, et al. Outcome and prognostic factors of desmoplastic medulloblastoma treated within a multidisciplinary treatment concept. BMC Cancer 2010;10:450.
203. Zeltzer PM, Boyett JM, Finlay JL, et al. Metastasis stage, adjuvant treatment, and residual tumor are prognostic factors for medulloblastoma in children: conclusions from the Children's Cancer Group 921 randomized phase III study. J Clin Oncol 1999;17:832-45.
204. Gajjar A, Chintagumpala M, Ashley D, et al. Risk-adapted craniospinal radiotherapy followed by high-dose chemotherapy and stem-cell rescue in children with newly diagnosed medulloblastoma (St Jude Medulloblastoma-96): long-term results from a prospective, multicentre trial. Lancet Oncol 2006;7:813-20.
205. Park TS, Hoffman HJ, Hendrick EB, Humphreys RP, Becker LE. Medulloblastoma: clinical presentation and management. Experience at the hospital for sick children, toronto, 1950-1980. J Neurosurg 1983;58:543-52.
206. Crawford JR, MacDonald TJ, Packer RJ. Medulloblastoma in childhood: new biological advances. Lancet Neurol 2007;6:1073-85.
207. Gottardo NG, Gajjar A. Current therapy for medulloblastoma. Curr Treat Options Neurol 2006;8:319-34.
208. Ribi K, Relly C, Landolt MA, Alber FD, Boltshauser E, Grotzer MA. Outcome of medulloblastoma in children: long-term complications and quality of life. Neuropediatrics 2005;36:357-65.
209. Taylor MD, Poppleton H, Fuller C, et al. Radial glia cells are candidate stem cells of ependymoma. Cancer Cell 2005;8:323-35.
210. Rickert CH, Paulus W. Comparative genomic hybridization in central and peripheral nervous system tumors of childhood and adolescence. J Neuropathol Exp Neurol 2004;63:399-417.
211. Gilbertson RJ, Bentley L, Hernan R, et al. ERBB receptor signaling promotes ependymoma cell proliferation and represents a potential novel therapeutic target for this disease. Clin Cancer Res 2002;8:3054-64.
212. Mendrzyk F, Korshunov A, Benner A, et al. Identification of gains on 1q and epidermal growth factor receptor overexpression as independent prognostic markers in intracranial ependymoma. Clin Cancer Res 2006;12:2070-9.
213. Tabori U, Ma J, Carter M, et al. Human telomere reverse transcriptase expression predicts progression and survival in pediatric intracranial ependymoma. J Clin Oncol 2006;24:1522-8.
214. Tabori U, Wong V, Ma J, et al. Telomere maintenance and dysfunction predict recurrence in paediatric ependymoma. Br J Cancer 2008;99:1129-35.
215. Godfraind C. Classification and controversies in pathology of ependymomas. Childs Nerv Syst 2009;25:1185-93.
216. Merchant TE, Li C, Xiong X, Kun LE, Boop FA, Sanford RA. Conformal radiotherapy after surgery for paediatric ependymoma: a prospective study. Lancet Oncol 2009;10:258-66.

217. Grundy RG, Wilne SA, Weston CL, et al. Primary postoperative chemotherapy without radiotherapy for intracranial ependymoma in children: the UKCCSG/SIOP prospective study. Lancet Oncol 2007;8:696-705.
218. Risau W. Mechanisms of angiogenesis. Nature 1997;386:671-4.
219. Oliver G, Detmar M. The rediscovery of the lymphatic system: old and new insights into the development and biological function of the lymphatic vasculature. Genes Dev 2002;16:773-83.
220. Sabin FR. On the origin of the lymphatic system from the veins and the development of the lymph hearts and thoracic duct in the pig. Am J Anat 1902;1:367-91.
221. Karkkainen MJ, Haiko P, Sainio K, et al. Vascular endothelial growth factor C is required for sprouting of the first lymphatic vessels from embryonic veins. NatImmunol 2004;5:74-80.
222. Wigle JT, Oliver G. Prox1 function is required for the development of the murine lymphatic system. Cell 1999;98:769-78.
223. Ferrara N. Role of vascular endothelial growth factor in physiologic and pathologic angiogenesis: therapeutic implications. SeminOncol 2002;29:10-4.
224. Ferrara N. VEGF and the quest for tumour angiogenesis factors. NatRevCancer 2002;2:795-803.
225. Ferrara N. Vascular endothelial growth factor: basic science and clinical progress. EndocrRev 2004;25:581-611.
226. Ide AG, Baker NH, Warren SL. Vascularization of the Brown Pearce rabbit epithelioma transplant as seen in the transparent ear chamber. Am J Roentgenol 1939;42:891-9.
227. Algire GH, Chalkley HW, Legallais FY, Park HD. Vascular reactions of normal and malignant tissues in vivo. I. Vascular reactions of mice to woundsand to normal and neoplastic transplants. J Natl Cancer Inst 1945:73-85.
228. Greenblatt M, Shubi P. Tumor angiogenesis: transfilter diffusion studies in the hamster by the transparent chamber technique. JNatlCancer Inst 1968;41:111-24.
229. Ehrmann RL, Knoth M. Choriocarcinoma. Transfilter stimulation of vasoproliferation in the hamster cheek pouch. Studied by light and electron microscopy. JNatlCancer Inst 1968;41:1329-41.
230. Folkman J. Tumor angiogenesis: therapeutic implications. NEnglJMed 1971;285:1182-6.
231. Folkman J, Klagsbrun M. Vascular physiology. A family of angiogenic peptides. Nature 1987;329:671-2.
232. Senger DR, Galli SJ, Dvorak AM, Perruzzi CA, Harvey VS, Dvorak HF. Tumor cells secrete a vascular permeability factor that promotes accumulation of ascites fluid. Science 1983;219:983-5.
233. Ferrara N, Henzel WJ. Pituitary follicular cells secrete a novel heparin-binding growth factor specific for vascular endothelial cells. BiochemBiophysResCommun 1989;161:851-8.
234. Keck PJ, Hauser SD, Krivi G, et al. Vascular permeability factor, an endothelial cell mitogen related to PDGF. Science 1989;246:1309-12.

235. Leung DW, Cachianes G, Kuang WJ, Goeddel DV, Ferrara N. Vascular endothelial growth factor is a secreted angiogenic mitogen. Science 1989;246:1306-9.
236. Maglione D, Guerriero V, Viglietto G, Delli-Bovi P, Persico MG. Isolation of a human placenta cDNA coding for a protein related to the vascular permeability factor. ProcNatlAcadSciUSA 1991;88:9267-71.
237. Maglione D, Guerriero V, Viglietto G, et al. Two alternative mRNAs coding for the angiogenic factor, placenta growth factor (PlGF), are transcribed from a single gene of chromosome 14. Oncogene 1993;8:925-31.
238. Olofsson B, Korpelainen E, Pepper MS, et al. Vascular endothelial growth factor B (VEGF-B) binds to VEGF receptor-1 and regulates plasminogen activator activity in endothelial cells. ProcNatlAcadSciUSA 1998;95:11709-14.
239. Olofsson B, Pajusola K, Kaipainen A, et al. Vascular endothelial growth factor B, a novel growth factor for endothelial cells. ProcNatlAcadSciUSA 1996;93:2576-81.
240. Joukov V, Pajusola K, Kaipainen A, et al. A novel vascular endothelial growth factor, VEGF-C, is a ligand for the Flt4 (VEGFR-3) and KDR (VEGFR-2) receptor tyrosine kinases. EMBO J 1996;15:1751.
241. Lee J, Gray A, Yuan J, Luoh SM, Avraham H, Wood WI. Vascular endothelial growth factor-related protein: a ligand and specific activator of the tyrosine kinase receptor Flt4. ProcNatlAcadSciUSA 1996;93:1988-92.
242. Achen MG, Jeltsch M, Kukk E, et al. Vascular endothelial growth factor D (VEGF-D) is a ligand for the tyrosine kinases VEGF receptor 2 (Flk1) and VEGF receptor 3 (Flt4). ProcNatlAcadSciUSA 1998;95:548-53.
243. Orlandini M, Marconcini L, Ferruzzi R, Oliviero S. Identification of a c-fos-induced gene that is related to the platelet-derived growth factor/vascular endothelial growth factor family. ProcNatlAcadSciUSA 1996;93:11675-80.
244. Kiba A, Yabana N, Shibuya M. A set of loop-1 and -3 structures in the novel vascular endothelial growth factor (VEGF) family member, VEGF-ENZ-7, is essential for the activation of VEGFR-2 signaling. JBiolChem 2003;278:13453-61.
245. Stacker SA, Caesar C, Baldwin ME, et al. VEGF-D promotes the metastatic spread of tumor cells via the lymphatics. NatMed 2001;7:186-91.
246. Hanahan D, Folkman J. Patterns and emerging mechanisms of the angiogenic switch during tumorigenesis. Cell 1996;86:353-64.
247. Carmeliet P. Angiogenesis in health and disease. NatMed 2003;9:653-60.
248. Jain RK. Molecular regulation of vessel maturation. NatMed 2003;9:685-93.
249. Yancopoulos GD, Davis S, Gale NW, Rudge JS, Wiegand SJ, Holash J. Vascular-specific growth factors and blood vessel formation. Nature 2000;407:242-8.
250. van Hinsbergh VW, Collen A, Koolwijk P. Role of fibrin matrix in angiogenesis. Ann N Y Acad Sci 2001;936:426-37.

251. Kalebic T, Garbisa S, Glaser B, Liotta LA. Basement membrane collagen: degradation by migrating endothelial cells. Science 1983;221:281-3.
252. Kahari VM, Saarialho-Kere U. Matrix metalloproteinases in skin. ExpDermatol 1997;6:199-213.
253. Lee CZ, Xu B, Hashimoto T, McCulloch CE, Yang GY, Young WL. Doxycycline suppresses cerebral matrix metalloproteinase-9 and angiogenesis induced by focal hyperstimulation of vascular endothelial growth factor in a mouse model. Stroke 2004;35:1715-9.
254. Cornelius LA, Nehring LC, Roby JD, Parks WC, Welgus HG. Human dermal microvascular endothelial cells produce matrix metalloproteinases in response to angiogenic factors and migration. JInvest Dermatol 1995;105:170-6.
255. Raza SL, Cornelius LA. Matrix metalloproteinases: pro- and anti-angiogenic activities. JInvestigDermatolSympProc 2000;5:47-54.
256. Hiraoka N, Allen E, Apel IJ, Gyetko MR, Weiss SJ. Matrix metalloproteinases regulate neovascularization by acting as pericellular fibrinolysins. Cell 1998;95:365-77.
257. Itoh T, Tanioka M, Yoshida H, Yoshioka T, Nishimoto H, Itohara S. Reduced angiogenesis and tumor progression in gelatinase A-deficient mice. Cancer Res 1998;58:1048-51.
258. Brooks PC, Clark RA, Cheresh DA. Requirement of vascular integrin alpha v beta 3 for angiogenesis. Science 1994;264:569-71.
259. Brooks PC, Montgomery AM, Rosenfeld M, et al. Integrin alpha v beta 3 antagonists promote tumor regression by inducing apoptosis of angiogenic blood vessels. Cell 1994;79:1157-64.
260. Senger DR, Ledbetter SR, Claffey KP, Papadopoulos-Sergiou A, Peruzzi CA, Detmar M. Stimulation of endothelial cell migration by vascular permeability factor/vascular endothelial growth factor through cooperative mechanisms involving the alphavbeta3 integrin, osteopontin, and thrombin. AmJPathol 1996;149:293-305.
261. Brooks PC, Stromblad S, Sanders LC, et al. Localization of matrix metalloproteinase MMP-2 to the surface of invasive cells by interaction with integrin alpha v beta 3. Cell 1996;85:683-93.
262. Brooks PC, Silletti S, von Schalscha TL, Friedlander M, Cheresh DA. Disruption of angiogenesis by PEX, a noncatalytic metalloproteinase fragment with integrin binding activity. Cell 1998;92:391-400.
263. Doherty MJ, Canfield AE. Gene expression during vascular pericyte differentiation. Crit RevEukaryotGene Expr 1999;9:1-17.
264. Lindahl P, Johansson BR, Leveen P, Betsholtz C. Pericyte loss and microaneurysm formation in PDGF-B-deficient mice. Science 1997;277:242-5.
265. Davis S, Yancopoulos GD. The angiopoietins: Yin and Yang in angiogenesis. CurrTopMicrobiolImmunol 1999;237:173-85.
266. Davis S, Aldrich TH, Jones PF, et al. Isolation of angiopoietin-1, a ligand for the TIE2 receptor, by secretion-trap expression cloning. Cell 1996;87:1161-9.

267. Suri C, McClain J, Thurston G, et al. Increased vascularization in mice overexpressing angiopoietin-1. Science 1998;282:468-71.
268. Maisonpierre PC, Suri C, Jones PF, et al. Angiopoietin-2, a natural antagonist for Tie2 that disrupts in vivo angiogenesis. Science 1997;277:55-60.
269. Holash J, Maisonpierre PC, Compton D, et al. Vessel cooption, regression, and growth in tumors mediated by angiopoietins and VEGF. Science 1999;284:1994-8.
270. Fiedler U, Reiss Y, Scharpfenecker M, et al. Angiopoietin-2 sensitizes endothelial cells to TNF-alpha and has a crucial role in the induction of inflammation. NatMed 2006;12:235-9.
271. Imhof BA, urrand-Lions M. Angiogenesis and inflammation face off. NatMed 2006;12:171-2.
272. Hughes DP, Marron MB, Brindle NP. The antiinflammatory endothelial tyrosine kinase Tie2 interacts with a novel nuclear factor-kappaB inhibitor ABIN-2. CircRes 2003;92:630-6.
273. Good DJ, Polverini PJ, Rastinejad F, et al. A tumor suppressor-dependent inhibitor of angiogenesis is immunologically and functionally indistinguishable from a fragment of thrombospondin. ProcNatlAcadSciUSA 1990;87:6624-8.
274. Wight TN, Raugi GJ, Mumby SM, Bornstein P. Light microscopic immunolocation of thrombospondin in human tissues. JHistochemCytochem 1985;33:295-302.
275. Taraboletti G, Roberts D, Liotta LA, Giavazzi R. Platelet thrombospondin modulates endothelial cell adhesion, motility, and growth: a potential angiogenesis regulatory factor. JCell Biol 1990;111:765-72.
276. Tolsma SS, Volpert OV, Good DJ, Frazier WA, Polverini PJ, Bouck N. Peptides derived from two separate domains of the matrix protein thrombospondin-1 have anti-angiogenic activity. JCell Biol 1993;122:497-511.
277. Streit M, Riccardi L, Velasco P, et al. Thrombospondin-2: a potent endogenous inhibitor of tumor growth and angiogenesis. ProcNatlAcadSciUSA 1999;96:14888-93.
278. Johnson MD, Kim HR, Chesler L, Tsao-Wu G, Bouck N, Polverini PJ. Inhibition of angiogenesis by tissue inhibitor of metalloproteinase. JCell Physiol 1994;160:194-202.
279. Moses MA. The regulation of neovascularization of matrix metalloproteinases and their inhibitors. Stem Cells 1997;15:180-9.
280. Gomez DE, Alonso DF, Yoshiji H, Thorgeirsson UP. Tissue inhibitors of metalloproteinases: structure, regulation and biological functions. EurJCell Biol 1997;74:111-22.
281. Vazquez F, Hastings G, Ortega MA, et al. METH-1, a human ortholog of ADAMTS-1, and METH-2 are members of a new family of proteins with angio-inhibitory activity. JBiolChem 1999;274:23349-57.
282. O'Reilly MS, Holmgren L, Shing Y, et al. Angiostatin: a novel angiogenesis inhibitor that mediates the suppression of metastases by a Lewis lung carcinoma. Cell 1994;79:315-28.

283. O'Reilly MS, Boehm T, Shing Y, et al. Endostatin: an endogenous inhibitor of angiogenesis and tumor growth. Cell 1997;88:277-85.
284. Dhanabal M, Ramchandran R, Waterman MJ, et al. Endostatin induces endothelial cell apoptosis. JBiolChem 1999;274:11721-6.
285. Neuwelt EA. Mechanisms of disease: the blood-brain barrier. Neurosurgery 2004;54:131-40; discussion 41-2.
286. Palmieri D, Chambers AF, Felding-Habermann B, Huang S, Steeg PS. The biology of metastasis to a sanctuary site. Clin Cancer Res 2007;13:1656-62.
287. Plate KH, Mennel HD. Vascular morphology and angiogenesis in glial tumors. ExpToxicolPathol 1995;47:89-94.
288. Yuan F, Salehi HA, Boucher Y, Vasthare US, Tuma RF, Jain RK. Vascular permeability and microcirculation of gliomas and mammary carcinomas transplanted in rat and mouse cranial windows. Cancer Res 1994;54:4564-8.
289. Jain RK. Barriers to drug delivery in solid tumors. Sci Am 1994;271:58-65.
290. Folkman J. Angiogenesis in cancer, vascular, rheumatoid and other disease. NatMed 1995;1:27-31.
291. Carmeliet P, Mackman N, Moons L, et al. Role of tissue factor in embryonic blood vessel development. Nature 1996;383:73-5.
292. Ferrara N, Carver-Moore K, Chen H, et al. Heterozygous embryonic lethality induced by targeted inactivation of the VEGF gene. Nature 1996;380:439-42.
293. Haigh JJ, Morelli PI, Gerhardt H, et al. Cortical and retinal defects caused by dosage-dependent reductions in VEGF-A paracrine signaling. DevBiol 2003;262:225-41.
294. Raab S, Beck H, Gaumann A, et al. Impaired brain angiogenesis and neuronal apoptosis induced by conditional homozygous inactivation of vascular endothelial growth factor. ThrombHaemost 2004;91:595-605.
295. Miquerol L, Langille BL, Nagy A. Embryonic development is disrupted by modest increases in vascular endothelial growth factor gene expression. Development 2000;127:3941-6.
296. Carmeliet P, Moons L, Luttun A, et al. Synergism between vascular endothelial growth factor and placental growth factor contributes to angiogenesis and plasma extravasation in pathological conditions. NatMed 2001;7:575-83.
297. Bellomo D, Headrick JP, Silins GU, et al. Mice lacking the vascular endothelial growth factor-B gene (Vegfb) have smaller hearts, dysfunctional coronary vasculature, and impaired recovery from cardiac ischemia. CircRes 2000;86:E29-E35.
298. Gerber HP, Hillan KJ, Ryan AM, et al. VEGF is required for growth and survival in neonatal mice. Development 1999;126:1149-59.
299. Kitamoto Y, Tokunaga H, Tomita K. Vascular endothelial growth factor is an essential molecule for mouse kidney development: glomerulogenesis and nephrogenesis. JClinInvest 1997;99:2351-7.
300. Ostendorf T, Kunter U, Eitner F, et al. VEGF(165) mediates glomerular endothelial repair. JClinInvest 1999;104:913-23.

301. Ryan AM, Eppler DB, Hagler KE, et al. Preclinical safety evaluation of rhuMAbVEGF, an antiangiogenic humanized monoclonal antibody. ToxicolPathol 1999;27:78-86.
302. Poole AR, Hall BK, Newman SA. The growth plate: cellular physiology, cartilage assembly and mineralization. In: Cartilage: molecular aspects. Boca Raton, FL: CRC Press; 1991:179-211.
303. Carlevaro MF, Cermelli S, Cancedda R, Descalzi CF. Vascular endothelial growth factor (VEGF) in cartilage neovascularization and chondrocyte differentiation: auto-paracrine role during endochondral bone formation. JCell Sci 2000;113 (Pt 1):59-69.
304. Zeleznik AJ, Schuler HM, Reichert LE, Jr. Gonadotropin-binding sites in the rhesus monkey ovary: role of the vasculature in the selective distribution of human chorionic gonadotropin to the preovulatory follicle. Endocrinology 1981;109:356-62.
305. Bassett DL. The changes in the vascular pattern of the ovary of the albino rat during the estrous cycle. Am J Anat 1943;73:251-78.
306. Carr BR, MacDonald PC, Simpson ER. The role of lipoproteins in the regulation of progesterone secretion by the human corpus luteum. FertilSteril 1982;38:303-11.
307. Goede V, Schmidt T, Kimmina S, Kozian D, Augustin HG. Analysis of blood vessel maturation processes during cyclic ovarian angiogenesis. Lab Invest 1998;78:1385-94.
308. Phillips HS, Hains J, Leung DW, Ferrara N. Vascular endothelial growth factor is expressed in rat corpus luteum. Endocrinology 1990;127:965-7.
309. Ravindranath N, Little-Ihrig L, Phillips HS, Ferrara N, Zeleznik AJ. Vascular endothelial growth factor messenger ribonucleic acid expression in the primate ovary. Endocrinology 1992;131:254-60.
310. Zimmermann RC, Xiao E, Husami N, et al. Short-term administration of antivascular endothelial growth factor antibody in the late follicular phase delays follicular development in the rhesus monkey. JClinEndocrinolMetab 2001;86:768-72.
311. Ferrara N, Chen H, Davis-Smyth T, et al. Vascular endothelial growth factor is essential for corpus luteum angiogenesis. NatMed 1998;4:336-40.
312. Zimmermann RC, Hartman T, Bohlen P, Sauer MV, Kitajewski J. Preovulatory treatment of mice with anti-VEGF receptor 2 antibody inhibits angiogenesis in corpora lutea. MicrovascRes 2001;62:15-25.
313. Fraser HM, Dickson SE, Lunn SF, et al. Suppression of luteal angiogenesis in the primate after neutralization of vascular endothelial growth factor. Endocrinology 2000;141:995-1000.
314. Hazzard TM, Xu F, Stouffer RL. Injection of soluble vascular endothelial growth factor receptor 1 into the preovulatory follicle disrupts ovulation and subsequent luteal function in rhesus monkeys. BiolReprod 2002;67:1305-12.
315. Rowe AJ, Morris KD, Bicknell R, Fraser HM. Angiogenesis in the corpus luteum of early pregnancy in the marmoset and the effects of vascular endothelial growth factor immunoneutralization on establishment of pregnancy. BiolReprod 2002;67:1180-8.

316. Lecouter J, Kowalski J, Foster J, et al. Identification of an angiogenic mitogen selective for endocrine gland endothelium. Nature 2001;412:877-84.
317. Kaser A, Winklmayr M, Lepperdinger G, Kreil G. The AVIT protein family. Secreted cysteine-rich vertebrate proteins with diverse functions. EMBO Rep 2003;4:469-73.
318. Lecouter J, Lin R, Ferrara N. The role of EG-VEGF in the regulation of angiogenesis in endocrine glands. Cold Spring HarbSympQuantBiol 2002;67:217-21.
319. Lammert E, Gu G, McLaughlin M, et al. Role of VEGF-A in vascularization of pancreatic islets. CurrBiol 2003;13:1070-4.
320. Nagy JA, Vasile E, Feng D, et al. Vascular permeability factor/vascular endothelial growth factor induces lymphangiogenesis as well as angiogenesis. JExpMed 2002;196:1497-506.
321. Saaristo A, Veikkola T, Enholm B, et al. Adenoviral VEGF-C overexpression induces blood vessel enlargement, tortuosity, and leakiness but no sprouting angiogenesis in the skin or mucous membranes. FASEB J 2002;16:1041-9.
322. Makinen T, Veikkola T, Mustjoki S, et al. Isolated lymphatic endothelial cells transduce growth, survival and migratory signals via the VEGF-C/D receptor VEGFR-3. EMBO J 2001;20:4762-73.
323. Cursiefen C, Chen L, Borges LP, et al. VEGF-A stimulates lymphangiogenesis and hemangiogenesis in inflammatory neovascularization via macrophage recruitment. JClinInvest 2004;113:1040-50.
324. Schoppmann SF, Birner P, Stockl J, et al. Tumor-associated macrophages express lymphatic endothelial growth factors and are related to peritumoral lymphangiogenesis. AmJPathol 2002;161:947-56.
325. Gale NW, Thurston G, Hackett SF, et al. Angiopoietin-2 is required for postnatal angiogenesis and lymphatic patterning, and only the latter role is rescued by Angiopoietin-1. DevCell 2002;3:411-23.
326. Breiteneder-Geleff S, Soleiman A, Kowalski H, et al. Angiosarcomas express mixed endothelial phenotypes of blood and lymphatic capillaries: podoplanin as a specific marker for lymphatic endothelium. AmJPathol 1999;154:385-94.
327. Schacht V, Ramirez MI, Hong YK, et al. T1alpha/podoplanin deficiency disrupts normal lymphatic vasculature formation and causes lymphedema. EMBO J 2003;22:3546-56.
328. Ayadi A, Zheng H, Sobieszczuk P, et al. Net-targeted mutant mice develop a vascular phenotype and up-regulate egr-1. EMBO J 2001;20:5139-52.
329. Wang JF, Zhang XF, Groopman JE. Stimulation of beta 1 integrin induces tyrosine phosphorylation of vascular endothelial growth factor receptor-3 and modulates cell migration. JBiolChem 2001;276:41950-7.
330. Karkkainen MJ, Saaristo A, Jussila L, et al. A model for gene therapy of human hereditary lymphedema. ProcNatlAcadSciUSA 2001;98:12677-82.
331. Alon T, Hemo I, Itin A, Pe'er J, Stone J, Keshet E. Vascular endothelial growth factor acts as a survival factor for newly formed retinal vessels and has implications for retinopathy of prematurity. NatMed 1995;1:1024-8.

332. Benjamin LE, Golijanin D, Itin A, Pode D, Keshet E. Selective ablation of immature blood vessels in established human tumors follows vascular endothelial growth factor withdrawal. JClinInvest 1999;103:159-65.
333. Davis-Smyth T, Presta LG, Ferrara N. Mapping the charged residues in the second immunoglobulin-like domain of the vascular endothelial growth factor/placenta growth factor receptor Flt-1 required for binding and structural stability. JBiolChem 1998;273:3216-22.
334. Ferrara N, Davis-Smyth T. The biology of vascular endothelial growth factor. EndocrRev 1997;18:4-25.
335. Gerber HP, Dixit V, Ferrara N. Vascular endothelial growth factor induces expression of the antiapoptotic proteins Bcl-2 and A1 in vascular endothelial cells. JBiolChem 1998;273:13313-6.
336. Gerber HP, McMurtrey A, Kowalski J, et al. Vascular endothelial growth factor regulates endothelial cell survival through the phosphatidylinositol 3'-kinase/Akt signal transduction pathway. Requirement for Flk-1/KDR activation. JBiolChem 1998;273:30336-43.
337. Yuan F, Chen Y, Dellian M, Safabakhsh N, Ferrara N, Jain RK. Time-dependent vascular regression and permeability changes in established human tumor xenografts induced by an anti-vascular endothelial growth factor/vascular permeability factor antibody. ProcNatlAcadSciUSA 1996;93:14765-70.
338. Fujio Y, Walsh K. Akt mediates cytoprotection of endothelial cells by vascular endothelial growth factor in an anchorage-dependent manner. JBiolChem 1999;274:16349-54.
339. Tran J, Rak J, Sheehan C, et al. Marked induction of the IAP family antiapoptotic proteins survivin and XIAP by VEGF in vascular endothelial cells. BiochemBiophysResCommun 1999;264:781-8.
340. Tran J, Master Z, Yu JL, Rak J, Dumont DJ, Kerbel RS. A role for survivin in chemoresistance of endothelial cells mediated by VEGF. ProcNatlAcadSciUSA 2002;99:4349-54.
341. Compernolle V, Brusselmans K, Acker T, et al. Loss of HIF-2alpha and inhibition of VEGF impair fetal lung maturation, whereas treatment with VEGF prevents fatal respiratory distress in premature mice. NatMed 2002;8:702-10.
342. Lambrechts D, Storkebaum E, Morimoto M, et al. VEGF is a modifier of amyotrophic lateral sclerosis in mice and humans and protects motoneurons against ischemic death. NatGenet 2003;34:383-94.
343. Clauss M, Gerlach M, Gerlach H, et al. Vascular permeability factor: a tumor-derived polypeptide that induces endothelial cell and monocyte procoagulant activity, and promotes monocyte migration. JExpMed 1990;172:1535-45.
344. Broxmeyer HE, Cooper S, Li ZH, et al. Myeloid progenitor cell regulatory effects of vascular endothelial cell growth factor. IntJHematol 1995;62:203-15.
345. Gabrilovich D, Ishida T, Oyama T, et al. Vascular endothelial growth factor inhibits the development of dendritic cells and dramatically affects the differentiation of multiple hematopoietic lineages in vivo. Blood 1998;92:4150-66.

346. Gabrilovich DI, Chen HL, Girgis KR, et al. Production of vascular endothelial growth factor by human tumors inhibits the functional maturation of dendritic cells. NatMed 1996;2:1096-103.
347. Hattori K, Dias S, Heissig B, et al. Vascular endothelial growth factor and angiopoietin-1 stimulate postnatal hematopoiesis by recruitment of vasculogenic and hematopoietic stem cells. JExpMed 2001;193:1005-14.
348. Morbidelli L, Chang CH, Douglas JG, Granger HJ, Ledda F, Ziche M. Nitric oxide mediates mitogenic effect of VEGF on coronary venular endothelium. AmJPhysiol 1996;270:H411-H5.
349. Parenti A, Morbidelli L, Cui XL, et al. Nitric oxide is an upstream signal of vascular endothelial growth factor-induced extracellular signal-regulated kinase1/2 activation in postcapillary endothelium. JBiolChem 1998;273:4220-6.
350. Ziche M, Morbidelli L, Choudhuri R, et al. Nitric oxide synthase lies downstream from vascular endothelial growth factor-induced but not basic fibroblast growth factor-induced angiogenesis. JClinInvest 1997;99:2625-34.
351. Roberts WG, Palade GE. Increased microvascular permeability and endothelial fenestration induced by vascular endothelial growth factor. JCell Sci 1995;108 (Pt 6):2369-79.
352. Dvorak HF, Brown LF, Detmar M, Dvorak AM. Vascular permeability factor/vascular endothelial growth factor, microvascular hyperpermeability, and angiogenesis. AmJPathol 1995;146:1029-39.
353. Dvorak HF, Harvey VS, Estrella P, Brown LF, McDonagh J, Dvorak AM. Fibrin containing gels induce angiogenesis. Implications for tumor stroma generation and wound healing. Lab Invest 1987;57:673-86.
354. Eliceiri BP, Paul R, Schwartzberg PL, Hood JD, Leng J, Cheresh DA. Selective requirement for Src kinases during VEGF-induced angiogenesis and vascular permeability. MolCell 1999;4:915-24.
355. Ku DD, Zaleski JK, Liu S, Brock TA. Vascular endothelial growth factor induces EDRF-dependent relaxation in coronary arteries. AmJPhysiol 1993;265:H586-H92.
356. Yang R, Thomas GR, Bunting S, et al. Effects of vascular endothelial growth factor on hemodynamics and cardiac performance. JCardiovascPharmacol 1996;27:838-44.
357. Henry TD, Annex BH, McKendall GR, et al. The VIVA trial: Vascular endothelial growth factor in Ischemia for Vascular Angiogenesis. Circulation 2003;107:1359-65.
358. Yang JC, Haworth L, Sherry RM, et al. A randomized trial of bevacizumab, an anti-vascular endothelial growth factor antibody, for metastatic renal cancer. NEnglJMed 2003;349:427-34.
359. Vincenti V, Cassano C, Rocchi M, Persico G. Assignment of the vascular endothelial growth factor gene to human chromosome 6p21.3. Circulation 1996;93:1493-5.
360. Houck KA, Ferrara N, Winer J, Cachianes G, Li B, Leung DW. The vascular endothelial growth factor family: identification of a fourth molecular species and characterization of alternative splicing of RNA. MolEndocrinol 1991;5:1806-14.

361. Tischer E, Mitchell R, Hartman T, et al. The human gene for vascular endothelial growth factor. Multiple protein forms are encoded through alternative exon splicing. JBiolChem 1991;266:11947-54.
362. Poltorak Z, Cohen T, Sivan R, et al. VEGF145, a secreted vascular endothelial growth factor isoform that binds to extracellular matrix. JBiolChem 1997;272:7151-8.
363. Jingjing L, Xue Y, Agarwal N, Roque RS. Human Muller cells express VEGF183, a novel spliced variant of vascular endothelial growth factor. Invest OphthalmolVisSci 1999;40:752-9.
364. Lange T, Guttmann-Raviv N, Baruch L, Machluf M, Neufeld G. VEGF162, a new heparin-binding vascular endothelial growth factor splice form that is expressed in transformed human cells. JBiolChem 2003;278:17164-9.
365. Bates DO, Cui TG, Doughty JM, et al. VEGF165b, an inhibitory splice variant of vascular endothelial growth factor, is down-regulated in renal cell carcinoma. Cancer Res 2002;62:4123-31.
366. Houck KA, Leung DW, Rowland AM, Winer J, Ferrara N. Dual regulation of vascular endothelial growth factor bioavailability by genetic and proteolytic mechanisms. JBiolChem 1992;267:26031-7.
367. Muller YA, Li B, Christinger HW, Wells JA, Cunningham BC, de Vos AM. Vascular endothelial growth factor: crystal structure and functional mapping of the kinase domain receptor binding site. ProcNatlAcadSciUSA 1997;94:7192-7.
368. McDonald NQ, Hendrickson WA. A structural superfamily of growth factors containing a cystine knot motif. Cell 1993;73:421-4.
369. Park JE, Keller GA, Ferrara N. The vascular endothelial growth factor (VEGF) isoforms: differential deposition into the subepithelial extracellular matrix and bioactivity of extracellular matrix-bound VEGF. MolBiolCell 1993;4:1317-26.
370. Keyt BA, Berleau LT, Nguyen HV, et al. The carboxyl-terminal domain (111-165) of vascular endothelial growth factor is critical for its mitogenic potency. JBiolChem 1996;271:7788-95.
371. Ruhrberg C, Gerhardt H, Golding M, et al. Spatially restricted patterning cues provided by heparin-binding VEGF-A control blood vessel branching morphogenesis. Genes Dev 2002;16:2684-98.
372. Neufeld G, Cohen T, Gengrinovitch S, Poltorak Z. Vascular endothelial growth factor (VEGF) and its receptors. FASEB J 1999;13:9-22.
373. Cao Y, Linden P, Farnebo J, et al. Vascular endothelial growth factor C induces angiogenesis in vivo. ProcNatlAcadSciUSA 1998;95:14389-94.
374. Karkkainen MJ, Makinen T, Alitalo K. Lymphatic endothelium: a new frontier of metastasis research. NatCell Biol 2002;4:E2-E5.
375. Matthews W, Jordan CT, Gavin M, Jenkins NA, Copeland NG, Lemischka IR. A receptor tyrosine kinase cDNA isolated from a population of enriched primitive hematopoietic cells and exhibiting close genetic linkage to c-kit. ProcNatlAcadSciUSA 1991;88:9026-30.
376. Shibuya M, Yamaguchi S, Yamane A, et al. Nucleotide sequence and expression of a novel human receptor-type tyrosine kinase gene (flt) closely related to the fms family. Oncogene 1990;5:519-24.

377. Terman BI, Carrion ME, Kovacs E, Rasmussen BA, Eddy RL, Shows TB. Identification of a new endothelial cell growth factor receptor tyrosine kinase. Oncogene 1991;6:1677-83.
378. Bussolati B, Dunk C, Grohman M, Kontos CD, Mason J, Ahmed A. Vascular endothelial growth factor receptor-1 modulates vascular endothelial growth factor-mediated angiogenesis via nitric oxide. AmJPathol 2001;159:993-1008.
379. Fong GH, Rossant J, Gertsenstein M, Breitman ML. Role of the Flt-1 receptor tyrosine kinase in regulating the assembly of vascular endothelium. Nature 1995;376:66-70.
380. Huang K, Andersson C, Roomans GM, Ito N, Claesson-Welsh L. Signaling properties of VEGF receptor-1 and -2 homo- and heterodimers. IntJBiochemCell Biol 2001;33:315-24.
381. Kendall RL, Thomas KA. Inhibition of vascular endothelial cell growth factor activity by an endogenously encoded soluble receptor. ProcNatlAcadSciUSA 1993;90:10705-9.
382. Waltenberger J, Claesson-Welsh L, Siegbahn A, Shibuya M, Heldin CH. Different signal transduction properties of KDR and Flt1, two receptors for vascular endothelial growth factor. JBiolChem 1994;269:26988-95.
383. Kendall RL, Wang G, Thomas KA. Identification of a natural soluble form of the vascular endothelial growth factor receptor, FLT-1, and its heterodimerization with KDR. BiochemBiophysResCommun 1996;226:324-8.
384. Fong GH, Zhang L, Bryce DM, Peng J. Increased hemangioblast commitment, not vascular disorganization, is the primary defect in flt-1 knock-out mice. Development 1999;126:3015-25.
385. Park JE, Chen HH, Winer J, Houck KA, Ferrara N. Placenta growth factor. Potentiation of vascular endothelial growth factor bioactivity, in vitro and in vivo, and high affinity binding to Flt-1 but not to Flk-1/KDR. JBiolChem 1994;269:25646-54.
386. Barleon B, Totzke F, Herzog C, et al. Mapping of the sites for ligand binding and receptor dimerization at the extracellular domain of the vascular endothelial growth factor receptor FLT-1. JBiolChem 1997;272:10382-8.
387. Davis-Smyth T, Chen H, Park J, Presta LG, Ferrara N. The second immunoglobulin-like domain of the VEGF tyrosine kinase receptor Flt-1 determines ligand binding and may initiate a signal transduction cascade. EMBO J 1996;15:4919-27.
388. Millauer B, Wizigmann-Voos S, Schnurch H, et al. High affinity VEGF binding and developmental expression suggest Flk-1 as a major regulator of vasculogenesis and angiogenesis. Cell 1993;72:835-46.
389. Quinn TP, Peters KG, de VC, Ferrara N, Williams LT. Fetal liver kinase 1 is a receptor for vascular endothelial growth factor and is selectively expressed in vascular endothelium. ProcNatlAcadSciUSA 1993;90:7533-7.
390. Terman BI, Dougher-Vermazen M, Carrion ME, et al. Identification of the KDR tyrosine kinase as a receptor for vascular endothelial cell growth factor. BiochemBiophysResCommun 1992;187:1579-86.
391. De Vries C, Escobedo JA, Ueno H, Houck K, Ferrara N, Williams LT. The fms-like tyrosine kinase, a receptor for vascular endothelial growth factor. Science 1992;255:989-91.

392. Gerber HP, Condorelli F, Park J, Ferrara N. Differential transcriptional regulation of the two vascular endothelial growth factor receptor genes. Flt-1, but not Flk-1/KDR, is up-regulated by hypoxia. JBiolChem 1997;272:23659-67.
393. Belgore FM, Blann AD, Li-Saw-Hee FL, Beevers DG, Lip GY. Plasma levels of vascular endothelial growth factor and its soluble receptor (SFlt-1) in essential hypertension. AmJCardiol 2001;87:805-7, A9.
394. Clark DE, Smith SK, He Y, et al. A vascular endothelial growth factor antagonist is produced by the human placenta and released into the maternal circulation. BiolReprod 1998;59:1540-8.
395. He Y, Smith SK, Day KA, Clark DE, Licence DR, Charnock-Jones DS. Alternative splicing of vascular endothelial growth factor (VEGF)-R1 (FLT-1) pre-mRNA is important for the regulation of VEGF activity. MolEndocrinol 1999;13:537-45.
396. Belgore FM, Blann AD, Lip GY. Measurement of free and complexed soluble vascular endothelial growth factor receptor, Flt-1, in fluid samples: development and application of two new immunoassays. ClinSci(Lond) 2001;100:567-75.
397. Belgore FM, Lip GY, Blann AD. Vascular endothelial growth factor and its receptor, Flt-1, in smokers and non-smokers. BrJBiomedSci 2000;57:207-13.
398. Gille H, Kowalski J, Yu L, et al. A repressor sequence in the juxtamembrane domain of Flt-1 (VEGFR-1) constitutively inhibits vascular endothelial growth factor-dependent phosphatidylinositol 3'-kinase activation and endothelial cell migration. EMBO J 2000;19:4064-73.
399. Landgren E, Schiller P, Cao Y, Claesson-Welsh L. Placenta growth factor stimulates MAP kinase and mitogenicity but not phospholipase C-gamma and migration of endothelial cells expressing Flt 1. Oncogene 1998;16:359-67.
400. Maru Y, Yamaguchi S, Shibuya M. Flt-1, a receptor for vascular endothelial growth factor, has transforming and morphogenic potentials. Oncogene 1998;16:2585-95.
401. Autiero M, Waltenberger J, Communi D, et al. Role of PlGF in the intra- and intermolecular cross talk between the VEGF receptors Flt1 and Flk1. NatMed 2003;9:936-43.
402. Lecouter J, Moritz DR, Li B, et al. Angiogenesis-independent endothelial protection of liver: role of VEGFR-1. Science 2003;299:890-3.
403. Adini A, Kornaga T, Firoozbakht F, Benjamin LE. Placental growth factor is a survival factor for tumor endothelial cells and macrophages. Cancer Res 2002;62:2749-52.
404. Shalaby F, Rossant J, Yamaguchi TP, et al. Failure of blood-island formation and vasculogenesis in Flk-1-deficient mice. Nature 1995;376:62-6.
405. Fuh G, Li B, Crowley C, Cunningham B, Wells JA. Requirements for binding and signaling of the kinase domain receptor for vascular endothelial growth factor. JBiolChem 1998;273:11197-204.
406. Byzova TV, Goldman CK, Pampori N, et al. A mechanism for modulation of cellular responses to VEGF: activation of the integrins. MolCell 2000;6:851-60.

407. Kaipainen A, Korhonen J, Mustonen T, et al. Expression of the fms-like tyrosine kinase 4 gene becomes restricted to lymphatic endothelium during development. ProcNatlAcadSciUSA 1995;92:3566-70.
408. Jeltsch M, Kaipainen A, Joukov V, et al. Hyperplasia of lymphatic vessels in VEGF-C transgenic mice. Science 1997;276:1423-5.
409. Oh SJ, Jeltsch MM, Birkenhager R, et al. VEGF and VEGF-C: specific induction of angiogenesis and lymphangiogenesis in the differentiated avian chorioallantoic membrane. DevBiol 1997;188:96-109.
410. Veikkola T, Jussila L, Makinen T, et al. Signalling via vascular endothelial growth factor receptor-3 is sufficient for lymphangiogenesis in transgenic mice. EMBO J 2001;20:1223-31.
411. Makinen T, Jussila L, Veikkola T, et al. Inhibition of lymphangiogenesis with resulting lymphedema in transgenic mice expressing soluble VEGF receptor-3. NatMed 2001;7:199-205.
412. Soker S, Fidder H, Neufeld G, Klagsbrun M. Characterization of novel vascular endothelial growth factor (VEGF) receptors on tumor cells that bind VEGF165 via its exon 7-encoded domain. JBiolChem 1996;271:5761-7.
413. Soker S, Takashima S, Miao HQ, Neufeld G, Klagsbrun M. Neuropilin-1 is expressed by endothelial and tumor cells as an isoform-specific receptor for vascular endothelial growth factor. Cell 1998;92:735-45.
414. Fuh G, Garcia KC, de Vos AM. The interaction of neuropilin-1 with vascular endothelial growth factor and its receptor flt-1. JBiolChem 2000;275:26690-5.
415. Yuan L, Moyon D, Pardanaud L, et al. Abnormal lymphatic vessel development in neuropilin 2 mutant mice. Development 2002;129:4797-806.
416. Dvorak HF. Vascular permeability factor/vascular endothelial growth factor: a critical cytokine in tumor angiogenesis and a potential target for diagnosis and therapy. JClinOncol 2002;20:4368-80.
417. Young HS, Summers AM, Bhushan M, Brenchley PE, Griffiths CE. Single-nucleotide polymorphisms of vascular endothelial growth factor in psoriasis of early onset. JInvest Dermatol 2004;122:209-15.
418. Reinders ME, Sho M, Izawa A, et al. Proinflammatory functions of vascular endothelial growth factor in alloimmunity. JClinInvest 2003;112:1655-65.
419. Carmeliet P. Mechanisms of angiogenesis and arteriogenesis. NatMed 2000;6:389-95.
420. Streit M, Velasco P, Riccardi L, et al. Thrombospondin-1 suppresses wound healing and granulation tissue formation in the skin of transgenic mice. EMBO J 2000;19:3272-82.
421. Braverman IM, Sibley J. Role of the microcirculation in the treatment and pathogenesis of psoriasis. JInvest Dermatol 1982;78:12-7.
422. Lange-Asschenfeldt B, Weninger W, Velasco P, et al. Increased and prolonged inflammation and angiogenesis in delayed-type hypersensitivity reactions elicited in the skin of thrombospondin-2--deficient mice. Blood 2002;99:538-45.
423. Stacker SA, Achen MG, Jussila L, Baldwin ME, Alitalo K. Lymphangiogenesis and cancer metastasis. NatRevCancer 2002;2:573-83.

424. Akagi K, Ikeda Y, Miyazaki M, et al. Vascular endothelial growth factor-C (VEGF-C) expression in human colorectal cancer tissues. BrJCancer 2000;83:887-91.
425. Niki T, Iba S, Tokunou M, Yamada T, Matsuno Y, Hirohashi S. Expression of vascular endothelial growth factors A, B, C, and D and their relationships to lymph node status in lung adenocarcinoma. ClinCancer Res 2000;6:2431-9.
426. Kurebayashi J, Otsuki T, Kunisue H, et al. Expression of vascular endothelial growth factor (VEGF) family members in breast cancer. JpnJCancer Res 1999;90:977-81.
427. Jenny B, Harrison JA, Baetens D, et al. Expression and localization of VEGF-C and VEGFR-3 in glioblastomas and haemangioblastomas. J Pathol 2006;209:34-43.
428. Grau SJ, Trillsch F, Herms J, et al. Expression of VEGFR3 in glioma endothelium correlates with tumor grade. J Neurooncol 2007;82:141-50.
429. Salven P, Lymboussaki A, Heikkila P, et al. Vascular endothelial growth factors VEGF-B and VEGF-C are expressed in human tumors. AmJPathol 1998;153:103-8.
430. Karpanen T, Egeblad M, Karkkainen MJ, et al. Vascular endothelial growth factor C promotes tumor lymphangiogenesis and intralymphatic tumor growth. Cancer Res 2001;61:1786-90.
431. Mattila MM, Ruohola JK, Karpanen T, Jackson DG, Alitalo K, Harkonen PL. VEGF-C induced lymphangiogenesis is associated with lymph node metastasis in orthotopic MCF-7 tumors. IntJCancer 2002;98:946-51.
432. Skobe M, Hawighorst T, Jackson DG, et al. Induction of tumor lymphangiogenesis by VEGF-C promotes breast cancer metastasis. NatMed 2001;7:192-8.
433. He Y, Kozaki K, Karpanen T, et al. Suppression of tumor lymphangiogenesis and lymph node metastasis by blocking vascular endothelial growth factor receptor 3 signaling. JNatlCancer Inst 2002;94:819-25.
434. Machein MR, Plate KH. VEGF in brain tumors. JNeurooncol 2000;50:109-20.
435. Huang H, Held-Feindt J, Buhl R, Mehdorn HM, Mentlein R. Expression of VEGF and its receptors in different brain tumors. Neurol Res 2005;27:371-7.
436. Plate KH, Breier G, Weich HA, Risau W. Vascular endothelial growth factor is a potential tumour angiogenesis factor in human gliomas in vivo. Nature 1992;359:845-8.
437. Wizigmann-Voos S, Breier G, Risau W, Plate KH. Up-regulation of vascular endothelial growth factor and its receptors in von Hippel-Lindau disease-associated and sporadic hemangioblastomas. Cancer Res 1995;55:1358-64.
438. Kalkanis SN, Carroll RS, Zhang J, Zamani AA, Black PM. Correlation of vascular endothelial growth factor messenger RNA expression with peritumoral vasogenic cerebral edema in meningiomas. JNeurosurg 1996;85:1095-101.

439. Huber H, Eggert A, Janss AJ, et al. Angiogenic profile of childhood primitive neuroectodermal brain tumours/medulloblastomas. EurJCancer 2001;37:2064-72.
440. Wagemakers M, Sie M, Hoving EW, Molema G, de Bont ES, den Dunnen WF. Tumor vessel biology in pediatric intracranial ependymoma. J Neurosurg Pediatr;5:335-41.
441. Brem S, Cotran R, Folkman J. Tumor angiogenesis: a quantitative method for histologic grading. JNatlCancer Inst 1972;48:347-56.
442. Plate KH, Breier G, Weich HA, Mennel HD, Risau W. Vascular endothelial growth factor and glioma angiogenesis: coordinate induction of VEGF receptors, distribution of VEGF protein and possible in vivo regulatory mechanisms. IntJCancer 1994;59:520-9.
443. Shweiki D, Itin A, Neufeld G, Gitay-Goren H, Keshet E. Patterns of expression of vascular endothelial growth factor (VEGF) and VEGF receptors in mice suggest a role in hormonally regulated angiogenesis. JClinInvest 1993;91:2235-43.
444. Machein MR, Kullmer J, Fiebich BL, Plate KH, Warnke PC. Vascular endothelial growth factor expression, vascular volume, and, capillary permeability in human brain tumors. Neurosurgery 1999;44:732-40.
445. Korkolopoulou P, Patsouris E, Kavantzas N, et al. Prognostic implications of microvessel morphometry in diffuse astrocytic neoplasms. NeuropatholApplNeurobiol 2002;28:57-66.
446. Korkolopoulou P, Patsouris E, Konstantinidou AE, et al. Hypoxia-inducible factor 1alpha/vascular endothelial growth factor axis in astrocytomas. Associations with microvessel morphometry, proliferation and prognosis. NeuropatholApplNeurobiol 2004;30:267-78.
447. Peles E, Lidar Z, Simon AJ, Grossman R, Nass D, Ram Z. Angiogenic factors in the cerebrospinal fluid of patients with astrocytic brain tumors. Neurosurgery 2004;55:562-7; discussion 7-8.
448. Kremer C, Breier G, Risau W, Plate KH. Up-regulation of flk-1/vascular endothelial growth factor receptor 2 by its ligand in a cerebral slice culture system. Cancer Res 1997;57:3852-9.
449. Abdulrauf SI, Edvardsen K, Ho KL, Yang XY, Rock JP, Rosenblum ML. Vascular endothelial growth factor expression and vascular density as prognostic markers of survival in patients with low-grade astrocytoma. JNeurosurg 1998;88:513-20.
450. Chaudhry IH, O'Donovan DG, Brenchley PE, Reid H, Roberts IS. Vascular endothelial growth factor expression correlates with tumour grade and vascularity in gliomas. Histopathology 2001;39:409-15.
451. Yao Y, Kubota T, Sato K, Kitai R, Takeuchi H, Arishima H. Prognostic value of vascular endothelial growth factor and its receptors Flt-1 and Flk-1 in astrocytic tumours. Acta Neurochir(Wien) 2001;143:159-66.
452. Zhou YH, Tan F, Hess KR, Yung WK. The expression of PAX6, PTEN, vascular endothelial growth factor, and epidermal growth factor receptor in gliomas: relationship to tumor grade and survival. ClinCancer Res 2003;9:3369-75.

453. Leung SY, Chan AS, Wong MP, Yuen ST, Cheung N, Chung LP. Expression of vascular endothelial growth factor and its receptors in pilocytic astrocytoma. AmJSurgPathol 1997;21:941-50.
454. Mukhopadhyay D, Tsiokas L, Sukhatme VP. Wild-type p53 and v-Src exert opposing influences on human vascular endothelial growth factor gene expression. Cancer Res 1995;55:6161-5.
455. Pietsch T, Wiestler OD. Molecular neuropathology of astrocytic brain tumors. J Neurooncol 1997;35:211-22.
456. Takeshita S, Pu LQ, Stein LA, et al. Intramuscular administration of vascular endothelial growth factor induces dose-dependent collateral artery augmentation in a rabbit model of chronic limb ischemia. Circulation 1994;90:II228-II34.
457. Takeshita S, Zheng LP, Brogi E, et al. Therapeutic angiogenesis. A single intraarterial bolus of vascular endothelial growth factor augments revascularization in a rabbit ischemic hind limb model. JClinInvest 1994;93:662-70.
458. Takeshita S, Tsurumi Y, Couffinahl T, et al. Gene transfer of naked DNA encoding for three isoforms of vascular endothelial growth factor stimulates collateral development in vivo. Lab Invest 1996;75:487-501.
459. Takeshita S, Weir L, Chen D, et al. Therapeutic angiogenesis following arterial gene transfer of vascular endothelial growth factor in a rabbit model of hindlimb ischemia. BiochemBiophysResCommun 1996;227:628-35.
460. Isner JM, Pieczek A, Schainfeld R, et al. Clinical evidence of angiogenesis after arterial gene transfer of phVEGF165 in patient with ischaemic limb. Lancet 1996;348:370-4.
461. Makinen K, Manninen H, Hedman M, et al. Increased vascularity detected by digital subtraction angiography after VEGF gene transfer to human lower limb artery: a randomized, placebo-controlled, double-blinded phase II study. MolTher 2002;6:127-33.
462. Dor Y, Djonov V, Abramovitch R, et al. Conditional switching of VEGF provides new insights into adult neovascularization and pro-angiogenic therapy. EMBO J 2002;21:1939-47.
463. Street J, Bao M, DeGuzman L, et al. Vascular endothelial growth factor stimulates bone repair by promoting angiogenesis and bone turnover. ProcNatlAcadSciUSA 2002;99:9656-61.
464. Tarkka T, Sipola A, Jamsa T, et al. Adenoviral VEGF-A gene transfer induces angiogenesis and promotes bone formation in healing osseous tissues. JGene Med 2003;5:560-6.
465. Garber K. Angiogenesis inhibitors suffer new setback. NatBiotechnol 2002;20:1067-8.
466. Hurwitz H, Fehrenbacher L, Novotny W, et al. Bevacizumab plus irinotecan, fluorouracil, and leucovorin for metastatic colorectal cancer. NEnglJMed 2004;350:2335-42.
467. Friedman HS, Prados MD, Wen PY, et al. Bevacizumab alone and in combination with irinotecan in recurrent glioblastoma. J Clin Oncol 2009;27:4733-40.

468. Kreisl TN, Kim L, Moore K, et al. Phase II trial of single-agent bevacizumab followed by bevacizumab plus irinotecan at tumor progression in recurrent glioblastoma. J Clin Oncol 2009;27:740-5.
469. Batchelor TT, Sorensen AG, di Tomaso E, et al. AZD2171, a pan-VEGF receptor tyrosine kinase inhibitor, normalizes tumor vasculature and alleviates edema in glioblastoma patients. Cancer Cell 2007;11:83-95.
470. Guo P, Hu B, Gu W, et al. Platelet-derived growth factor-B enhances glioma angiogenesis by stimulating vascular endothelial growth factor expression in tumor endothelia and by promoting pericyte recruitment. Am J Pathol 2003;162:1083-93.
471. Stupp R, Hegi ME, Neyns B, et al. Phase I/IIa study of cilengitide and temozolomide with concomitant radiotherapy followed by cilengitide and temozolomide maintenance therapy in patients with newly diagnosed glioblastoma. J Clin Oncol 2010;28:2712-8.
472. Chakravarti A. Population genetics--making sense out of sequence. Nat Genet 1999;21:56-60.
473. Lander ES, Schork NJ. Genetic dissection of complex traits. Science 1994;265:2037-48.
474. Risch N, Merikangas K. The future of genetic studies of complex human diseases. Science 1996;273:1516-7.
475. Cargill M, Altshuler D, Ireland J, et al. Characterization of single-nucleotide polymorphisms in coding regions of human genes. Nat Genet 1999;22:231-8.
476. Lander ES. The new genomics: global views of biology. Science 1996;274:536-9.
477. Pritchard JK. Are rare variants responsible for susceptibility to complex diseases? Am J Hum Genet 2001;69:124-37.
478. Pritchard JK, Cox NJ. The allelic architecture of human disease genes: common disease-common variant...or not? Hum Mol Genet 2002;11:2417-23.
479. Reich DE, Lander ES. On the allelic spectrum of human disease. Trends Genet 2001;17:502-10.
480. Smith DJ, Lusis AJ. The allelic structure of common disease. Hum Mol Genet 2002;11:2455-61.
481. Brookes AJ. The essence of SNPs. Gene 1999;234:177-86.
482. Kruglyak L, Nickerson DA. Variation is the spice of life. Nat Genet 2001;27:234-6.
483. Sachidanandam R, Weissman D, Schmidt SC, et al. A map of human genome sequence variation containing 1.42 million single nucleotide polymorphisms. Nature 2001;409:928-33.
484. Taillon-Miller P, Gu Z, Li Q, Hillier L, Kwok PY. Overlapping genomic sequences: a treasure trove of single-nucleotide polymorphisms. Genome Res 1998;8:748-54.
485. Gray IC, Campbell DA, Spurr NK. Single nucleotide polymorphisms as tools in human genetics. Hum Mol Genet 2000;9:2403-8.
486. Risch NJ. Searching for genetic determinants in the new millennium. Nature 2000;405:847-56.

487. Tabor HK, Risch NJ, Myers RM. Candidate-gene approaches for studying complex genetic traits: practical considerations. Nat Rev Genet 2002;3:391-7.
488. Cardon LR, Bell JI. Association study designs for complex diseases. Nat Rev Genet 2001;2:91-9.
489. Peltonen L, Palotie A, Lange K. Use of population isolates for mapping complex traits. Nat Rev Genet 2000;1:182-90.
490. Kere J. Human population genetics: lessons from Finland. Annu Rev Genomics Hum Genet 2001;2:103-28.
491. Spielman RS, Ewens WJ. The TDT and other family-based tests for linkage disequilibrium and association. Am J Hum Genet 1996;59:983-9.
492. Spielman RS, McGinnis RE, Ewens WJ. Transmission test for linkage disequilibrium: the insulin gene region and insulin-dependent diabetes mellitus (IDDM). Am J Hum Genet 1993;52:506-16.
493. Haines JL, Pericak-Vance MA. Approaches to gene mapping in complex human diseases. New York ; Chichester: Wiley-Liss; 1998.
494. Ott J. Analysis of human genetic linkage. Baltimore ; London: Johns Hopkins University Press; 1985.
495. Strachan T, Read AP. Human molecular genetics. 2nd ed. ed. New York: Wiley; 1999.
496. Morton NE. Sequential tests for the detection of linkage. Am J Hum Genet 1955;7:277-318.
497. Risch N. Linkage strategies for genetically complex traits. I. Multilocus models. Am J Hum Genet 1990;46:222-8.
498. Kruglyak L, Daly MJ, Reeve-Daly MP, Lander ES. Parametric and nonparametric linkage analysis: a unified multipoint approach. Am J Hum Genet 1996;58:1347-63.
499. Lander E, Kruglyak L. Genetic dissection of complex traits: guidelines for interpreting and reporting linkage results. Nat Genet 1995;11:241-7.
500. Morton NE. Significance levels in complex inheritance. Am J Hum Genet 1998;62:690-7.
501. Gabriel SB, Schaffner SF, Nguyen H, et al. The structure of haplotype blocks in the human genome. Science 2002;296:2225-9.
502. Cavalli-Sforza LL. The DNA revolution in population genetics. Trends Genet 1998;14:60-5.
503. Daly MJ, Rioux JD, Schaffner SF, Hudson TJ, Lander ES. High-resolution haplotype structure in the human genome. NatGenet 2001;29:229-32.
504. Reich DE, Schaffner SF, Daly MJ, et al. Human genome sequence variation and the influence of gene history, mutation and recombination. Nat Genet 2002;32:135-42.
505. Kruglyak L. Prospects for whole-genome linkage disequilibrium mapping of common disease genes. Nat Genet 1999;22:139-44.
506. Heutink P, Oostra BA. Gene finding in genetically isolated populations. Hum Mol Genet 2002;11:2507-15.
507. Mattei MG, Borg JP, Rosnet O, Marme D, Birnbaum D. Assignment of vascular endothelial growth factor (VEGF) and placenta growth factor (PLGF)

genes to human chromosome 6p12-p21 and 14q24-q31 regions, respectively. Genomics 1996;32:168-9.
508. Brogan IJ, Khan N, Isaac K, Hutchinson JA, Pravica V, Hutchinson IV. Novel polymorphisms in the promoter and 5' UTR regions of the human vascular endothelial growth factor gene. HumImmunol 1999;60:1245-9.
509. Renner W, Kotschan S, Hoffmann C, Obermayer-Pietsch B, Pilger E. A common 936 C/T mutation in the gene for vascular endothelial growth factor is associated with vascular endothelial growth factor plasma levels. JVascRes 2000;37:443-8.
510. Goldstein DB. Islands of linkage disequilibrium. NatGenet 2001;29:109-11.
511. Awata T, Inoue K, Kurihara S, et al. A common polymorphism in the 5'-untranslated region of the VEGF gene is associated with diabetic retinopathy in type 2 diabetes. Diabetes 2002;51:1635-9.
512. Summers AM, Coupes BM, Brennan MF, Ralph SA, Short CD, Brenchley PE. VEGF -460 genotype plays an important role in progression to chronic kidney disease stage 5. NephrolDialTransplant 2005;20:2427-32.
513. Brusselmans K, Bono F, Maxwell P, et al. Hypoxia-inducible factor-2alpha (HIF-2alpha) is involved in the apoptotic response to hypoglycemia but not to hypoxia. JBiolChem 2001;276:39192-6.
514. Pugh CW, Ratcliffe PJ. Regulation of angiogenesis by hypoxia: role of the HIF system. NatMed 2003;9:677-84.
515. Soh EY, Sobhi SA, Wong MG, et al. Thyroid-stimulating hormone promotes the secretion of vascular endothelial growth factor in thyroid cancer cell lines. Surgery 1996;120:944-7.
516. Shifren JL, Mesiano S, Taylor RN, Ferrara N, Jaffe RB. Corticotropin regulates vascular endothelial growth factor expression in human fetal adrenal cortical cells. JClinEndocrinolMetab 1998;83:1342-7.
517. Christenson LK, Stouffer RL. Follicle-stimulating hormone and luteinizing hormone/chorionic gonadotropin stimulation of vascular endothelial growth factor production by macaque granulosa cells from pre- and periovulatory follicles. JClinEndocrinolMetab 1997;82:2135-42.
518. Mueller MD, Vigne JL, Minchenko A, Lebovic DI, Leitman DC, Taylor RN. Regulation of vascular endothelial growth factor (VEGF) gene transcription by estrogen receptors alpha and beta. ProcNatlAcadSciUSA 2000;97:10972-7.
519. Mueller MD, Vigne JL, Pritts EA, Chao V, Dreher E, Taylor RN. Progestins activate vascular endothelial growth factor gene transcription in endometrial adenocarcinoma cells. FertilSteril 2003;79:386-92.
520. Xie K, Wei D, Shi Q, Huang S. Constitutive and inducible expression and regulation of vascular endothelial growth factor. Cytokine Growth Factor Rev 2004;15:297-324.
521. Reisinger K, Kaufmann R, Gille J. Increased Sp1 phosphorylation as a mechanism of hepatocyte growth factor (HGF/SF)-induced vascular endothelial growth factor (VEGF/VPF) transcription. J Cell Sci 2003;116:225-38.

522. Ryuto M, Ono M, Izumi H, et al. Induction of vascular endothelial growth factor by tumor necrosis factor alpha in human glioma cells. Possible roles of SP-1. JBiolChem 1996;271:28220-8.
523. Buettner R, Mora LB, Jove R. Activated STAT signaling in human tumors provides novel molecular targets for therapeutic intervention. Clin Cancer Res 2002;8:945-54.
524. Renner W, Kotschan S, Hoffmann C, Obermayer-Pietsch B, Pilger E. A common 936 C/T mutation in the gene for vascular endothelial growth factor is associated with vascular endothelial growth factor plasma levels. J Vasc Res 2000;37:443-8.
525. Shahbazi M, Fryer AA, Pravica V, et al. Vascular endothelial growth factor gene polymorphisms are associated with acute renal allograft rejection. JAmSocNephrol 2002;13:260-4.
526. McCarron SL, Edwards S, Evans PR, et al. Influence of cytokine gene polymorphisms on the development of prostate cancer. Cancer Res 2002;62:3369-72.
527. Howell WM, Bateman AC, Turner SJ, Collins A, Theaker JM. Influence of vascular endothelial growth factor single nucleotide polymorphisms on tumour development in cutaneous malignant melanoma. Genes Immun 2002;3:229-32.
528. Stevens A, Soden J, Brenchley PE, Ralph S, Ray DW. Haplotype analysis of the polymorphic human vascular endothelial growth factor gene promoter. Cancer Res 2003;63:812-6.
529. Chen WC, Chen HY, Wu HC, Wu MC, Hsu CD, Tsai FJ. Vascular endothelial growth factor gene polymorphism is associated with calcium oxalate stone disease. UrolRes 2003;31:218-22.
530. Yang B, Cross DF, Ollerenshaw M, Millward BA, Demaine AG. Polymorphisms of the vascular endothelial growth factor and susceptibility to diabetic microvascular complications in patients with type 1 diabetes mellitus. JDiabetes Complications 2003;17:1-6.
531. Lin CC, Wu HC, Tsai FJ, Chen HY, Chen WC. Vascular endothelial growth factor gene-460 C/T polymorphism is a biomarker for prostate cancer. Urology 2003;62:374-7.
532. Ray D, Mishra M, Ralph S, Read I, Davies R, Brenchley P. Association of the VEGF gene with proliferative diabetic retinopathy but not proteinuria in diabetes. Diabetes 2004;53:861-4.
533. Hsieh YY, Chang CC, Tsai FJ, Yeh LS, Lin CC, Peng CT. T allele for VEGF gene-460 polymorphism at the 5'-untranslated region: association with a higher susceptibility to endometriosis. JReprodMed 2004;49:468-72.
534. Boiardi L, Casali B, Nicoli D, et al. Vascular endothelial growth factor gene polymorphisms in giant cell arteritis. JRheumatol 2003;30:2160-4.
535. Cooke RW, Drury JA, Mountford R, Clark D. Genetic polymorphisms and retinopathy of prematurity. Invest OphthalmolVisSci 2004;45:1712-5.
536. Papazoglou D, Galazios G, Koukourakis MI, et al. Vascular endothelial growth factor gene polymorphisms and pre-eclampsia. MolHumReprod 2004;10:321-4.
537. Papazoglou D, Galazios G, Koukourakis MI, Kontomanolis EN, Maltezos E. Association of -634G/C and 936C/T polymorphisms of the vascular

endothelial growth factor with spontaneous preterm delivery. Acta ObstetGynecolScand 2004;83:461-5.
538. Salvarani C, Boiardi L, Casali B, et al. Vascular endothelial growth factor gene polymorphisms in Behcet's disease. JRheumatol 2004;31:1785-9.
539. Szeto CC, Chow KM, Poon P, Szeto CY, Wong TY, Li PK. Genetic polymorphism of VEGF: Impact on longitudinal change of peritoneal transport and survival of peritoneal dialysis patients. Kidney Int 2004;65:1947-55.
540. Kariyazono H, Ohno T, Khajoee V, et al. Association of vascular endothelial growth factor (VEGF) and VEGF receptor gene polymorphisms with coronary artery lesions of Kawasaki disease. PediatrRes 2004;56:953-9.
541. Koukourakis MI, Papazoglou D, Giatromanolaki A, Bougioukas G, Maltezos E, Siviridis E. VEGF gene sequence variation defines VEGF gene expression status and angiogenic activity in non-small cell lung cancer. Lung Cancer 2004;46:293-8.
542. Wolf G, Aigner RM, Schaffler G, et al. The 936C>T polymorphism of the gene for vascular endothelial growth factor is associated with 18F-fluorodeoxyglucose uptake. Breast Cancer ResTreat 2004;88:205-8.
543. Lu H, Shu XO, Cui Y, et al. Association of genetic polymorphisms in the VEGF gene with breast cancer survival. Cancer Res 2005;65:5015-9.
544. Jin Q, Hemminki K, Enquist K, et al. Vascular endothelial growth factor polymorphisms in relation to breast cancer development and prognosis. Clin Cancer Res 2005;11:3647-53.
545. Kim EJ, Jeong P, Quan C, et al. Genotypes of TNF-alpha, VEGF, hOGG1, GSTM1, and GSTT1: useful determinants for clinical outcome of bladder cancer. Urology 2005;65:70-5.
546. Medford AR, Keen LJ, Bidwell JL, Millar AB. Vascular endothelial growth factor gene polymorphism and acute respiratory distress syndrome. Thorax 2005;60:244-8.
547. Bhanoori M, Arvind Babu K, Pavankumar Reddy NG, et al. The vascular endothelial growth factor (VEGF) +405G>C 5'-untranslated region polymorphism and increased risk of endometriosis in South Indian women: a case control study. Hum Reprod 2005;20:1844-9.
548. Tzanakis N, Gazouli M, Rallis G, et al. Vascular endothelial growth factor polymorphisms in gastric cancer development, prognosis, and survival. J Surg Oncol 2006;94:624-30.
549. Rueda B, Perez-Armengol C, Lopez-Lopez S, Garcia-Porrua C, Martin J, Gonzalez-Gay MA. Association between functional haplotypes of vascular endothelial growth factor and renal complications in Henoch-Schonlein purpura. J Rheumatol 2006;33:69-73.
550. Sfar S, Hassen E, Saad H, Mosbah F, Chouchane L. Association of VEGF genetic polymorphisms with prostate carcinoma risk and clinical outcome. Cytokine 2006;35:21-8.
551. Hefler LA, Mustea A, Konsgen D, et al. Vascular endothelial growth factor gene polymorphisms are associated with prognosis in ovarian cancer. Clin Cancer Res 2007;13:898-901.

552. Kim JG, Sohn SK, Chae YS, et al. Vascular endothelial growth factor gene polymorphisms associated with prognosis for patients with gastric cancer. Ann Oncol 2007;18:1030-6.
553. Kong SY, Park JW, Lee JA, et al. Association between vascular endothelial growth factor gene polymorphisms and survival in hepatocellular carcinoma patients. Hepatology 2007;46:446-55.
554. Langsenlehner U, Wolf G, Langsenlehner T, et al. Genetic polymorphisms in the vascular endothelial growth factor gene and breast cancer risk. The Austrian "tumor of breast tissue: incidence, genetics, and environmental risk factors" study. Breast Cancer Res Treat 2008;109:297-304.
555. Kim DH, Lee NY, Lee MH, Sohn SK, Do YR, Park JY. Vascular endothelial growth factor (VEGF) gene (VEGFA) polymorphism can predict the prognosis in acute myeloid leukaemia patients. Br J Haematol 2008;140:71-9.
556. Kim JG, Chae YS, Sohn SK, et al. Vascular endothelial growth factor gene polymorphisms associated with prognosis for patients with colorectal cancer. Clin Cancer Res 2008;14:62-6.
557. Lurje G, Hendifar AE, Schultheis AM, et al. Polymorphisms in interleukin 1 beta and interleukin 1 receptor antagonist associated with tumor recurrence in stage II colon cancer. Pharmacogenet Genomics 2009;19:95-102.
558. Maltese P, Canestrari E, Ruzzo A, et al. VEGF gene polymorphisms and susceptibility to colorectal cancer disease in Italian population. Int J Colorectal Dis 2009;24:165-70.
559. Masago K, Fujita S, Kim YH, et al. Effect of vascular endothelial growth factor polymorphisms on survival in advanced-stage non-small-cell lung cancer. Cancer Sci 2009;100:1917-22.
560. Pastuszczak M, Branicka A, Jakiela B, et al. The +405 GG variant of vascular endothelial growth factor polymorphism is associated with poor prognosis in patients undergoing coronary artery bypass graft surgery. Pol Arch Med Wewn 2009;119:719-25.
561. Smerdel MP, Waldstrom M, Brandslund I, Steffensen KD, Andersen RF, Jakobsen A. Prognostic importance of vascular endothelial growth factor-A expression and vascular endothelial growth factor polymorphisms in epithelial ovarian cancer. Int J Gynecol Cancer 2009;19:578-84.
562. Lambrechts D, Poesen K, Fernandez-Santiago R, et al. Meta-analysis of vascular endothelial growth factor variations in amyotrophic lateral sclerosis: increased susceptibility in male carriers of the -2578AA genotype. J Med Genet 2009;46:840-6.
563. Bradbury PA, Zhai R, Ma C, et al. Vascular endothelial growth factor polymorphisms and esophageal cancer prognosis. Clin Cancer Res 2009;15:4680-5.
564. Dassoulas K, Gazouli M, Rizos S, et al. Common polymorphisms in the vascular endothelial growth factor gene and colorectal cancer development, prognosis, and survival. Mol Carcinog 2009;48:563-9.
565. Steffensen KD, Waldstrom M, Brandslund I, Jakobsen A. The relationship of VEGF polymorphisms with serum VEGF levels and

progression-free survival in patients with epithelial ovarian cancer. Gynecol Oncol 2010;117:109-16.
566. Hansen TF, Garm Spindler KL, Andersen RF, Lindebjerg J, Brandslund I, Jakobsen A. The predictive value of genetic variations in the vascular endothelial growth factor A gene in metastatic colorectal cancer. Pharmacogenomics J.
567. Kim DH, Xu W, Kamel-Reid S, et al. Clinical relevance of vascular endothelial growth factor (VEGFA) and VEGF receptor (VEGFR2) gene polymorphism on the treatment outcome following imatinib therapy. Ann Oncol 2010;21:1179-88.
568. Hansen TF, Spindler KL, Lorentzen KA, et al. The importance of -460 C/T and +405 G/C single nucleotide polymorphisms to the function of vascular endothelial growth factor A in colorectal cancer. J Cancer Res Clin Oncol 2010;136:751-8.
569. Ruiz MT, Biselli PM, Maniglia JV, Pavarino-Bertelli EC, Goloni-Bertollo EM. Genetic variability of vascular endothelial growth factor and prognosis of head and neck cancer in a Brazilian population. Braz J Med Biol Res 2010;43:127-33.
570. Lorenzen S, Panzram B, Keller G, et al. Association of the VEGF 936C>T Polymorphism with FDG Uptake, Clinical, Histopathological, and Metabolic Response in Patients with Adenocarcinomas of the Esophagogastric Junction. Mol Imaging Biol 2010.
571. Webb NJ, Bottomley MJ, Watson CJ, Brenchley PE. Vascular endothelial growth factor (VEGF) is released from platelets during blood clotting: implications for measurement of circulating VEGF levels in clinical disease. ClinSci(Lond) 1998;94:395-404.
572. Huez I, Creancier L, Audigier S, Gensac MC, Prats AC, Prats H. Two independent internal ribosome entry sites are involved in translation initiation of vascular endothelial growth factor mRNA. MolCell Biol 1998;18:6178-90.
573. Huez I, Bornes S, Bresson D, Creancier L, Prats H. New vascular endothelial growth factor isoform generated by internal ribosome entry site-driven CUG translation initiation. MolEndocrinol 2001;15:2197-210.
574. Yu ZY, Wrange O, Haglund B, Granholm L, Gustafsson JA. Estrogen and progestin receptors in intracranial meningiomas. J Steroid Biochem 1982;16:451-6.
575. Blaauw G, Koudstaal J, Blankenstein MA, Debets-Te Baerts M, Gijzen AH. Progestin receptors in meningiomas. Comparison of cytosolic assays with immunocytochemical identification in cryostat and paraffin sections. Acta Neurochir (Wien) 1995;134:83-7.
576. Olson JJ, Beck DW, Schlechte JA, Loh PM. Effect of the antiprogesterone RU-38486 on meningioma implanted into nude mice. J Neurosurg 1987;66:584-7.
577. Davis C. Surgical and non-surgical treatment of symptomatic intracranial meningiomas. Br J Neurosurg 1995;9:295-302.
578. Goodwin JW, Crowley J, Eyre HJ, Stafford B, Jaeckle KA, Townsend JJ. A phase II evaluation of tamoxifen in unresectable or refractory meningiomas: a Southwest Oncology Group study. J Neurooncol 1993;15:75-7.

579. Grunberg SM. Role of antiprogestational therapy for meningiomas. Hum Reprod 1994;9 Suppl 1:202-7.
580. Grunberg SM, Daniels AM, Muensch H, et al. Correlation of meningioma hormone receptor status with hormone sensitivity in a tumor stem-cell assay. J Neurosurg 1987;66:405-8.
581. Grunberg SM, Weiss MH, Russell CA, et al. Long-term administration of mifepristone (RU486): clinical tolerance during extended treatment of meningioma. Cancer Invest 2006;24:727-33.
582. Qiu M, Lange CA. MAP kinases couple multiple functions of human progesterone receptors: degradation, transcriptional synergy, and nuclear association. J Steroid Biochem Mol Biol 2003;85:147-57.
583. Qiu M, Olsen A, Faivre E, Horwitz KB, Lange CA. Mitogen-activated protein kinase regulates nuclear association of human progesterone receptors. Mol Endocrinol 2003;17:628-42.
584. Rodriguez D, Cheung MC, Housri N, Quinones-Hinojosa A, Camphausen K, Koniaris LG. Outcomes of malignant CNS ependymomas: an examination of 2408 cases through the Surveillance, Epidemiology, and End Results (SEER) database (1973-2005). J Surg Res 2009;156:340-51.
585. Godard S, Getz G, Delorenzi M, et al. Classification of human astrocytic gliomas on the basis of gene expression: a correlated group of genes with angiogenic activity emerges as a strong predictor of subtypes. Cancer Res 2003;63:6613-25.
586. Karcher S, Steiner HH, Ahmadi R, et al. Different angiogenic phenotypes in primary and secondary glioblastomas. Int J Cancer 2006;118:2182-9.
587. Sonoda Y, Kanamori M, Deen DF, Cheng SY, Berger MS, Pieper RO. Overexpression of vascular endothelial growth factor isoforms drives oxygenation and growth but not progression to glioblastoma multiforme in a human model of gliomagenesis. Cancer Res 2003;63:1962-8.
588. Lamszus K, Ulbricht U, Matschke J, Brockmann MA, Fillbrandt R, Westphal M. Levels of soluble vascular endothelial growth factor (VEGF) receptor 1 in astrocytic tumors and its relation to malignancy, vascularity, and VEGF-A. Clin Cancer Res 2003;9:1399-405.
589. Xu B, Feng NH, Tong N, et al. VEGF -460C>T polymorphism and cancer risk: a meta-analysis. Med Oncol 2009.
590. Zhai R, Liu G, Asomaning K, et al. Genetic polymorphisms of VEGF, interactions with cigarette smoking exposure and esophageal adenocarcinoma risk. Carcinogenesis 2008;29:2330-4.
591. Bhowmick DA, Zhuang Z, Wait SD, Weil RJ. A functional polymorphism in the EGF gene is found with increased frequency in glioblastoma multiforme patients and is associated with more aggressive disease. Cancer Res 2004;64:1220-3.
592. Hansen TF, Sorensen FB, Spindler KL, et al. Microvessel density and the association with single nucleotide polymorphisms of the vascular endothelial growth factor receptor 2 in patients with colorectal cancer. Virchows Arch 2010;456:251-60.

593. Young HS, Summers AM, Read IR, et al. Interaction between genetic control of vascular endothelial growth factor production and retinoid responsiveness in psoriasis. J Invest Dermatol 2006;126:453-9.
594. Schneider BP, Wang M, Radovich M, et al. Association of vascular endothelial growth factor and vascular endothelial growth factor receptor-2 genetic polymorphisms with outcome in a trial of paclitaxel compared with paclitaxel plus bevacizumab in advanced breast cancer: ECOG 2100. J Clin Oncol 2008;26:4672-8.
595. Schneider BP, Radovich M, Miller KD. The role of vascular endothelial growth factor genetic variability in cancer. Clin Cancer Res 2009;15:5297-302.
596. Murukesh N, Dive C, Jayson GC. Biomarkers of angiogenesis and their role in the development of VEGF inhibitors. Br J Cancer 2010;102:8-18.
597. Burstein HJ, Chen YH, Parker LM, et al. VEGF as a marker for outcome among advanced breast cancer patients receiving anti-VEGF therapy with bevacizumab and vinorelbine chemotherapy. Clin Cancer Res 2008;14:7871-7.

Appendix I – Meningioma receptor study data table

Number	Date of Birth	Age at Presentation	Sex	Histology Category	PRn Negative	PRc Negative	ERn Negative	ERc Negative	Date of Operation	Simpson Grade	Recurrence	Date Recurrence	Died	Final Follow Up
1	06/08/1927	68	F	Transitional	No	No	Yes	No	28/06/1996	1	No	14/04/2010	No	14/04/2010
2	24/01/1940	52	F	Transitional	No	No	Yes	No	01/01/1992	4	No	21/03/2001	No	21/03/2001
3	16/12/1953	42	M	Transitional	Yes	No	Yes	Yes	04/10/1996	4	No	11/02/2004	No	11/02/2004
4	31/07/1911	82	M	Microcystic	Yes	No	Yes	No	26/03/1993	4	No	01/06/2002	No	01/06/2002
5	27/08/1943	47	F	Meningothelial	Yes	Yes	Yes	No	07/03/1991	1	No	01/06/2002	No	01/06/2002
6	24/07/1929	65	F	Transitional	No	No	Yes	Yes	16/06/1995	4	No	02/05/2007	No	02/05/2007
7	07/11/1923	70	F	Transitional	Yes	No	Yes	No	01/01/1994		No	01/06/2002	No	01/06/2002
8	13/03/1959	31	M	Transitional	Yes	No	Yes	No	22/11/1990	1	No	21/02/1997	No	21/02/1997
9	21/03/1921	73	F	Angiomatous	No	No	Yes	No	24/03/1995	1	Yes	04/04/1996	No	19/04/2006
10	21/04/1947	49	M	Transitional	No	No	Yes	No	03/10/1996	4	No	23/07/2004	No	23/07/2004
11	15/01/1965	32	F	Microcystic	No	No	Yes	No	14/02/1997	2	No	14/04/2010	No	14/04/2010
12	15/05/1937	53	F	Transitional	No	No	Yes	No	28/03/1991	4	Yes	28/08/1996	Yes	14/03/1999
13	27/09/1926	65	F	Meningothelial	No	No	Yes	No	10/09/1992	4	No	09/06/2000	No	01/06/2002
14	09/01/1924	63	M	Anaplastic	Yes	No	Yes	No	15/05/1987	4	Yes	23/02/1996	No	22/10/2003
15	12/09/1931	62	F	Transitional	No	No	Yes	No	25/07/1994	4	No	31/03/1999	No	31/03/1999
16	11/02/1934	61	F	Fibroblastic	Yes	No	Yes	No	18/05/1995	2	Yes	10/02/2000	No	25/01/2006
17	04/09/1924	67	M	Transitional	No	No	Yes	No	16/04/1992	3	No	04/12/1996	No	04/12/1996
18	18/08/1908	87	F	Psammomatous	Yes	No	Yes	No	01/01/1995	4	No	24/04/1996	No	24/04/1996
19	02/04/1941	55	F	Transitional	Yes	Yes	Yes	No	19/07/1996	1	No	01/06/2002	No	01/06/2002
20	19/04/1919	77	M	Transitional	Yes	No	Yes	Yes	26/11/1996	2	No	07/10/1998	No	07/10/1998
21	17/03/1923	71	F	Atypical	Yes	Yes	Yes	Yes	18/04/1994	4	Yes	28/07/1995	Yes	14/09/1995
22	09/01/1941	51	F	Psammomatous	Yes	No	Yes	Yes	20/08/1992	4	No	31/03/1999	No	31/03/1999
23	12/04/1938	56	F	Psammomatous	Yes	Yes	Yes	Yes	29/11/1994	2	No	01/06/2002	No	01/06/2002
24	02/11/1941	51	F	Meningothelial	No	No	Yes	Yes	21/01/1993	5	No	09/03/2010	No	09/03/2010
25	25/08/1912	79	M	Fibroblastic	Yes	No	Yes	No	01/01/1992		No	25/08/1995	Yes	25/08/1995
26	15/07/1925	70	F	Metaplastic	No	No	No	No	23/11/1995	2	No	01/06/2002	No	01/06/2002
27	19/05/1927	64	F	Transitional	No	No	Yes	No	04/07/1991	2	No	18/06/1997	No	18/06/1997
28	21/08/1944	49	F	Transitional	No	No	Yes	No	28/06/1994	3	No	07/12/2001	No	07/12/2001
29	30/11/1936	59	F	Transitional	No	No	Yes	No	12/09/1996	4	No	30/11/2006	Yes	30/11/2006
30	25/04/1952	44	F	Atypical	Yes	No	Yes	Yes	11/10/1996	1	No	15/11/2006	No	15/11/2006
31	03/03/1936	54	F	Meningothelial	No	No	Yes	Yes	23/10/1990	4	No	01/06/2002	No	01/06/2002
32	05/10/1926	67	F	Atypical	Yes	No	Yes	Yes	28/07/1994	2	No	01/06/2002	No	01/06/2002
33	09/02/1962	28	F	Transitional	No	No	Yes	No	28/09/1990	1	No	10/01/1997	No	10/01/1997
34	13/03/1920	71	M	Atypical	Yes	Yes	Yes	Yes	28/11/1991	4	No	01/06/2002	No	01/06/2002
35	09/10/1924	71	M	Transitional	No	No	Yes	No	25/01/1996	4	No	01/06/2002	No	01/06/2002
36	12/10/1913	76	F	Atypical	Yes	Yes	Yes	Yes	21/12/1988	4	Yes	21/05/1990	Yes	11/07/1990
37	16/04/1947	49	F	Meningothelial	Yes	Yes	Yes	Yes	06/03/1997	1	No	12/12/2001	No	12/12/2001
38	10/04/1930	64	F	Transitional	No	No	Yes	No	06/10/1994	5	No	22/10/1994	Yes	22/10/1994

Number	Date of Birth	Age at Presentation	Sex	Histology Category	PRn Negative	PRc Negative	ERn Negative	ERc Negative	Date of Operation	Simpson Grade	Recurrence	Date Recurrence	Died	Final Follow Up
39	30/11/1937	54	F	Transitional	Yes	No	Yes	No	28/02/1992	2	No	03/12/1997	No	03/12/1997
40	28/05/1921	72	M	Atypical	Yes	No	Yes	No	29/11/1993	4	No	01/10/1996	Yes	01/10/1996
41	07/02/1933	58	F	Transitional	No	No	Yes	Yes	17/07/1991	3	No	01/06/2002	No	01/06/2002
42	09/09/1942	52	F	Transitional	Yes	No	Yes	No	02/02/1995	2	No	09/09/2009	Yes	09/09/2009
43	11/01/1940	56	F	Transitional	No	No	Yes	Yes	16/02/1996	3	No	25/10/1996	Yes	25/10/1996
44	28/04/1915	77	F	Meningothelial	No	No	Yes	No	19/01/1993	1	No	01/06/2002	No	01/06/2002
45	26/02/1927	67	F	Meningothelial	Yes	No	Yes	Yes	02/08/1994	4	No	01/06/2002	No	01/06/2002
46	12/06/1918	76	F	Psammomatous	Yes	Yes	Yes	Yes	29/09/1994	1	No	15/02/1995	No	15/02/1995
47	22/05/1935	60	M	Transitional	Yes	No	Yes	No	02/11/1995	4	No	07/07/1997	Yes	07/07/1997
48	05/10/1937	56	F	Transitional	No	No	Yes	Yes	24/02/1994	3	No	12/04/1995	No	12/04/1995
49	22/04/1936	59	F	Psammomatous	No	No	Yes	No	04/08/1995	2	No	11/11/1998	No	11/11/1998
50	19/11/1939	51	M	Benign	Yes	No	Yes	No	01/01/1991	1	No	01/06/2002	No	01/06/2002
51	27/08/1920	73	F	Transitional	Yes	No	Yes	Yes	24/06/1994	4	No	17/09/1997	Yes	17/09/1997
52	28/02/1947	48	F	Transitional	No	No	Yes	Yes	15/12/1995	3	No	01/06/2002	No	01/06/2002
53	21/06/1904	83	F	Transitional	No	No	Yes	Yes	02/12/1987	2	No	01/06/2002	No	01/06/2002
54	31/07/1922	65	F	Transitional	Yes	No	Yes	Yes	09/12/1987	2	No	01/06/2002	No	01/06/2002
55	29/11/1923	64	F	Transitional	No	No	Yes	Yes	29/07/1988	2	No	01/06/2002	No	01/06/2002
56	09/10/1945	43	F	Benign	Yes	No	Yes	Yes	16/09/1988	2	No	01/06/2002	No	01/06/2002
57	09/05/1944	45	M	Atypical	Yes	No	Yes	No	20/10/1989	1	No	01/06/2002	No	01/06/2002
58	09/01/1932	57	F	Transitional	No	No	Yes	No	06/04/1989	1	No	03/06/1998	No	03/06/1998
59	24/02/1920	69	M	Transitional	No	No	Yes	No	21/12/1989	2	No	01/06/2002	No	01/06/2002
60	06/07/1938	50	M	Transitional	No	No	Yes	No	25/01/1989	1	No	01/06/2002	No	01/06/2002
61	03/08/1921	75	F	Transitional	No	No	Yes	Yes	20/05/1997	2	No	01/06/2002	No	01/06/2002
62	31/03/1957	40	M	Transitional	No	No	Yes	Yes	20/05/1997	1	No	19/04/1999	No	19/04/1999
63	23/09/1958	39	F	Transitional	No	No	Yes	Yes	06/11/1997	1	No	23/12/1998	No	23/12/1998
64	30/08/1917	70	F	Transitional	Yes	Yes	Yes	Yes	04/08/1987	2	No	30/04/1997	No	30/04/1997
65	28/12/1926	61	M	Transitional	No	No	Yes	No	10/03/1988	1	No	01/06/2002	No	01/06/2002
66	10/06/1923	64	F	Transitional	No	No	Yes	No	28/01/1988	1	No	01/06/2002	No	01/06/2002
67	06/03/1931	66	M	Psammomatous	Yes	No	Yes	No	17/10/1997	1	No	01/06/2002	No	01/06/2002
68	18/10/1918	79	M	Transitional	No	No	Yes	No	05/03/1998	2	No	17/03/1998	Yes	17/03/1998
69	22/08/1910	87	F	Transitional	No	No	Yes	Yes	07/10/1997	2	No	07/12/1997	Yes	07/12/1997
70	28/06/1943	54	M	Transitional	No	No	Yes	No	18/09/1997	1	No	26/11/1997	Yes	26/11/1997
71	29/01/1913	76	F	Transitional	No	No	Yes	No	20/07/1989	1	No	29/01/2005	Yes	29/01/2005
72	04/08/1926	62	F	Transitional	No	No	Yes	Yes	01/07/1988	2	No	01/06/2002	No	01/06/2002
73	07/09/1928	59	F	Transitional	No	Yes	Yes	Yes	11/12/1987	2	No	01/06/2002	No	01/06/2002
74	01/01/1962	35	F	Fibroblastic	No	No	Yes	Yes	25/11/1997	2	No	01/02/1998	Yes	01/02/1998
75	11/05/1925	72	F	Meningothelial	Yes	Yes	Yes	Yes	15/04/1997	2	No	11/05/2008	Yes	11/05/2008
76	29/09/1940	55	M	Transitional	No	No	Yes	No	26/07/1996	1	No	29/07/1998	No	29/07/1998
77	05/12/1922	74	F	Transitional	No	No	Yes	Yes	24/01/1997	2	No	14/12/2005	No	14/12/2005
78	24/08/1923	70	F	Psammomatous	No	No	Yes	Yes	01/01/1994	2	No	03/12/2001	Yes	03/12/2001
79	03/09/1933	59	M	Anaplastic	Yes	No	Yes	No	01/04/1993		No	18/08/1993	Yes	18/08/1993
80	02/12/1939	49	F	Transitional	No	No	Yes	Yes	14/11/1990	2	No	01/06/2002	No	01/06/2002

Number	Date of Birth	Age at Presentation	Sex	Histology Category	PRn Negative	PRc Negative	ERn Negative	ERc Negative	Date of Operation	Simpson Grade	Recurrence	Date Recurrence	Died	Final Follow Up
81	06/03/1926	71	F	Atypical	Yes	No	Yes	No	01/03/1997	3	Yes	01/11/2000	No	04/04/2001
82	30/01/1926	67	M	Atypical	Yes	Yes	Yes	Yes	01/01/1993		No	05/01/1996	Yes	05/01/1996
83	24/05/1910	82	F	Benign	No	No	Yes	No	01/01/1993		No	01/06/2002	No	01/06/2002
84	30/08/1908	83	F	Meningothelial	Yes	No	Yes	No	01/01/1992		No	17/02/1993	No	17/02/1993
85	29/01/1932	58	M	Transitional	Yes	No	Yes	No	06/02/1992	1	No	01/06/2002	No	01/06/2002
86	24/09/1944	48	F	Transitional	No	No	Yes	No	04/02/1993	2	No	26/03/2003	No	26/03/2003
87	08/01/1954	43	M	Atypical	Yes	No	Yes	Yes	13/05/1997	1	No	18/11/2004	No	18/11/2004
88	12/03/1932	60	F	Meningothelial	Yes	No	Yes	Yes	18/03/1997	4	Yes	07/06/1999	No	06/02/2003
89	20/06/1915	81	F	Benign	No	No	Yes	Yes	22/04/1997	4	No	21/07/1999	No	21/07/1999
90	21/11/1935	61	M	Atypical	Yes	No	Yes	Yes	15/07/1997	1	No	12/03/1999	No	12/03/1999
91	21/02/1922	75	F	Fibroblastic	Yes	No	Yes	Yes	24/12/1997	4	No	14/04/2005	No	14/04/2005
92	28/07/1958	40	F	Microcystic	No	No	Yes	Yes	01/01/1989		No	01/06/2002	No	01/06/2002
93	30/04/1923	66	F	Transitional	No	No	Yes	No	01/01/1989		No	09/10/1996	No	09/10/1996
94	29/01/1913	76	F	Transitional	No	No	Yes	Yes	01/01/1989		No	01/06/2002	No	01/06/2002
95	15/10/1936	51	F	Benign	No	No	Yes	Yes	01/01/1988		No	21/09/1998	No	21/09/1998
96	12/11/1913	75	F	Transitional	No	No	Yes	Yes	05/08/1988	1	No	21/01/1999	No	21/01/1999
97	13/12/1940	47	F	Transitional	Yes	No	Yes	No	01/01/1988		No	01/06/2002	No	01/06/2002
98	09/02/1920	68	F	Benign	Yes	No	Yes	Yes	01/01/1988		No	09/02/1989	Yes	09/02/1989
99	08/08/1936	62	F	Fibroblastic	No	No	Yes	Yes	30/04/1998	4	No	19/08/2009	No	19/08/2009
100	28/10/1964	34	M	Transitional	No	No	Yes	No	24/04/1998	1	No	23/01/2002	No	23/01/2002
101	13/12/1928	69	F	Anaplastic	Yes	No	Yes	No	01/01/1998	4	Yes	20/04/1999	No	02/02/2000
102	07/06/1969	27	F	Psammomatous	No	No	Yes	No	19/03/1998	1	No	28/07/1999	No	28/07/1999

Appendix II – Choroid plexus carcinoma data table

ID	Gender	DOB	Age Months	Group	GTR	Follow Up Date	Survival Years	Died	Op Date	Op Age	Op Weight	% Debulking	Blood Vol	Blood Loss
1	Female	10/04/1986	23	2	1	23/03/1990	1.97	Yes	12/04/1988	2	13	33	1040	354.08
1	Female	10/04/1986	23	2	1	23/03/1990	1.97	Yes	16/08/1988	2	16	100	1280	125.6
1	Female	10/04/1986	23	2	1	23/03/1990	1.97	Yes	31/10/1989	3	18	100	1440	150.76
2	Female	22/01/1974	154	1	1	09/07/1993	6.58	No	13/12/1986	12	49	95	3920	680.68
2	Female	22/01/1974	154	1	1	09/07/1993	6.58	No	06/07/1993	19	50	100	4000	750
3	Male	12/07/1988	28	2	0	24/04/1992	1.45	Yes	21/11/1990	2	12	5	960	356.04
3	Male	12/07/1988	28	2	0	24/04/1992	1.45	Yes	08/05/1991	2	13	95	1040	217.92
4	Male	09/09/1988	0	2	1	16/07/2004	15.79	No	14/10/1988	0	4	20	320	286.56
4	Male	09/09/1988	0	2	1	16/07/2004	15.79	No	20/12/1988	0	5	100	400	54
5	Female	31/12/1992	6	1	1	24/07/1993	0.00	Yes	24/07/1994	0	7	100	560	1500
6	Male	24/04/1993	0	1	0	20/12/1993	0.59	Yes	25/05/1993	0	2.7	95	216	330.424
7	Male	20/07/1984	8	1	0	08/07/1985	0.22	Yes	22/04/1985	0	8	80	640	168.64
8	Male	07/11/1984	19	1	0	17/06/1987	0.99	Yes	03/07/1986	1	10.33	0	826.4	2848.965
9	Female	24/11/1993	63	3	1	28/04/2004	5.15	No	08/03/1999	5	17	5	1360	247.6
9	Female	24/11/1993	63	3	1	28/04/2004	5.15	No	17/06/1999	5	22.6	100	1808	244.576
10	Female	15/11/1988	7	2	1	13/07/1994	5.05	Yes	02/07/1989	0	8.1	55	648	541.576
10	Female	15/11/1988	7	2	1	13/07/1994	5.05	Yes	09/11/1989	0	9	95	720	215.44
10	Female	15/11/1988	7	2	1	13/07/1994	5.05	Yes	02/11/1990	1	10.1	100	808	79.992
10	Female	15/11/1988	7	2	1	13/07/1994	5.05	Yes	30/01/1991	2	10.2	100	816	86.496
11	Female	06/05/1995	4	2	1	31/08/2004	8.92	No	04/10/1995	0	7	50	560	223.44
11	Female	06/05/1995	4	2	1	31/08/2004	8.92	No	07/02/1996	0	8.7	100	696	55.68
12	Male	16/10/1993	39	1	0	29/07/2003	6.47	No	25/02/1997	3	14.7	95	1176	75.264
13	Male	16/08/2003	2	3	1	25/08/2004	0.79	No	03/08/2004	0	7.2	100	576	489.056
14	Male	15/02/2000	52	1	1	22/10/2004	0.30	No	07/07/2004	4	15.7	100	1256	3799.56
15	Male	07/02/1999	65	3	1	22/10/2004	0.28	No	23/07/2004	5	18.2	5	1456	133.952
15	Male	07/02/1999	65	3	1	22/10/2004	0.28	No	04/10/2004	5	22	100	1760	495.84
16	Male	11/01/1978	59	1	1	20/08/2003	20.70	No	20/12/1982	4	12.3	100	984	568.08

Appendix III – Adult glioma VEGF data table

ID	Cases	Diagnosis	Histology	Died	FU Date	FU Years	p405 gen	m460 gen	Hap1	Hap2
1	study	04-Apr-93	Oligodendroglioma	NO	26-Feb-04	10.90411	GC	CT	TC	CG
2	study	11-Mar-94	Oligodendroglioma	NO	03-Sep-99	5.484932	CC	TT	TC	TC
3	Study	07-May-91	Neuroepithelial	YES	17-Mar-95	3.863014	GG	CT	TG	CG
4	study	17-Sep-90	Astrocytoma	YES	01-Jun-91	0.70411	GC	TT	TG	TC
5	study	04-Sep-93	Astrocytoma	YES	21-Aug-96	2.964384	CC	TT	TC	TC
6	study	10-Aug-94	Glioblastoma	NO	19-Aug-94	0.024658	GC	CT	TC	CG
7	study	21-Oct-92	Glioblastoma	YES	02-Jul-93	0.69589	GG	CT	TG	CG
8	study	15-Jul-92	Astrocytoma	YES	09-Feb-94	1.572603	GC	CT	TC	CG
9	study	09-Nov-95	Oligodendroglioma	YES	04-Mar-00	4.320548	CC	TT	TC	TC
10	study	21-Oct-92	Astrocytoma	YES	27-Nov-94	2.10137	GG	CT	TG	CG
11	study	29-Mar-93	Oligodendroglioma	NO	12-Dec-03	10.71233	GG	CC	CG	CG
12	study	20-Sep-93	Astrocytoma	YES	08-Sep-97	3.969863	GG	CC	CG	CG
13	study	29-Sep-92	Astrocytoma	NO	01-Oct-99	7.008219	GG	CC	CG	CG
14	study	30-May-92	Astrocytoma	YES	27-Sep-00	8.334247	GG	CC	CG	CG
15	study	26-Dec-90	Glioblastoma	YES	10-Sep-92	1.709589	GC	CT	TC	CG
16	study	15-Dec-88	Astrocytoma	YES	05-Mar-94	5.221918	GC	CT	TC	CG
17	study	16-Jan-93	Astrocytoma	NO	03-Sep-96	3.632877	GC	CT	TC	CG
18	study	07-Jul-94	Glioblastoma	YES	06-May-95	0.830137	GC	CT	TC	CG
19	study	25-Feb-94	Astrocytoma	NO	09-Nov-01	7.709589	CC	TT	TC	TC
20	study	13-Mar-90	Glioblastoma	YES	16-Mar-92	2.010959	GG	TT	TG	TG
21	study	15-Apr-95	Mixed Glioma	YES	07-Jul-98	3.230137	GC	CT	TC	CG
22	study	11-Aug-92	Glioblastoma	YES	31-Aug-93	1.054795	GG	TT	TG	TG
23	study	13-Mar-95	Mixed Glioma	YES	28-Nov-95	0.712329	GC	CT	TC	CG
24	study	22-Mar-93	Glioblastoma	YES	12-Feb-94	0.89589	GG	CC	CG	CG
25	study	16-Nov-94	Astrocytoma	NO	28-Nov-00	6.038356	GG	CT	TG	CG
26	study	15-Mar-89	Astrocytoma	YES	18-Dec-89	0.761644	CC	TT	TC	TC
27	study	25-Mar-92	Astrocytoma	YES	04-Jan-95	2.780822	GG	CC	CG	CG
28	study	01-Feb-93	Astrocytoma	NO	17-Jul-98	5.457534	GG	CC	CG	CG
29	study	23-Mar-92	Astrocytoma	YES	24-Jun-94	2.254795	GC	TT	TG	TC
30	study	01-Sep-92	Mixed Glioma	YES	10-May-95	2.687671	GC	CT	TC	CG
31	study	25-Aug-88	Mixed Glioma	YES	21-Mar-93	4.572603	GG	CT	TG	CG
32	study	07-Mar-88	Oligodendroglioma	YES	22-Jul-94	6.378082	CC	TT	TC	TC
33	study	21-Jan-94	Astrocytoma	NO	28-Jan-94	0.019178	GC	CT	TC	CG
34	study	01-Feb-91	Glioblastoma	YES	25-Sep-92	1.649315	GC	CT	TC	CG
35	study	25-Jun-91	Astrocytoma	NO	02-Oct-03	12.27945	GG	CT	TG	CG
36	study	03-Aug-92	Glioblastoma	YES	18-Jan-93	0.460274	GC	CT	TC	CG
37	study	10-Jan-92	Astrocytoma	YES	15-Oct-98	6.767123	GG	CC	CG	CG
38	study	09-Mar-94	Astrocytoma	NO	11-Mar-03	9.010959	GG	CC	CG	CG

ID	Cases	Diagnosis	Histology	Died	FU Date	FU Years	p405 gen	m460 gen	Hap1	Hap2
39	study	07-Sep-94	Mixed Glioma	YES	14-Jul-96	1.852055	GC	CT	TC	CG
40	study	02-May-94	Mixed Glioma	YES	08-Mar-96	1.852055	CC	TT	TC	TC
41	study	02-Jun-92	Mixed Glioma	NO	12-Feb-99	6.70137	GG	CC	CG	CG
42	study	03-Apr-95	Mixed Glioma	YES	20-Sep-96	1.468493	CC	TT	TC	TC
43	study	11-Aug-92	Glioblastoma	YES	11-Feb-95	2.50411	CC	TT	TC	TC
44	study	25-Feb-91	Glioblastoma	YES	21-Feb-93	1.991781	GG	CC	CG	CG
45	study	19-Jun-96	Oligodendroglioma	YES	23-Jul-98	2.093151	GC	CT	TC	CG
46	study	08-Apr-95	Astrocytoma	YES	03-Nov-95	0.572603	GG	CT	TG	CG
47	study	05-Mar-91	Glioblastoma	YES	11-Nov-91	0.687671	GG	CT	TG	CG
48	study	10-Sep-96	Oligodendroglioma	NO	17-Jul-03	6.852055	GC	CT	TC	CG
49	study	29-Jul-94	Astrocytoma	NO	21-Apr-04	9.736986	GG	TT	TG	TG
50	study	01-Jun-94	Astrocytoma	YES	17-May-95	0.958904	GC	CT	TC	CG
51	study	11-Jan-91	Oligodendroglioma	YES	29-Dec-95	4.967123	GC	TT	TG	TC
52	study	26-Jan-96	Oligodendroglioma	YES	05-Jun-00	4.361644	GC	CT	TC	CG
53	study	06-Jun-94	Glioblastoma	YES	20-Dec-94	0.539726	GG	CT	TG	CG
54	study	05-Jul-88	Glioblastoma	YES	01-Jan-89	0.493151	GG	TT	TG	TG
55	study	05-Nov-92	Astrocytoma	NO	11-Dec-92	0.09863	GG	CT	TG	CG
56	study	18-Sep-89	Oligodendroglioma	YES	15-Dec-01	12.24932	GG	CT	TG	CG
57	study	06-May-94	Glioblastoma	YES	25-Oct-94	0.471233	GC	TT	TG	TC
58	study	18-Jul-89	Glioblastoma	YES	12-May-91	1.816438	GG	CT	TG	CG
59	study	13-Jul-92	Astrocytoma	YES	20-Jan-95	2.523288	GC	TT	TG	TC
60	study	31-Jul-89	Glioblastoma	YES	04-Jul-91	1.926027	GC	TT	TG	TC
61	study	14-Jul-92	Astrocytoma	NO	02-Jul-03	10.9726	GG	CC	CG	CG
62	study	09-Feb-95	Astrocytoma	NO	20-Feb-04	9.035616	GG	CC	CG	CG
63	study	10-Jun-92	Mixed Glioma	YES	04-Jul-97	5.068493	GG	CC	CG	CG
64	study	11-Oct-91	Oligodendroglioma	YES	30-Aug-94	2.887671	GC	CT	TC	CG
65	study	01-May-92	Glioblastoma	YES	27-Nov-92	0.575342	GC	TT	TG	TC
66	study	14-Feb-94	Neuroepithelial	YES	11-Jun-96	2.323288	GG	CC	CG	CG
67	study	29-Sep-92	Glioblastoma	YES	14-Aug-94	1.873973	GG	CC	CG	CG
68	study	03-Feb-93	Oligodendroglioma	YES	10-Nov-94	1.767123	CC	TT	TC	TC
69	study	14-Dec-92	Astrocytoma	NO	02-Apr-99	6.30137	CC	TT	TC	TC
70	study	18-Jan-91	Glioblastoma	YES	15-May-91	0.320548	GG	TT	TG	TG
71	study	28-Dec-90	Astrocytoma	YES	02-Sep-95	4.682192	GC	TT	TG	TC
72	study	12-Sep-94	Neuroepithelial	YES	04-Apr-01	6.564384	GC	CT	TC	CG
73	study	04-Jan-93	Astrocytoma	YES	07-Nov-95	2.841096	GC	CT	TC	CG
74	study	04-Dec-91	Glioblastoma	YES	12-Dec-92	1.024658	GG	CC	CG	CG
75	study	02-Dec-94	Glioblastoma	YES	24-May-95	0.473973	GG	CC	CG	CG
76	study	15-Jul-94	Oligodendroglioma	NO	09-Sep-03	9.158904	CC	TT	TC	TC
77	study	20-Mar-92	Astrocytoma	YES	17-Apr-02	10.08219	GC	CT	TC	CG
78	study	20-Nov-92	Astrocytoma	NO	04-Aug-93	0.70411	GC	TT	TG	TC
79	study	09-May-89	Glioblastoma	YES	13-Sep-93	4.350685	CC	TT	TC	TC
80	study	09-Aug-92	Astrocytoma	YES	04-Feb-93	0.490411	GC	CT	TC	CG

ID	Cases	Diagnosis	Histology	Died	FU Date	FU Years	p405 gen	m460 gen	Hap1	Hap2
81	study	23-Oct-95	Astrocytoma	YES	15-Aug-96	0.813699	CC	TT	TC	TC
82	study	28-Sep-92	Astrocytoma	NO	02-Jun-95	2.676712	GG	CT	TG	CG
83	study	14-Nov-90	Astrocytoma	YES	05-Apr-93	2.391781	GG	CC	CG	CG
84	study	22-May-92	Glioblastoma	YES	16-Oct-92	0.40274	GC	TT	TG	TC
85	study	01-Feb-94	Astrocytoma	NO	10-Jul-98	4.438356	GG	CT	TG	CG
86	study	05-Nov-91	Neuroepithelial	NO	22-Nov-93	2.049315	GC	CT	TC	CG
87	study	09-Dec-91	Glioblastoma	YES	24-Jan-93	1.128767	GG	CT	TG	CG
88	study	16-Oct-92	Mixed Glioma	NO	17-Oct-93	1.00274	CC	TT	TC	TC
89	study	07-Aug-90	Mixed Glioma	NO	23-Jul-03	12.96712	GG	CC	CG	CG
90	study	08-Feb-93	Astrocytoma	NO	01-Oct-93	0.643836	GC	TT	TG	TC
91	study	20-Jun-91	Mixed Glioma	YES	16-Jul-93	2.073973	GG	CC	CG	CG
92	study	23-Jun-95	Astrocytoma	NO	14-Oct-03	8.315068	GG	CC	CG	CG
93	study	22-Jul-96	Oligodendroglioma	YES	15-Oct-02	6.235616	GC	CT	TC	CG
94	study	25-Nov-92	Glioblastoma	YES	07-Jan-94	1.117808	CC	TT	TC	TC
95	study	15-Nov-93	Oligodendroglioma	NO	19-May-98	4.509589	GG	TT	TG	TG
96	study	02-Feb-94	Oligodendroglioma	NO	03-Feb-94	0.00274	GC	CT	TC	CG
97	study	07-Jun-93	Oligodendroglioma	YES	30-Apr-01	7.90137	CC	TT	TC	TC
98	study	13-Mar-92	Astrocytoma	NO	10-Jan-04	11.83562	GC	TT	TG	TC
99	study	02-Apr-93	Astrocytoma	NO	02-Apr-93	0.00274	GC	CT	TC	CG
100	study	31-Mar-95	Oligodendroglioma	YES	05-Jul-97	2.265753	GG	CT	TG	CG
101	study	01-Jan-80	Mixed Glioma	YES	12-Dec-95	15.95616	GC	TT	TG	TC
102	study	06-Aug-93	Glioblastoma	YES	17-Nov-95	2.282192	GG	CC	CG	CG
103	study	03-Jul-96	Astrocytoma	NO	13-Jan-00	3.531507	GC	TT	TG	TC
104	study	28-Jun-89	Glioblastoma	YES	02-Aug-90	1.09589	GC	TT	TG	TC
105	study	11-Jul-96	Oligodendroglioma	NO	24-Feb-04	7.627397	GC	TT	TG	TC
106	study	19-Sep-95	Mixed Glioma	YES	12-Apr-96	0.564384	GG	TT	TG	TG
107	study	22-Mar-91	Glioblastoma	YES	04-Dec-91	0.70411	GC	CT	TC	CG
108	study	28-Dec-90	Oligodendroglioma	NO	16-May-04	13.39178	GG	TT	TG	TG
109	study	09-Dec-92	Mixed Glioma	NO	18-Oct-01	8.863014	GG	CC	CG	CG
110	study	01-Apr-95	Mixed Glioma	NO	10-Apr-01	6.030137	GC	CT	TC	CG
111	study	11-Jul-94	Glioblastoma	YES	05-Jun-95	0.90137	GC	CT	TC	CG
112	study	26-Feb-94	Glioblastoma	YES	24-Oct-94	0.657534	GG	CC	CG	CG
113	study	01-Mar-93	Astrocytoma	YES	07-Apr-95	2.10137	GG	CT	TG	CG
114	study	23-Nov-90	Astrocytoma	NO	20-Apr-04	13.41644	GC	CT	TC	CG
115	study	05-Jun-90	Glioblastoma	NO	06-Jun-90	0.00274	GC	CT	TC	CG
116	study	12-Mar-88	Astrocytoma	YES	05-Sep-97	9.490411	GC	CT	TC	CG
117	study	16-Jul-90	Mixed Glioma	NO	06-Apr-94	3.726027	CC	TT	TC	TC
118	study	02-Aug-93	Astrocytoma	YES	10-Oct-94	1.189041	GC	CT	TC	CG
119	study	21-May-96	Astrocytoma	NO	26-Jul-96	0.180822	GG	CC	CG	CG
120	study	15-Oct-96	Oligodendroglioma	YES	16-Feb-98	1.339726	GC	TT	TG	TC
121	study	11-Oct-96	Astrocytoma	YES	29-Jan-99	2.30137	GC	TT	TG	TC
122	study	16-Nov-96	Glioblastoma	YES	05-Jun-97	0.550685	GG	CC	CG	CG

ID	Cases	Diagnosis	Histology	Died	FU Date	FU Years	p405 gen	m460 gen	Hap1	Hap2
123	study	13-Aug-91	Mixed Glioma	NO	10-Jan-97	5.416438	GG	CC	CG	CG
124	study	07-Apr-97	Mixed Glioma	NO	25-Sep-97	0.468493	GG	CT	TG	CG
125	study	01-Apr-96	Astrocytoma	YES	15-Sep-98	2.457534	GG	CT	TG	CG
126	study	05-Aug-91	Glioblastoma	YES	10-Jul-98	6.934247	GC	TT	TG	TC
127	study	09-Sep-91	Astrocytoma	YES	26-May-93	1.712329	GG	CT	TG	CG
128	study	18-Nov-96	Astrocytoma	YES	17-Nov-97	0.99726	CC	TT	TC	TC
129	study	01-Mar-97	Astrocytoma	YES	03-Feb-99	1.928767	GG	CC	CG	CG
130	control	.			.	.	GC	CT	TC	CG
131	control	.			.	.	GG	CC	CG	CG
132	control	.			.	.	GC	CT	TC	CG
133	control	.			.	.	GC	TT	TG	TC
134	control	.			.	.	GC	CT	TC	CG
135	control	.			.	.	GG	CC	CG	CG
136	control	.			.	.	GC	CT	TC	CG
137	control	.			.	.	GG	CC	CG	CG
138	control	.			.	.	GG	CC	CG	CG
139	control	.			.	.	GC	TT	TG	TC
140	control	.			.	.	GC	TT	TG	TC
141	control	.			.	.	GG	CC	CG	CG
142	control	.			.	.	GC	CT	TC	CG
143	control	.			.	.	GC	CT	TC	CG
144	control	.			.	.	GC	TT	TG	TC
145	control	.			.	.	GC	CT	TC	CG
146	control	.			.	.	GG	CT	TG	CG
147	control	.			.	.	GC	CT	TC	CG
148	control	.			.	.	GG	CC	CG	CG
149	control	.			.	.	GG	CC	CG	CG
150	control	.			.	.	GC	CT	TC	CG
151	control	.			.	.	GC	CT	TC	CG
152	control	.			.	.	GC	TT	TG	TC
153	control	.			.	.	GG	CT	TG	CG
154	control	.			.	.	GG	CC	CG	CG
155	control	.			.	.	GC	CT	TC	CG
156	control	.			.	.	GG	CT	TG	CG
157	control	.			.	.	GC	CT	TC	CG
158	control	.			.	.	GC	TT	TG	TC
159	control	.			.	.	GG	CC	CG	CG
160	control	.			.	.	GG	CC	CG	CG
161	control	.			.	.	GC	CT	TC	CG
162	control	.			.	.	GC	CT	TC	CG
163	control	.			.	.	GG	CC	CG	CG
164	control	.			.	.	GC	CT	TC	CG

ID	Cases	Diagnosis	Histology	Died	FU Date	FU Years	p405 gen	m460 gen	Hap1	Hap2
165	control	.			.	.	CC	TT	TC	TC
166	control	.			.	.	CC	TT	TC	TC
167	control	.			.	.	GC	TT	TG	TC
168	control	.			.	.	GG	CT	TG	CG
169	control	.			.	.	GC	CT	TC	CG
170	control	.			.	.	GG	CC	CG	CG
171	control	.			.	.	GC	TT	TG	TC
172	control	.			.	.	GG	CC	CG	CG
173	control	.			.	.	GC	CT	TC	CG
174	control	.			.	.	GC	CT	TC	CG
175	control	.			.	.	GC	TT	TG	TC
176	control	.			.	.	GG	CC	CG	CG
177	control	.			.	.	GC	CT	TC	CG
178	control	.			.	.	GG	CT	TG	CG
179	control	.			.	.	GG	CT	TG	CG
180	control	.			.	.	GC	TT	TG	TC
181	control	.			.	.	GC	CT	TC	CG
182	control	.			.	.	GG	CT	TG	CG
183	control	.			.	.	GG	CT	TG	CG
184	control	.			.	.	GC	CT	TC	CG
185	control	.			.	.	GC	CT	TC	CG
186	control	.			.	.	GG	CT	TG	CG
187	control	.			.	.	GG	CT	TG	CG
188	control	.			.	.	GC	CT	TC	CG
189	control	.			.	.	GG	CC	CG	CG
190	control	.			.	.	GC	CT	TC	CG
191	control	.			.	.	GC	CT	TC	CG
192	control	.			.	.	GC	CT	TC	CG
193	control	.			.	.	GC	CT	TC	CG
194	control	.			.	.	GC	CT	TC	CG
195	control	.			.	.	GG	CT	TG	CG
196	control	.			.	.	GC	CT	TC	CG
197	control	.			.	.	GG	CT	TG	CG
198	control	.			.	.	CC	TT	TC	TC
199	control	.			.	.	GG	CC	CG	CG
200	control	.			.	.	GG	CT	TG	CG
201	control	.			.	.	GC	CT	TC	CG
202	control	.			.	.	CC	TT	TC	TC
203	control	.			.	.	GG	CT	TG	CG
204	control	.			.	.	GC	CT	TC	CG
205	control	.			.	.	GC	TT	TG	TC
206	control	.			.	.	GG	CT	TG	CG

ID	Cases	Diagnosis	Histology	Died	FU Date	FU Years	p405 gen	m460 gen	Hap1	Hap2
207	control	.			.	.	GC	CT	TC	CG
208	control	.			.	.	GC	CT	TC	CG
209	control	.			.	.	GC	CT	TC	CG
210	control	.			.	.	GG	TT	TG	TG
211	control	.			.	.	GG	CC	CG	CG
212	control	.			.	.	CC	TT	TC	TC
213	control	.			.	.	GG	CC	CG	CG
214	control	.			.	.	CC	TT	TC	TC
215	control	.			.	.	GG	CC	CG	CG
216	control	.			.	.	GG	CT	TG	CG
217	control	.			.	.	GG	CT	TG	CG
218	control	.			.	.	GG	CT	TG	CG
219	control	.			.	.	GG	CT	TG	CG
220	control	.			.	.	GC	CT	TC	CG
221	control	.			.	.	GG	CT	TG	CG
222	control	.			.	.	CC	TT	TC	TC
223	control	.			.	.	GG	CC	CG	CG
224	control	.			.	.	GC	CT	TC	CG
225	control	.			.	.	GG	CT	TG	CG
226	control	.			.	.	GG	CT	TG	CG
227	control	.			.	.	GG	CT	TG	CG
228	control	.			.	.	GG	TT	TG	TG
229	control	.			.	.	GC	CT	TC	CG
230	control	.			.	.	GG	CC	CG	CG

Appendix IV – Paediatric tumour VEGF data table

ID	dob	Gend	Date Diagnosis	Diagnosis Category	Pres Age	Rec	Date Rec	Time Rec	Died	Date FU	Time FU	GTR	grade	m460 Gen	p405 Gen	Hap 1	Hap 2
1	26/09/1984	M	26/06/1998	PITUITARY	13.76							0	1	CC	GG	CG	CG
2	25/03/1985	F	25/08/1998	MEDULLOBLASTOMA	13.43	No	12/12/2005	7.30	No	12/12/2005	7.30	1	4	TT	GG	TG	TG
3	28/11/1985	M	04/11/1998	CRANIOPHARYNGIOMA	12.94	No	30/08/2004	5.82	No	30/08/2004	5.82	1	1	CC	GG	CG	CG
4	01/03/1986	M	07/01/2004	NEUROCYTOMA	17.87	Yes	07/01/2004	0.00	No	26/04/2004	0.30	0	3	CT	CG	CG	TC
5	25/05/1987	M	01/11/2001	GANGLIOGLIOMA	14.45	No	31/05/2005	3.58	No	31/05/2005	3.58	0	1	TT	CC	TC	TC
6	06/12/1987	F	11/09/1998	ASTROCYTOMA	10.77	No	06/05/2003	4.65	No	06/05/2003	4.72	1	1	TT	CC	TC	TC
7	21/11/1988	M	16/02/2000	ASTROCYTOMA	11.24	Yes	21/07/2004	4.43	No	30/05/2005	5.29	0	1	CT	CG	CG	TC
8	10/11/1984	M	30/05/1998	SARCOMA	13.56							0	3	TT	CC	TC	TC
9	05/05/1988	M	26/06/2000	GERM CELL TUMOUR	12.15	No	28/10/2004	4.34	No	28/10/2004	4.34	0	3	CT	CG	CG	TC
10	30/06/1987	F	07/05/1999	ASTROCYTOMA	11.86	Yes	01/03/2004	4.82	No	04/03/2004	4.90	0	1	CT	GG	CG	TG
11	27/05/1990	M	25/09/2001	DPG	11.34	Yes	19/10/2001	0.07	Yes	30/12/2001	0.26	0	4	TT	CG	TC	TG
12	17/07/1990	M	22/05/1998	PNET	7.85	No	13/05/1999	0.98	No	13/05/1999	0.98	0	3	CT	CG	CG	TC
13	01/05/1990	M	19/05/1998	ASTROCYTOMA	8.05	No	30/06/2004	6.12	No	30/06/2004	6.21	0	1	CT	CG	CG	TC
14	10/11/1984	M	26/03/1999	GERMINOMA	14.38	No	20/06/2002	3.24	No	20/06/2002	3.24	0	1	TT	GG	TG	TG
15	04/06/1991	M	31/08/2000	GERMINOMA	9.25	No	12/09/2000	0.03	No	12/09/2000	0.03	0	1	CT	CG	CG	TC
16	14/05/1991	M	19/12/2001	ASTROCYTOMA	10.61	No	01/12/2004	2.95	No	01/12/2004	2.99	0	1	CT	GG	CG	TG
17	25/04/1991	F	18/03/1998	ASTROCYTOMA	6.90	No	12/11/2002	4.66	No	12/11/2002	4.72	1	1	CT	CG	CG	TC
18		M	01/01/2001	GERMINOMA		No	21/04/2005	4.30	No	21/04/2005	4.30	0		CC	GG	CG	CG
19	15/02/1992	M	13/04/2000	ASTROCYTOMA	8.16	No	29/01/2004	3.80	No	29/01/2004	3.85	0	1	TT	CC	TC	TC
20	06/10/1992	M	12/10/2002	ASTROCYTOMA	10.02							0	1	TT	GG	TG	TG
21	02/05/1988	F	18/02/2000	ASTROCYTOMA	11.81	No	22/06/2004	4.35	No	22/06/2004	4.41	1	1	CT	GG	CG	TG
22	18/05/1992	F	18/12/1999	ASTROCYTOMA	7.59							0	1	CT	CG	CG	TC
23	05/12/1986	F	25/06/2001	GANGLIOGLIOMA	14.56	No	07/04/2004	2.79	No	07/04/2004	2.79	0	1	TT	CC	TC	TC
24	01/03/1993	F	18/06/2002	ASTROCYTOMA	9.30	No	29/06/2004	2.03	No	29/06/2004	2.06	1	1	CT	CG	CG	TC
25		F	19/03/1999											TT	CG	TG	TC
26	08/07/1986	M	05/12/1996	DNET	10.42	Yes	18/12/1997	1.04	No	13/04/2004	7.36	0	1	TT	CC	TC	TC
27	15/12/1997	F	26/02/1999	EPENDYMOMA	1.20	Yes	30/05/2000	1.26	Yes	05/01/2001	1.86	0	3	CT	GG	CG	TG
28	14/08/1993	F	03/12/2001	ASTROCYTOMA	8.31	No	31/05/2005	3.49	No	31/05/2005	3.54	1	1	CT	CG	CG	TC
29	14/07/1992	F	11/12/2000	ASTROCYTOMA	8.42	No	05/04/2004	3.32	No	05/04/2004	3.36	0	1	CT	CG	CG	TC
30	30/06/1992		21/11/1997	CRANIOPHARYNGIOMA	5.40	No	18/01/2005	7.16	No	18/01/2005	7.16	1	1	TT	GG	TG	TG
31	16/12/1993	M	11/02/2000	MEDULLOBLASTOMA	6.16	No	22/03/2005	5.11	No	22/03/2005	5.11	1	4	TT	CC	TC	TC
32	19/03/1990	M	19/03/2002	OLIGODENDROGLIOMA	12.01	No	05/08/2004	2.38	No	05/08/2004	2.38	1	2	TT	CG	TG	TC
33	14/06/1983	F	24/02/1998	GANGLIOGLIOMA	14.71	No	14/06/2001	3.30	No	14/06/2001	3.30	0	1	CT	CG	CG	TC
34	20/02/1986	F	28/04/1998	GANGLIOGLIOMA	12.19	No	04/02/2004	5.78	No	04/02/2004	5.78	1	1	CT	CG	CG	TC
35	11/03/1986	M	06/07/2000	PITUITARY	14.33	Yes	21/12/2001	1.46	No	10/03/2004	3.68	0	1	CC	GG	CG	CG
36	27/02/1989	M	24/12/2002	MEDULLOBLASTOMA	13.83	No	06/04/2005	2.28	No	06/04/2005	2.28	1	4	TT	CG	TG	TC
37	01/08/1991	M	08/06/2000	DNET	8.86	No	02/03/2005	4.73	No	02/03/2005	4.73	0	1	CT	GG	CG	TG
38	01/11/1986	M	10/09/1998	ASTROCYTOMA	11.87	No	06/12/2004	6.24	No	06/12/2004	6.33	1	1	CT	GG	CG	TG
39	17/12/1993	M	21/03/2001	MEDULLOBLASTOMA	7.26	Yes	05/03/2002	0.96	Yes	15/05/2002	1.15	0	4	CT	CG	CG	TC
40	01/10/1995	F	03/04/1998	ASTROCYTOMA	2.51	No	30/09/2003	5.50	No	30/09/2003	5.57	1	1	CT	GG	CG	TG
41	01/04/1995	M	11/09/2002	ASTROCYTOMA	7.45	No	04/10/2004	2.07	No	04/10/2004	2.09	1	1	CT	CG	CG	TC
42	17/04/1994	M	15/04/1999	MEDULLOBLASTOMA	5.00	No	31/03/2005	5.96	No	31/03/2005	5.96	1	4	CT	GG	CG	TG
43	31/12/1995	F	19/02/2002	ASTROCYTOMA	6.14	No	22/11/2004	2.76	No	22/11/2004	2.80	1	1	CT	CG	CG	TC
44	24/08/1995	M	22/01/2002	ASTROCYTOMA	6.42	No	03/05/2005	3.28	No	03/05/2005	3.33	1	1	CT	CG	CG	TC
45	16/06/1995	M	18/10/1997	MEDULLOBLASTOMA	2.34	Yes	21/05/2000	2.59	Yes	15/08/2000	2.83	0	3	CT	CG	CG	TC
46	29/09/1982	M	03/11/1997	GERMINOMA	15.11	No	21/06/2000	2.63	No	21/06/2000	2.63	0	1	CT	GG	CG	TG
47	02/10/1992	M	20/03/2001	ASTROCYTOMA	8.47	No	07/11/2003	2.64	No	07/11/2003	2.67	1	1	CT	GG	CG	TG
48	04/03/199	M	27/09/199	DNET	8.57	No	23/03/2004	4.49	No	23/03/2004	4.49	1	1	CC	GG	CG	CG

ID	dob	Gend	Date Diagnosis	Diagnosis Category	Pres Age	Rec	Date Rec	Time Rec	Died	Date FU	Time FU	GTR	grade	m460 Gen	p405 Gen	Hap 1	Hap 2
	1		9														
49	26/08/1996	M	17/12/1997	ASTROCYTOMA	1.31	No	18/11/2004	6.93	No	18/11/2004	7.02	0	1	CT	CG	CG	TC
50	10/02/1995	F	16/07/2001	MEDULLOBLASTOMA	6.43	No	19/01/2005	3.52	No	19/01/2005	3.52	1	4	TT	CG	TG	TC
51	17/09/1996	F	09/01/2002	ASTROCYTOMA	5.32	No	21/02/2005	3.12	No	21/02/2005	3.16	1	1	TT	CG	TG	TC
52	05/05/1996	M	13/11/1998	ASTROCYTOMA	2.53	No	28/07/2004	5.71	No	28/07/2004	5.79	1	1	CT	CG	CG	TC
53	18/12/1993	M	04/02/1999	GANGLIOGLIOMA	5.13	No	17/11/2004	5.79	No	17/11/2004	5.79	0	1	CT	CG	CG	TC
54	20/11/1993	F	19/12/1997	OLIGOASTROCYTOMA	4.08	No	17/03/2005	7.25	No	17/03/2005	7.25	1	2	CC	GG	CG	CG
55	13/01/1987	F	17/11/1997	DNET	10.85	No	27/01/2005	7.20	No	27/01/2005	7.20	1	1	CT	CG	CG	TC
56	19/08/1986	F	03/11/1997	GANGLIOGLIOMA	11.22	No	15/01/2002	4.20	No	15/01/2002	4.20	1	1	TT	GG	TG	TG
57	21/06/1994	M	02/07/1998	ATRT	4.03	Yes	04/10/1998	0.26	Yes	04/10/1998	0.26	0	4	CC	GG	CG	CG
58	15/05/1990	M	16/10/1997	ASTROCYTOMA	7.43	No	04/12/1997	0.13	No	04/12/1997	0.14	1	3	CC	GG	CG	CG
59		M	24/02/1998	ASTROCYTOMA		No	14/09/2004	6.56	No	14/09/2004	6.56		1	CC	GG	CG	CG
60	30/10/1982	F	11/11/1997	EPENDYMOMA	15.04	No	28/06/2001	3.63	No	28/06/2001	3.63	1	2	CT	CG	CG	TC
61	13/05/1993	M	24/12/1997	ASTROCYTOMA	4.62	No	18/07/2002	4.57	No	18/07/2002	4.63	1	1	TT	CC	TC	TC
62	13/05/1993	F	11/09/1998	PINEOBLASTOMA	5.33	Yes	26/02/2001	2.46	Yes	02/04/2001	2.56	0	3	TT	CC	TC	TC
63	16/01/1994		26/02/1998	ASTROCYTOMA	4.12	No	23/02/2005	7.00	No	23/02/2005	7.00	1	1	CT	CG	CG	TC
64	07/04/1987	M	26/02/1998	ASTROCYTOMA	10.90	No	23/02/2005	7.00	No	23/02/2005	7.09	1	1	CC	GG	CG	CG
65	10/01/1998	F	23/11/2001	ASTROCYTOMA	3.87	No	10/05/2005	3.46	No	10/05/2005	3.46	1	1	CC	GG	CG	CG
66	04/11/1985	M	28/04/1999	DNET	13.49							0	1	CT	CG	CG	TC
67	18/10/1983	F	06/04/1998	GANGLIOGLIOMA	14.48	No	16/08/2001	3.36	No	16/08/2001	3.36	1	1	CC	GG	CG	CG
68	26/07/1996	M	14/04/1998	EPENDYMOMA	1.72	Yes	27/05/2000	2.12	Yes	27/05/2000	2.12	1	3	TT	GG	TG	TG
69	21/04/1994	M	14/05/1998	PNET	4.07							1	4	CC	GG	CG	CG
70	24/08/1992	F	09/06/1998	HAMARTOMA	5.79	No	31/05/2004	5.98	No	31/05/2004	5.98	0	1	CC	GG	CG	CG
71	01/05/1997	M	28/07/2000	MEDULLOBLASTOMA	3.24	No	01/12/2004	4.35	No	01/12/2004	4.35	1	4	CT	CG	CG	TC
72	12/11/1984	F	16/07/1998	MEDULLOBLASTOMA	13.68	No	17/06/2003	4.92	No	17/06/2003	4.92	1	4	CT	CG	CG	TC
73	03/03/1997	M	02/06/1998	ASTROCYTOMA	1.25	No	25/01/2005	6.65	No	25/01/2005	6.75	1	1	TT	CG	TG	TC
74	08/04/1994	M	10/06/1998	MEDULLOBLASTOMA	4.18	No	24/03/2004	5.79	No	24/03/2004	5.79	1	4	TT	CC	TC	TC
75	04/08/1995	M	31/01/2003	MEDULLOBLASTOMA	7.50	No	21/05/2005	2.30	No	21/05/2005	2.30	0	4	CC	GG	CG	CG
76	02/09/1994	M	28/01/1999	ASTROCYTOMA	4.41	No	23/11/2004	5.82	No	23/11/2004	5.91	0	1	CT	CG	CG	TC
77	20/05/1996	F	10/08/1998	ATRT	2.22							0	4	CT	CG	CG	TC
78	22/11/1994	F	14/08/1998	ASTROCYTOMA	3.73	Yes	21/02/2005	6.53	No	01/06/2005	6.90	1	1	CT	CG	CG	TC
79	25/10/1992	F	21/08/1998	LYMPHOMA	5.82	No	01/04/2005	6.62	No	01/04/2005	6.62	0	3	CT	CG	CG	TC
80		F	16/06/1998	ASTROCYTOMA									3	CC	GG	CG	CG
81	19/06/1991	F	04/04/2001	CRANIOPHARYNGIOMA	9.80	Yes	04/04/2001	0.00	No	05/04/2004	3.01	0	1	CC	GG	CG	CG
82	27/08/1994	F	24/09/1998	OLIGODENDROGLIOMA	4.08	Yes	10/02/2005	6.39	No	08/06/2005	6.71	0	2	CC	GG	CG	CG
83	28/06/1996	M	09/09/1998	MEDULLOBLASTOMA	2.20	No	30/08/2004	5.98	No	30/08/2004	5.98	0		TT	CC	TC	TC
84	19/08/1998	M	18/10/2002	EPENDYMOMA	4.17	No	08/05/2003	0.55	No	08/05/2003	0.55	1	3	CT	CG	CG	TC
85	07/08/1984	M	14/10/1998	SCHWANNOMA	14.19	No	20/01/2002	3.27	No	20/01/2002	3.27	1	1	TT	GG	TG	TG
86	10/05/1998	M	13/11/1998	CPP	0.51	No	10/05/2005	6.49	No	10/05/2005	6.49	1	2	CT	CG	CG	TC
87	14/06/1994	M	19/11/1998	ASTROCYTOMA	4.44	No	11/08/2004	5.73	No	11/08/2004	5.81	0	1	CT	GG	CG	TG
88	21/09/1990	M	03/12/1998	CRANIOPHARYNGIOMA	8.21	No	11/01/2005	6.11	No	11/01/2005	6.11	0	1	CT	CG	CG	TC
89	26/07/1992	F	15/12/1998	MEDULLOBLASTOMA	6.39	No	16/12/2004	6.01	No	16/12/2004	6.01	1	4	CC	GG	CG	CG
90	20/05/1988	M	21/12/1998	ASTROCYTOMA	10.59	No	26/10/2004	5.85	No	26/10/2004	5.93	1	1	CT	GG	CG	TG
91	24/09/1984	M	21/04/1999		14.58	No	30/09/2002	3.45	No	30/09/2002	3.45			CC	GG	CG	CG
92	07/06/1985	F	12/01/1999	ASTROCYTOMA	13.61	No	17/10/2002	3.76	No	17/10/2002	3.82	1	1	TT	CC	TC	TC
93	28/01/1986	M	02/02/1999	MEDULLOBLASTOMA	13.02	No	11/06/2004	5.36	No	11/06/2004	5.36	1	4	TT	CG	TG	TC
94	15/12/1997	F	04/02/1999	EPENDYMOMA	1.14							1	3	CC	GG	CG	CG
95	01/07/1996	F	08/03/1999	SARCOMA	2.68	Yes	22/06/1999	0.29	No	22/06/1999	0.29	0	3	CT	CG	CG	TC
96	24/11/1993	F	08/03/1999	CPC	5.29	Yes	16/10/2000	1.61	No	28/04/2004	5.15	1	3	CT	CG	CG	TC
97	07/01/1994	F	12/03/1999	ASTROCYTOMA	5.18	No	18/05/2004	5.19	No	18/05/2004	5.26	1	1	CT	CG	CG	TC
98	26/02/1990	M	03/08/2000	CRANIOPHARYNGIOMA	10.44	No	05/08/2004	4.01	No	05/08/2004	4.01	0	1	CC	GG	CG	CG
99	08/01/1998	M	08/04/1999	HAEMANGIOENDOTHELIOMA	1.25	No	18/11/2003	4.62	No	18/11/2003	4.62	0	1	CC	GG	CG	CG

ID	dob	Gend	Date Diagnosis	Diagnosis Category	Pres Age	Rec	Date Rec	Time Rec	Died	Date FU	Time FU	GTR	grade	m460 Gen	p405 Gen	Hap 1	Hap 2
100		F	15/12/1999	ASTROCYTOMA		Yes	24/05/2003	3.44	No	24/11/2004	4.95	0	1	CC	GG	CG	CG
101	19/01/1992	F	31/05/1999	CRANIOPHARYNGIOMA	7.37	No	08/06/2004	5.03	No	08/06/2004	5.03	0	1	CT	CG	CG	TC
102	17/08/1985	F	04/03/2003	ASTROBLASTOMA	17.56	No	26/05/2003	0.23	No	26/05/2003	0.23	0	2	CC	GG	CG	CG
103	15/06/1989	M	27/07/1999	GANGLIOGLIOMA	10.12	Yes	01/11/2000	1.27	Yes	18/01/2001	1.48	0	3	CC	GG	CG	CG
104	25/07/1993	M	31/08/1999	EPENDYMOMA	6.10	Yes	20/07/2004	4.89	No	12/05/2005	5.70	0		CT	GG	CG	TG
105	06/03/1997	F	01/09/1999	EPENDYMOMA	2.49	Yes	01/02/2000	0.42	Yes	18/05/2000	0.71	0	3	CT	CG	CG	TC
106	02/06/1995	M	25/10/1999	MEDULLOBLASTOMA	4.40	No	05/03/2005	5.36	No	05/03/2005	5.36	1	4	CC	GG	CG	CG
107	20/04/1989	F	10/10/2001	OLIGOASTROCYTOMA	12.48	No	26/08/2004	2.88	No	26/08/2004	2.88	0	2	CT	GG	CG	TG
108	01/12/1990	M	02/12/1999	GERMINOMA	9.01	No	12/05/2005	5.45	No	12/05/2005	5.45	0	1	TT	CC	TC	TC
109	28/01/1997	F	22/12/1999	ASTROCYTOMA	2.90	No	07/12/2004	4.96	No	07/12/2004	5.03	1	1	CT	GG	CG	TG
110	29/06/1992	M	26/12/1999	CRANIOPHARYNGIOMA	7.50	Yes	04/11/2002	2.86	No	11/01/2005	5.05	0	1	CT	GG	CG	TG
111	23/06/1998	F	29/01/2000	ASTROCYTOMA	1.60	Yes	14/08/2000	0.54	No	01/12/2004	4.84	0	1	CT	CG	CG	TC
112	23/06/1998	F	29/01/2000	ASTROCYTOMA	1.60	Yes	14/08/2000	0.54	No	01/12/2004	4.91	1	1	CT	CG	CG	TC
113	26/08/1994	M	27/04/2000	GANGLIOGLIOMA	5.67	No	28/07/2004	4.25	No	28/07/2004	4.25	1		TT	CC	TC	TC
114	04/01/1992	M	07/02/2000	CRANIOPHARYNGIOMA	8.10	No	04/09/2002	2.58	No	04/09/2002	2.58	1	1	TT	CG	TG	TC
115	29/01/1998	F	25/02/2000	MEDULLOBLASTOMA	2.07	Yes	15/06/2000	0.30	Yes	08/08/2000	0.45	0	4	CT	GG	CG	TG
116	02/03/1992	M	10/03/2000	EPENDYMOMA	8.03	No	17/05/2005	5.19	No	17/05/2005	5.19	1	2	CT	GG	CG	TG
117	16/08/1986	F	15/05/2000	PITUITARY	13.76	No	22/06/2001	1.10	No	22/06/2001	1.10	1	1	CT	CG	CG	TC
118	07/10/1998	M	06/04/2000	ASTROCYTOMA	1.50	No	13/05/2005	5.10	No	13/05/2005	5.18	0	1	TT	CG	TG	TC
119	25/12/1993	F	11/04/2000	ASTROCYTOMA	6.30	Yes	17/05/2005	5.10	No	17/05/2005	5.17	0	1	TT	GG	TG	TG
120	08/08/1996	M	27/04/2000	ASTROCYTOMA	3.72	Yes	05/08/2004	4.28	No	05/08/2004	4.34	1	2	TT	CC	TC	TC
121	21/08/1997	M	29/04/2000	ASTROCYTOMA	2.69	No	17/02/2004	3.81	No	17/02/2004	3.86	1	1	CC	GG	CG	CG
122	12/09/1998	M	30/05/2000	MEDULLOBLASTOMA	1.72	Yes	12/03/2002	1.78	No	17/03/2005	4.80	0	4	CC	GG	CG	CG
123	22/09/1991	F	03/06/2000	EPENDYMOMA	8.70	No	16/12/2004	4.54	No	16/12/2004	4.54	0	3	CC	GG	CG	CG
124	04/02/1997	M	16/06/2000	ASTROCYTOMA	3.36	No	02/09/2004	4.22	No	02/09/2004	4.28	1	1	TT	CC	TC	TC
125	01/12/1990	M	29/06/2000	ASTROCYTOMA	9.58	Yes	01/10/2003	3.26	No	16/05/2005	4.95	1	1	TT	CC	TC	TC
126	01/12/1995	M	24/05/2002	MEDULLOBLASTOMA	6.48	Yes	20/02/2005	2.75	No	25/04/2005	2.92	0	4	CT	CG	CG	TC
127	20/10/1995	F	20/07/2000	ASTROCYTOMA	4.75	No	10/06/2002	1.89	No	10/06/2002	1.92	1	1	TT	GG	TG	TG
128	20/10/1995	M	29/07/2000	MEDULLOBLASTOMA	4.78	No	01/12/2004	4.35	No	01/12/2004	4.35	0	4	CT	CG	CG	TC
129	10/08/2000	M	26/04/2001	MELANOCYTOMA	0.71	Yes	25/07/2001	0.25	Yes	25/07/2001	0.25	0		TT	CC	TC	TC
130	14/12/1997	M	01/09/2000	GANGLIOGLIOMA	2.72	Yes	16/01/2001	0.38	Yes	12/03/2001	0.53	0	3	CT	CG	CG	TC
131	18/05/1992	F	19/01/2005	GERMINOMA	12.68	No	14/03/2005	0.15	No	14/03/2005	0.15	0		TT	CG	TG	TC
132	31/12/1986	F	24/10/2000	DIG	13.82	No	12/08/2004	3.80	No	12/08/2004	3.80	0	1	CC	GG	CG	CG
133	09/02/1994	F	25/10/2000	MEDULLOBLASTOMA	6.71	No	16/12/2004	4.15	No	16/12/2004	4.15	0	4	CT	CG	CG	TC
134	10/03/1990	F	02/11/2000	CRANIOPHARYNGIOMA	10.66	No	28/06/2004	3.65	No	28/06/2004	3.65	1	1	TT	CG	TG	TC
135	18/01/1995	F	13/11/2000	CRANIOPHARYNGIOMA	5.82	No	01/12/2004	4.05	No	01/12/2004	4.05	0	1	TT	GG	TG	TG
136	21/09/1998	F	19/12/2000	MEDULLOBLASTOMA	2.25	Yes	21/02/2003	2.18	Yes	02/10/2003	2.79	0	4	CT	GG	CG	TG
137	04/08/1994	M	20/01/2001	MEDULLOBLASTOMA	6.47	Yes	04/02/2005	4.04	No	04/04/2005	4.21	0	4	TT	CC	TC	TC
138	07/12/1990	M	26/02/2001	EPENDYMOMA	10.23	No	14/10/2004	3.63	No	14/10/2004	3.63	1	3	CC	GG	CG	CG
139	10/03/1993	M	27/02/2001	ASTROCYTOMA	7.98	No	18/08/2004	3.47	No	18/08/2004	3.47	1	1	CT	CG	CG	TC
140	10/03/1993	M	27/02/2001	ASTROCYTOMA	7.98	No	18/08/2004	3.47	No	18/08/2004	3.52	1	1	CT	CG	CG	TC
141	09/12/1992	F	16/04/2001	ASTROCYTOMA	8.36	No	29/03/2005	3.95	No	29/03/2005	4.01	0	1	CT	GG	CG	TG
142	07/08/1992	M	09/03/2001	PITUITARY	8.59	No	22/02/2005	3.96	No	22/02/2005	3.96	0	1	CT	CG	CG	TC
143	22/01/1996	M	22/03/2001	DPG	5.17	Yes	01/06/2002	1.19	Yes	18/10/2002	1.58	0	4	CT	CG	CG	TC
144	21/06/1993	M	26/03/2001	CRANIOPHARYNGIOMA	7.77	No	10/05/2005	4.13	No	10/05/2005	4.13	0	1	CT	GG	CG	TG
145		F	14/05/2001	DPG		Yes	12/10/2001	0.41	Yes	25/11/2001	0.53	0	4	CT	GG	CG	TG
146	23/06/1995	M	16/05/2001	DPG	5.90	Yes	02/05/2002	0.96	Yes	18/05/2002	1.01	0	4	TT	CG	TC	TG
147	02/07/1989	M	29/05/2001	ASTROCYTOMA	11.92	No	18/10/2004	3.39	No	18/10/2004	3.44	1	1	CT	GG	CG	TG
148		M	13/08/2001	ATRT		Yes	23/07/2002	0.94	Yes	03/08/2002	0.97	0	4	CC	GG	CG	CG
149	08/02/1988	F	12/06/2001	GERM CELL TUMOUR	13.35	No	10/03/2005	3.75	No	10/03/2005	3.75	0	1	TT	CG	TG	TC
150	31/07/1989	F	26/11/2001	OLIGOASTROCYTOMA	12.33	Yes	05/04/2004	2.36	No	17/11/2004	2.98	0	2	CT	CG	CG	TC
151	18/04/2000	M	29/06/2001	EPENDYMOMA	1.20	No	07/12/2001	0.44	No	16/05/2005	3.88	1	3	TT	CC	TC	TC

ID	dob	Gend	Date Diagnosis	Diagnosis Category	Pres Age	Rec	Date Rec	Time Rec	Died	Date FU	Time FU	GTR	grade	m460 Gen	p405 Gen	Hap 1	Hap 2
152	04/12/1996	M	20/07/2001	PNET	4.63	Yes	05/10/2001	0.21	Yes	28/01/2002	0.53	0	3	CC	GG	CG	CG
153	19/03/2001	M	07/08/2001	CPP	0.39	No	01/12/2004	3.32	No	01/12/2004	3.32	1	1	CC	GG	CG	CG
154	26/09/1996	M	18/08/2001	MEDULLOBLASTOMA	4.90	No	08/03/2005	3.56	No	08/03/2005	3.56	0	4	CC	GG	CG	CG
155	12/11/1987	F	11/12/2001	GANGLIOGLIOMA	14.09	No	20/09/2004	2.78	No	20/09/2004	2.78	0	1	TT	CC	TC	TC
156	26/08/1994	M	07/11/2001	PNET	7.21	Yes	03/09/2002	0.82	Yes	13/11/2002	1.02	0		CT	GG	CG	TG
157	01/10/1989	M	14/09/2001	ASTROCYTOMA	11.96	No	22/11/2004	3.19	No	22/11/2004	3.24	1	1	CT	CG	CG	TC
158			08/10/2001	DPG		Yes	02/06/2002	0.65	Yes	05/08/2002	0.82	0		CC	GG	CG	CG
159	21/06/1989	F	09/10/2001	ASTROCYTOMA	12.31	No	12/08/2004	2.84	No	12/08/2004	2.88	1	1	CT	CG	CG	TC
160	20/07/1990	F	22/10/2001	CRANIOPHARYNGIOMA	11.27	No	07/06/2005	3.63	No	07/06/2005	3.63	0	1	TT	CC	TC	TC
161	13/04/2000	M	21/10/2001	ASTROCYTOMA	1.52	No	07/11/2003	2.05	No	07/11/2003	2.08	1	1	TT	CG	TG	TC
162	02/04/1997	M	14/01/2002	MEDULLOBLASTOMA	4.79	No	13/04/2005	3.25	No	13/04/2005	3.25	1	4	CT	GG	CG	TG
163	22/10/1987	M	27/11/2001	CRANIOPHARYNGIOMA	14.11	No	25/11/2004	3.00	No	25/11/2004	3.00	0	1	TT	CG	TG	TC
164	26/02/1988	F	07/11/2001	CRANIOPHARYNGIOMA	13.71	No	17/05/2004	2.53	No	17/05/2004	2.53	0	1	CC	GG	CG	CG
165	13/07/2000	F	18/11/2001	MEDULLOBLASTOMA	1.35	Yes	04/02/2002	0.21	Yes	08/03/2002	0.30	0	4	TT	CG	TG	TC
166	07/03/1993	M	18/12/2001	ASTROCYTOMA	8.79	Yes	05/01/2005	3.05	No	05/01/2005	3.09	0	1	CT	GG	CG	TG
167	05/06/1994	F	28/12/2001	ASTROCYTOMA	7.57	No	26/05/2005	3.41	No	26/05/2005	3.46	0	1	CT	CG	CG	TC
168	04/05/1987	M	31/12/2001	ASTROCYTOMA	14.67	No	02/09/2003	1.67	Yes	02/09/2003	1.69	0	1	CT	CG	CG	TC
169	23/06/1999	M	09/01/2002	MEDULLOBLASTOMA	2.55	No	20/06/2005	3.45	No	20/06/2005	3.45	1	4	CT	GG	CG	TG
170		M	15/01/2002	PNET		No	09/03/2005	3.15		09/03/2005	3.15	1		CT	GG	CG	TG
171	14/03/1995	M	10/01/2002	ASTROCYTOMA	6.83	No	31/08/2004	2.64	No	31/08/2004	2.68	1	1	CT	GG	CG	TG
172	25/04/1988	M	13/01/2002	ASTROCYTOMA	13.73	No	01/11/2004	2.80	No	01/11/2004	2.84	1	1	TT	CC	TC	TC
173	17/03/2001	M	22/01/2002	ASTROCYTOMA	0.85	No	09/05/2005	3.30	No	09/05/2005	3.34	0	1	CT	CG	CG	TC
174	24/03/2001	M	06/03/2002	MEDULLOEPITHELIOMA	0.95	Yes	01/07/2003	1.32	Yes	28/07/2003	1.39	1	4	TT	CG	TG	TC
175	09/04/1989	F	24/02/2002	ASTROCYTOMA	12.89	No	03/10/2004	2.61	No	03/10/2004	2.64	1	1	TT	GG	TG	TG
176	26/10/1990	F	11/04/2002	ASTROCYTOMA	11.47	No	30/03/2005	2.97	No	30/03/2005	3.01	1	1	CT	CG	CG	TC
177	14/10/1992	M	09/05/2002	MEDULLOBLASTOMA	9.57	Yes	14/10/2002	0.43	No	14/10/2002	0.43	0	4	CC	GG	CG	CG
178		F	11/07/2002	GERMINOMA		No	21/04/2005	2.78	No	21/04/2005	2.78	0	1	TT	CG	TG	TC
179	14/02/1999	F	30/06/2002	CRANIOPHARYNGIOMA	3.38	No	08/04/2005	2.78	No	08/04/2005	2.78	0		TT	CG	TG	TC
180	25/11/1999	M	22/06/2002	EPENDYMOMA	2.58	No	17/03/2005	2.74	No	17/03/2005	2.74	0	2	TT	CG	TG	TC
181	13/09/1998	F	29/06/2002	MEDULLOBLASTOMA	3.79	No	08/06/2005	2.95	No	08/06/2005	2.95	1	4	CC	CG	CC	CG
182	04/05/1992	F	05/07/2002	MEDULLOBLASTOMA	10.18	No	24/01/2005	2.56	No	24/01/2005	2.56	0	4	TT	CG	TG	TC
183			15/07/2002	DPG		Yes	13/12/2002	0.41	Yes	08/01/2003	0.48	0		CT	CG	CG	TC
184	11/10/1999	M	29/07/2002	ASTROCYTOMA	2.80	No	18/11/2004	2.31	No	18/11/2004	2.34	1	1	CT	GG	CG	TG
185	26/08/1993	F	01/08/2002	ASTROCYTOMA	8.94	No	21/05/2005	2.81	No	21/05/2005	2.84	0	1	CT	CG	CG	TC
186	19/03/2002	F	22/08/2002	EPENDYMOMA	0.43	Yes	17/03/2003	0.57	Yes	21/03/2003	0.58	0		CT	CG	CG	TC
187	28/04/1993	F	17/09/2002	ASTROCYTOMA	9.39	No	03/08/2004	1.88	No	03/08/2004	1.91	1	1	CT	CG	CG	TC
188	03/07/2001	F	30/09/2002	CPP	1.24	No	10/05/2005	2.61	No	10/05/2005	2.61	1	1	CC	GG	CG	CG
189	21/06/1997	M	16/10/2002	MEDULLOBLASTOMA	5.32	No	17/01/2005	2.26	No	17/01/2005	2.26	1	4	CC	GG	CG	CG
190	08/10/1995	M	07/11/2002	ASTROCYTOMA	7.09	No	17/11/2004	2.03	No	17/11/2004	2.06	1	1	CT	CG	CG	TC
191	27/02/2001	M	12/11/2002	CPP	1.71	No	23/03/2005	2.36	No	23/03/2005	2.36	1	1	CT	CG	CG	TC
192	31/08/1997	M	19/12/2002	ASTROCYTOMA	5.30	No	26/04/2005	2.35	No	26/04/2005	2.39	0	1	CT	CG	CG	TC
193	20/08/1990	M	31/12/2002	MEDULLOBLASTOMA	12.37	No	21/04/2005	2.31	No	21/04/2005	2.31	1	4	TT	CC	TC	TC
194				CONTROL										TT	CC	TC	TC
195				CONTROL										CT	CG	CG	TC
196				CONTROL										CT	CG	CG	TC
197				CONTROL										CT	CG	CG	TC
198				CONTROL										TT	GG	TG	TG
199				CONTROL										TT	CG	TC	TG
200				CONTROL										TT	CG	TC	TG
201				CONTROL										CT	CG	CG	TC
202				CONTROL										TT	CG	TC	TG
203				CONTROL										CC	GG	CG	CG

ID	dob	Gend	Date Diagnosis	Diagnosis Category	Pres Age	Rec	Date Rec	Time Rec	Died	Date FU	Time FU	GTR	grade	m460 Gen	p405 Gen	Hap 1	Hap 2
204				CONTROL										CC	GG	CG	CG
205				CONTROL										CT	GG	TG	CG
206				CONTROL										TT	CG	TC	TG
207				CONTROL										CT	GG	TG	CG
208				CONTROL										CT	GG	TG	CG
209				CONTROL										TT	CG	TC	TG
210				CONTROL										CT	CG	CG	TC
211				CONTROL										TT	CC	TC	TC
212				CONTROL										TT	GG	TG	TG
213				CONTROL										CT	GG	TG	CG
214				CONTROL										CT	CG	CG	TC
215				CONTROL										CC	GG	CG	CG
216				CONTROL										CT	GG	TG	CG
217				CONTROL										TT	CG	TC	TG
218				CONTROL										CT	CG	CG	TC
219				CONTROL										CT	CG	CG	TC
220				CONTROL										CC	GG	CG	CG
221				CONTROL										TT	GG	TG	TG
222				CONTROL										CC	GG	CG	CG
223				CONTROL										TT	CC	TC	TC
224				CONTROL										CT	GG	TG	CG
225				CONTROL										CT	GG	TG	CG
226				CONTROL										CT	CG	CG	TC
227				CONTROL										TT	CG	TC	TG
228				CONTROL										CT	CG	CG	TC
229				CONTROL										CC	GG	CG	CG
230				CONTROL										CT	CG	CG	TC
231				CONTROL										CT	GG	TG	CG
232				CONTROL										CT	GG	TG	CG
233				CONTROL										TT	CG	TC	TG
234				CONTROL										CC	GG	CG	CG
235				CONTROL										CT	CG	CG	TC
236				CONTROL										CT	CG	CG	TC
237				CONTROL										CT	CG	CG	TC
238				CONTROL										CT	GG	TG	CG
239				CONTROL										TT	CG	TC	TG
240				CONTROL										CC	GG	CG	CG
241				CONTROL										CT	GG	TG	CG
242				CONTROL										CT	GG	TG	CG
243				CONTROL										CT	GG	TG	CG
244				CONTROL										CT	CG	CG	TC
245				CONTROL										TT	GG	TG	TG
246				CONTROL										CT	GG	TG	CG
247				CONTROL										TT	CC	TC	TC
248				CONTROL										TT	GG	TG	TG
249				CONTROL										CT	CG	CG	TC
250				CONTROL										TT	CC	TC	TC
251				CONTROL										CC	GG	CG	CG
252				CONTROL										CC	GG	CG	CG
253				CONTROL										TT	CG	TC	TG
254				CONTROL										CT	CG	CG	TC
255				CONTROL										CT	GG	TG	CG

ID	dob	Gend	Date Diagnosis	Diagnosis Category	Pres Age	Rec	Date Rec	Time Rec	Died	Date FU	Time FU	GTR	grade	m460 Gen	p405 Gen	Hap 1	Hap 2
256				CONTROL										CC	GG	CG	CG
257				CONTROL										TT	CC	TC	TC
258				CONTROL										CT	CG	CG	TC
259				CONTROL										CT	CG	CG	TC
260				CONTROL										CT	GG	TG	CG
261				CONTROL										CT	GG	TG	CG
262				CONTROL										TT	CG	TC	TG
263				CONTROL										TT	GG	TG	TG
264				CONTROL										CT	CG	CG	TC
265				CONTROL										TT	CG	TC	TG
266				CONTROL										CT	CG	CG	TC
267				CONTROL										CC	GG	CG	CG
268				CONTROL										TT	CG	TC	TG
269				CONTROL										TT	CG	TC	TG
270				CONTROL										TT	CG	TC	TG
271				CONTROL										TT	CC	TC	TC
272				CONTROL										CT	CG	CG	TC
273				CONTROL										TT	CG	TC	TG
274				CONTROL										CC	GG	CG	CG
275				CONTROL										TT	CG	TC	TG
276				CONTROL										CT	GG	TG	CG
277				CONTROL										CC	GG	CG	CG
278				CONTROL										CT	CG	CG	TC
279				CONTROL										CC	GG	CG	CG
280				CONTROL										CT	GG	TG	CG
281				CONTROL										TT	CG	TC	TG
282				CONTROL										CT	GG	TG	CG
283				CONTROL										CT	CG	CG	TC
284				CONTROL										TT	CG	TC	TG
285				CONTROL										TT	CC	TC	TC
286				CONTROL										CT	CG	CG	TC
287				CONTROL										TT	CG	TC	TG
288				CONTROL										CT	CG	CG	TC
289				CONTROL										TT	CG	TC	TG
290				CONTROL										TT	CC	TC	TC
291				CONTROL										CT	CG	CG	TC
292				CONTROL										TT	CC	TC	TC
293				CONTROL										TT	CG	TC	TG
294				CONTROL										TT	CG	TC	TG
295				CONTROL										CT	CG	CG	TC
296				CONTROL										CT	CG	CG	TC
297				CONTROL										CT	GG	TG	CG
298				CONTROL										CT	GG	TG	CG
299				CONTROL										CT	GG	TG	CG
300				CONTROL										TT	CC	TC	TC
301				CONTROL										CC	GG	CG	CG
302				CONTROL										TT	CC	TC	TC
303				CONTROL										TT	CG	TC	TG
304				CONTROL										CT	GG	TG	CG
305				CONTROL										CT	GG	TG	CG
306				CONTROL										CT	GG	TG	CG
307				CONTROL										CT	GG	TG	CG

ID	dob	Gend	Date Diagnosis	Diagnosis Category	Pres Age	Rec	Date Rec	Time Rec	Died	Date FU	Time FU	GTR	grade	m460 Gen	p405 Gen	Hap 1	Hap 2
308				CONTROL										TT	GG	TG	TG
309				CONTROL										CT	GG	TG	CG
310				CONTROL										CT	CG	CG	TC
311				CONTROL										CC	GG	CG	CG
312				CONTROL										TT	CG	TC	TG
313				CONTROL										CT	GG	TG	CG
314				CONTROL										CT	CG	CG	TC
315				CONTROL										CC	GG	CG	CG
316				CONTROL										CT	GG	TG	CG
317				CONTROL										CT	GG	TG	CG
318				CONTROL										CT	CG	CG	TC
319				CONTROL										CC	GG	CG	CG
320				CONTROL										TT	CG	TC	TG
321				CONTROL										TT	CG	TC	TG
322				CONTROL										CT	CG	CG	TC
323				CONTROL										TT	CG	TC	TG
324				CONTROL										TT	CC	TC	TC
325				CONTROL										CC	GG	CG	CG
326				CONTROL										TT	CG	TC	TG
327				CONTROL										CT	CG	CG	TC
328				CONTROL										CC	GG	CG	CG
329				CONTROL										CT	GG	TG	CG
330				CONTROL										CC	GG	CG	CG
331				CONTROL										CT	GG	TG	CG
332				CONTROL										TT	CC	TC	TC
333				CONTROL										CT	GG	TG	CG
334				CONTROL										CT	CG	CG	TC
335				CONTROL										TT	CC	TC	TC
336				CONTROL										CC	GG	CG	CG
337				CONTROL										CT	GG	TG	CG
338				CONTROL										CC	GG	CG	CG
339				CONTROL										CT	CG	CG	TC
340				CONTROL										CT	GG	TG	CG
341				CONTROL										TT	CG	TC	TG
342				CONTROL										CC	GG	CG	CG
343				CONTROL										TT	GG	TG	TG
344				CONTROL										TT	CG	TC	TG
345				CONTROL										CT	CG	CG	TC
346				CONTROL										CT	CG	CG	TC
347				CONTROL										CC	GG	CG	CG
348				CONTROL										CT	GG	TG	CG
349				CONTROL										TT	CC	TC	TC
350				CONTROL										CT	GG	TG	CG
351				CONTROL										CT	CG	CG	TC
352				CONTROL										CT	CG	CG	TC
353				CONTROL										TT	GG	TG	TG
354				CONTROL										CT	CG	CG	TC
355				CONTROL										CT	CG	CG	TC
356				CONTROL										CT	GG	TG	CG
357				CONTROL										CT	GG	TG	CG
358				CONTROL										TT	CG	TC	TG
359				CONTROL										TT	CC	TC	TC

ID	dob	Gend	Date Diagnosis	Diagnosis Category	Pres Age	Rec	Date Rec	Time Rec	Died	Date FU	Time FU	GTR	grade	m460 Gen	p405 Gen	Hap 1	Hap 2
360				CONTROL										TT	CG	TC	TG
361				CONTROL										CC	GG	CG	CG
362				CONTROL										CC	GG	CG	CG
363				CONTROL										CC	GG	CG	CG
364				CONTROL										CT	CG	CG	TC
365				CONTROL										TT	CG	TC	TG
366				CONTROL										CT	GG	TG	CG
367				CONTROL										TT	CG	TC	TG
368				CONTROL										TT	CG	TC	TG
369				CONTROL										CT	GG	TG	CG
370				CONTROL										TT	CG	TC	TG
371				CONTROL										CC	GG	CG	CG
372				CONTROL										TT	CG	TC	TG
373				CONTROL										CC	GG	CG	CG
374				CONTROL										TT	CC	TC	TC
375				CONTROL										TT	CG	TC	TG
376				CONTROL										TT	CG	TC	TG
377				CONTROL										CT	CG	CG	TC
378				CONTROL										CT	CG	CG	TC
379				CONTROL										CT	GG	TG	CG
380				CONTROL										CT	CG	CG	TC
381				CONTROL										CT	GG	TG	CG
382				CONTROL										TT	CG	TC	TG
383				CONTROL										CT	CG	CG	TC
384				CONTROL										TT	GG	TG	TG
385				CONTROL										TT	GG	TG	TG
386				CONTROL										TT	CC	TC	TC
387				CONTROL										TT	CG	TC	TG
388				CONTROL										CC	GG	CG	CG
389				CONTROL										CT	GG	TG	CG
390				CONTROL										TT	CG	TC	TG
391				CONTROL										CT	CG	CG	TC
392				CONTROL										CT	CG	CG	TC
393				CONTROL										CC	GG	CG	CG
394				CONTROL										CT	GG	TG	CG
395				CONTROL										CC	GG	CG	CG
396				CONTROL										TT	CC	TC	TC
397				CONTROL										TT	CC	TC	TC
398				CONTROL										CT	GG	TG	CG
399				CONTROL										CC	GG	CG	CG
400				CONTROL										CC	GG	CG	CG
401				CONTROL										CT	CG	CG	TC
402				CONTROL										TT	CC	TC	TC
403				CONTROL										CC	GG	CG	CG
404				CONTROL										CT	GG	TG	CG
405				CONTROL										CT	CG	CG	TC
406				CONTROL										CT	CG	CG	TC
407				CONTROL										CT	CG	CG	TC
408				CONTROL										TT	CG	TC	TG
409				CONTROL										CC	GG	CG	CG
410				CONTROL										CT	CG	CG	TC
411				CONTROL										CT	CG	CG	TC

ID	dob	Gend	Date Diagnosis	Diagnosis Category	Pres Age	Rec	Date Rec	Time Rec	Died	Date FU	Time FU	GTR	grade	m460 Gen	p405 Gen	Hap 1	Hap 2
412				CONTROL										TT	CC	TC	TC
413				CONTROL										CT	GG	TG	CG
414				CONTROL										CC	GG	CG	CG
415				CONTROL										CC	GG	CG	CG
416				CONTROL										CT	GG	TG	CG
417				CONTROL										TT	CC	TC	TC
418				CONTROL										CT	GG	TG	CG
419				CONTROL										TT	CG	TC	TG
420				CONTROL										CT	CG	CG	TC
421				CONTROL										TT	GG	TG	TG
422				CONTROL										CC	GG	CG	CG
423				CONTROL										CC	GG	CG	CG
424				CONTROL										CT	CG	CG	TC
425				CONTROL										TT	CG	TC	TG
426				CONTROL										TT	CC	TC	TC
427				CONTROL										CT	GG	TG	CG
428				CONTROL										CT	CG	CG	TC
429				CONTROL										TT	CG	TC	TG
430				CONTROL										CT	CG	CG	TC
431				CONTROL										CT	GG	TG	CG
432				CONTROL										CC	GG	CG	CG
433				CONTROL										TT	CG	TC	TG
434				CONTROL										CC	GG	CG	CG
435				CONTROL										CT	CG	CG	TC
436				CONTROL										TT	CG	TC	TG
437				CONTROL										CC	GG	CG	CG
438				CONTROL										CT	GG	TG	CG
439				CONTROL										CC	GG	CG	CG
440				CONTROL										CT	CG	CG	TC
441				CONTROL										CC	GG	CG	CG
442				CONTROL										CC	GG	CG	CG
443				CONTROL										TT	CG	TC	TG
444				CONTROL										TT	GG	TG	TG
445				CONTROL										TT	GG	TG	TG
446				CONTROL										CT	GG	TG	CG
447				CONTROL										CT	GG	TG	CG
448				CONTROL										TT	GG	TG	TG
449				CONTROL										CC	GG	CG	CG
450				CONTROL										CT	CG	CG	TC
451				CONTROL										CT	CG	CG	TC
452				CONTROL										CT	CG	CG	TC
453				CONTROL										CT	GG	TG	CG
454				CONTROL										TT	GG	TG	TG
455				CONTROL										CC	GG	CG	CG
456				CONTROL										CT	CG	CG	TC
457				CONTROL										TT	CG	TC	TG
458				CONTROL										CT	CG	CG	TC
459				CONTROL										CT	CG	CG	TC
460				CONTROL										CC	GG	CG	CG
461				CONTROL										TT	GG	TG	TG
462				CONTROL										TT	GG	TG	TG
463				CONTROL										CT	GG	TG	CG

ID	dob	Gend	Date Diagnosis	Diagnosis Category	Pres Age	Rec	Date Rec	Time Rec	Died	Date FU	Time FU	GTR	grade	m460 Gen	p405 Gen	Hap 1	Hap 2
464				CONTROL										TT	GG	TG	TG
465				CONTROL										CT	CG	CG	TC
466				CONTROL										CC	GG	CG	CG
467				CONTROL										CC	GG	CG	CG
468				CONTROL										CT	CG	CG	TC
469				CONTROL										CT	CG	CG	TC
470				CONTROL										CT	CG	CG	TC
471				CONTROL										CC	GG	CG	CG
472				CONTROL										TT	CC	TC	TC
473				CONTROL										CT	CG	CG	TC
474				CONTROL										TT	CC	TC	TC
475				CONTROL										CC	GG	CG	CG
476				CONTROL										TT	CG	TC	TG
477				CONTROL										TT	GG	TG	TG
478				CONTROL										CC	GG	CG	CG
479				CONTROL										CT	CG	CG	TC
480				CONTROL										CT	GG	TG	CG
481				CONTROL										CT	CG	CG	TC
482				CONTROL										CC	GG	CG	CG
483				CONTROL										CT	CG	CG	TC
484				CONTROL										CT	GG	TG	CG
485				CONTROL										CC	GG	CG	CG
486				CONTROL										CT	CG	CG	TC
487				CONTROL										TT	CG	TC	TG
488				CONTROL										TT	CG	TC	TG
489				CONTROL										CC	GG	CG	CG
490				CONTROL										CC	GG	CG	CG
491				CONTROL										CT	GG	TG	CG
492				CONTROL										CT	GG	TG	CG
493				CONTROL										CT	CG	CG	TC
494				CONTROL										CT	CG	CG	TC
495				CONTROL										CT	CG	CG	TC
496				CONTROL										CT	GG	TG	CG
497				CONTROL										CT	GG	TG	CG
498				CONTROL										CT	GG	TG	CG
499				CONTROL										TT	CC	TC	TC
500				CONTROL										TT	CG	TC	TG
501				CONTROL										TT	CC	TC	TC
502				CONTROL										CT	GG	TG	CG
503				CONTROL										CT	GG	TG	CG
504				CONTROL										TT	CC	TC	TC
505				CONTROL										TT	CG	TC	TG
506				CONTROL										CC	GG	CG	CG
507				CONTROL										CT	CG	CG	TC
508				CONTROL										TT	CG	TC	TG
509				CONTROL										CT	GG	TG	CG
510				CONTROL										CT	CG	CG	TC
511				CONTROL										CT	GG	TG	CG
512				CONTROL										TT	CG	TC	TG
513				CONTROL										CT	CG	CG	TC
514				CONTROL										TT	CG	TC	TG
515				CONTROL										TT	CG	TC	TG

ID	dob	Gend	Date Diagnosis	Diagnosis Category	Pres Age	Rec	Date Rec	Time Rec	Died	Date FU	Time FU	GTR	grade	m460 Gen	p405 Gen	Hap 1	Hap 2
516				CONTROL										TT	CC	TC	TC
517				CONTROL										CC	GG	CG	CG
518				CONTROL										CT	CG	CG	TC
519				CONTROL										CC	GG	CG	CG
520				CONTROL										TT	CG	TC	TG
521				CONTROL										CT	GG	TG	CG
522				CONTROL										CC	GG	CG	CG
523				CONTROL										CT	CG	CG	TC
524				CONTROL										CT	GG	TG	CG
525				CONTROL										TT	CG	TC	TG
526				CONTROL										TT	CC	TC	TC
527				CONTROL										TT	GG	TG	TG
528				CONTROL										CT	CG	CG	TC
529				CONTROL										CC	GG	CG	CG
530				CONTROL										CT	GG	TG	CG
531				CONTROL										CC	GG	CG	CG
532				CONTROL										CC	GG	CG	CG
533				CONTROL										TT	CG	TC	TG
534				CONTROL										CT	GG	TG	CG
535				CONTROL										CT	GG	TG	CG
536				CONTROL										CT	GG	TG	CG
537				CONTROL										TT	CG	TC	TG
538				CONTROL										CC	GG	CG	CG
539				CONTROL										CT	CG	CG	TC
540				CONTROL										CT	GG	TG	CG
541				CONTROL										TT	CC	TC	TC
542				CONTROL										TT	CG	TC	TG
543				CONTROL										CC	GG	CG	CG
544				CONTROL										CT	CG	CG	TC
545				CONTROL										CT	GG	TG	CG
546				CONTROL										CT	CG	CG	TC
547				CONTROL										CT	CG	CG	TC
548				CONTROL										CT	CG	CG	TC
549				CONTROL										CC	GG	CG	CG
550				CONTROL										CC	GG	CG	CG
551				CONTROL										CC	GG	CG	CG
552				CONTROL										CC	GG	CG	CG
553				CONTROL										TT	GG	TG	TG
554				CONTROL										CT	CG	CG	TC
555				CONTROL										TT	GG	TG	TG
556				CONTROL										CT	CG	CG	TC
557				CONTROL										CT	CG	CG	TC
558				CONTROL										CT	GG	TG	CG
559				CONTROL										CT	CG	CG	TC
560				CONTROL										TT	CG	TC	TG
561				CONTROL										CT	GG	TG	CG
562				CONTROL										CT	CG	CG	TC
563				CONTROL										CT	CG	CG	TC
564				CONTROL										TT	CG	TC	TG
565				CONTROL										TT	CG	TC	TG
566				CONTROL										CT	CG	CG	TC
567				CONTROL										CC	GG	CG	CG

ID	dob	Gend	Date Diagnosis	Diagnosis Category	Pres Age	Rec	Date Rec	Time Rec	Died	Date FU	Time FU	GTR	grade	m460 Gen	p405 Gen	Hap 1	Hap 2
568				CONTROL										CT	GG	TG	CG
569				CONTROL										TT	GG	TG	TG
570				CONTROL										CT	CG	CG	TC
571				CONTROL										CC	GG	CG	CG
572				CONTROL										TT	CC	TC	TC
573				CONTROL										CT	GG	TG	CG
574				CONTROL										CT	GG	TG	CG
575				CONTROL										TT	CC	TC	TC
576				CONTROL										TT	CG	TC	TG
577				CONTROL										CC	GG	CG	CG
578				CONTROL										CT	CG	CG	TC
579				CONTROL										CT	CG	CG	TC
580				CONTROL										TT	CG	TC	TG
581				CONTROL										CC	GG	CG	CG
582				CONTROL										CT	CG	CG	TC
583				CONTROL										TT	GG	TG	TG
584				CONTROL										CT	CG	CG	TC
585				CONTROL										TT	CC	TC	TC
586				CONTROL										CT	GG	TG	CG
587				CONTROL										CT	CG	CG	TC
588				CONTROL										CT	CG	CG	TC
589				CONTROL										CT	GG	TG	CG
590				CONTROL										CC	GG	CG	CG
591				CONTROL										CT	CG	CG	TC
592				CONTROL										CT	CG	CG	TC
593				CONTROL										CC	GG	CG	CG
594				CONTROL										CT	CG	CG	TC
595				CONTROL										CT	GG	TG	CG
596				CONTROL										CT	CG	CG	TC
597				CONTROL										TT	CG	TC	TG
598				CONTROL										CC	GG	CG	CG
599				CONTROL										CT	GG	TG	CG
600				CONTROL										CT	GG	TG	CG
601				CONTROL										CC	GG	CG	CG
602				CONTROL										TT	GG	TG	TG
603				CONTROL										CT	GG	TG	CG
604				CONTROL										CC	GG	CG	CG
605				CONTROL										CT	GG	TG	CG
606				CONTROL										CT	CG	CG	TC
607				CONTROL										TT	CG	TC	TG
608				CONTROL										TT	CC	TC	TC
609				CONTROL										TT	CG	TC	TG
610				CONTROL										CT	GG	TG	CG
611				CONTROL										TT	CC	TC	TC
612				CONTROL										TT	CC	TC	TC
613				CONTROL										TT	CC	TC	TC
614				CONTROL										CT	CG	CG	TC
615				CONTROL										CT	CG	CG	TC
616				CONTROL										CC	GG	CG	CG
617				CONTROL										CT	CG	CG	TC
618				CONTROL										CC	GG	CG	CG
619				CONTROL										CC	GG	CG	CG

ID	dob	Gend	Date Diagnosis	Diagnosis Category	Pres Age	Rec	Date Rec	Time Rec	Died	Date FU	Time FU	GTR	grade	m460 Gen	p405 Gen	Hap 1	Hap 2
620				CONTROL										TT	CC	TC	TC
621				CONTROL										CC	GG	CG	CG
622				CONTROL										CC	GG	CG	CG
623				CONTROL										TT	CG	TC	TG
624				CONTROL										CT	CG	CG	TC
625				CONTROL										TT	CG	TC	TG
626				CONTROL										CT	GG	TG	CG
627				CONTROL										CC	GG	CG	CG
628				CONTROL										CT	CG	CG	TC
629				CONTROL										CT	CG	CG	TC
630				CONTROL										CT	GG	TG	CG
631				CONTROL										CC	GG	CG	CG
632				CONTROL										CT	GG	TG	CG
633				CONTROL										CT	CG	CG	TC
634				CONTROL										CC	GG	CG	CG
635				CONTROL										CT	CG	CG	TC
636				CONTROL										CC	GG	CG	CG
637				CONTROL										CT	CG	CG	TC
638				CONTROL										CT	GG	TG	CG
639				CONTROL										CC	GG	CG	CG
640				CONTROL										CT	CG	CG	TC
641				CONTROL										TT	CG	TC	TG
642				CONTROL										TT	CG	TC	TG
643				CONTROL										TT	CC	TC	TC
644				CONTROL										CT	CG	CG	TC
645				CONTROL										TT	CG	TC	TG
646				CONTROL										CC	GG	CG	CG
647				CONTROL										CC	GG	CG	CG
648				CONTROL										CC	GG	CG	CG
649				CONTROL										CT	CG	CG	TC
650				CONTROL										TT	CG	TC	TG
651				CONTROL										CT	CG	CG	TC
652				CONTROL										CT	GG	TG	CG
653				CONTROL										TT	CG	TC	TG
654				CONTROL										CT	CG	CG	TC
655				CONTROL										CC	GG	CG	CG
656				CONTROL										CC	GG	CG	CG
657				CONTROL										CC	GG	CG	CG
658				CONTROL										CC	GG	CG	CG
659				CONTROL										CT	CG	CG	TC
660				CONTROL										CT	CG	CG	TC
661				CONTROL										TT	CG	TC	TG
662				CONTROL										CT	GG	TG	CG
663				CONTROL										CT	CG	CG	TC
664				CONTROL										CT	GG	TG	CG
665				CONTROL										TT	GG	TG	TG
666				CONTROL										CC	GG	CG	CG
667				CONTROL										CT	CG	CG	TC

Appendix V – Journal of Neurosurgery Pediatrics paper

Use of ifosfamide, carboplatin, and etoposide chemotherapy in choroid plexus carcinoma

Clinical article

Lucie Lafay-Cousin, M.D., M.Sc.,[1] Donald J. Mabbott, Ph.D.,[2,5] William Halliday, M.D.,[3] Michael D. Taylor, M.D., Ph.D.,[4] Uri Tabori, M.D.,[5] Ian D. Kamaly-Asl, M.B.Ch.B., F.R.C.S.(SN),[4] Abhaya V. Kulkarni, M.D., Ph.D.,[4] Ute Bartels, M.D.,[5] Mark Greenberg, M.D.,[6] and Eric Bouffet, M.D.[5]

[1]Department of Pediatric Oncology and Bone Marrow Transplantation, Alberta Children's Hospital, Calgary, Alberta; Departments of [2]Psychology and [3]Pathology, Divisions of [4]Pediatric Neurosurgery and [6]Pediatric Hematology/Oncology, and [5]Pediatric Brain Tumor Program, Division of Pediatric Hematology/Oncology, Hospital for Sick Children, Toronto, Ontario, Canada

Object. Choroid plexus carcinomas (CPCs) are rare pediatric tumors with a generally poor prognosis. Although the role of surgery is well recognized, the role of adjuvant chemotherapy and radiation therapy remains unclear. In this paper, the authors' goal was to assess the role of second-look surgery and neoadjuvant ifosfamide, carboplatin, etoposide (ICE) chemotherapy in the management of CPC and to study neurocognitive outcome.

Methods. The authors performed an institutional retrospective review of patients in whom CPC was diagnosed between 1985 and 2006 at the Hospital for Sick Children in Toronto. Fourteen patients (7 boys and 7 girls) were included. The median age at diagnosis was 18.6 months (range 1.1–65.3 months). Four patients had evidence of metastatic disease at diagnosis. Two of the 14 patients underwent gross-total resection during initial surgery; 12 of the patients received neoadjuvant chemotherapy, 10 of whom underwent second surgery. In total, of 12 patients who received chemotherapy with a curative intent, 11 underwent a greater than 95% resection. Neoadjuvant ICE chemotherapy was given prior to second surgery (median 4 cycles, range 2–5 cycles) and was continued after second resection for a median total of 7 cycles (range 4–16 cycles).

Results. No tumor progression was observed during chemotherapy prior to second surgery. Five patients subsequently experienced tumor progression/relapse. At a median follow-up of 6.9 years (range 1.9–18.5 years), 8 patients are alive. None of the survivors received radiation therapy. However, 6 of 8 display significant neurocognitive and/or sensorial deficit.

Conclusions. In this experience, second surgery following neoadjuvant ICE chemotherapy led to a high rate of complete or near-complete resection. Chemotherapy appears to facilitate second-look surgery, in particular through a reduction of intraoperative blood loss. Despite radiation avoidance, the majority of survivors experienced significant neurocognitive impairment. *(DOI: 10.3171/2010.3.PEDS09354)*

Key Words • **choroid plexus carcinoma** • **second look surgery** • **adjuvant chemotherapy**

Choroid plexus tumors account for 0.4–0.6% of all intracranial tumors. However, the incidence of choroid plexus tumors is disproportionally high in children younger than 2 years, in whom they represent up to 12% of CNS tumors. Twenty to 40% of all choroid plexus tumors are CPCs, and 70% of all CPCs occur in patients younger than 24 months.[2,10,16,18,23] Unlike choroid plexus papillomas, which have a high cure rate after complete resection, CPCs carry a dismal prognosis.[11,22] Nonsurgical management of a CPC has included a variety of postoperative chemotherapy regimens with or without radiation therapy. Information collected from small series most often includes various modalities of postsurgical treatment, encompassing several eras of surgical and imaging techniques.[2,5,23–25] The role of surgery has been well reported, and it is now established that complete resection is a key prognostic factor in CPCs.[8,16]

Abbreviations used in this paper: CPC = choroid plexus carcinoma; GTR = gross-total resection; ICE = ifosfamide, carboplatin, etoposide; ICP = intracranial pressure; NTR = near-total resection; PFS = progression-free survival; STR = subtotal resection.

However, aggressive resection may be limited because of the very invasive nature and the extreme vascularity of CPCs. Some authors have therefore suggested a 2-stage surgical approach with an initial biopsy followed by neoadjuvant chemotherapy and definitive surgery,[10,23] but this experience has not been reported in a series of consecutive patients. We report herein a 20-year institutional experience during which patients with CPC were homogeneously treated, and we analyze the outcome in relation to surgical management, chemotherapy, and irradiation.

Methods

This is a retrospective review of an institutional experience between 1985 and 2006 at the Hospital for Sick Children, where the philosophy of treatment for CPCs has been consistent throughout the study period. In brief, the aim of initial surgery was maximal resection whenever possible without causing further neurological deficit or massive blood loss. When initial surgery was incomplete or was limited to a biopsy, second-look surgery was considered after neoadjuvant ICE chemotherapy. Finally, because of the very young age of patients with CPCs, radiation therapy was never considered as part of the frontline therapy.

This approach was initially approved by the clinical practice committee of the Division of Pediatric Hematology/Oncology, and the retrospective study was approved by the research ethics board of the hospital. We performed a comprehensive chart review of all pediatric patients (< 18 years at the time of diagnosis) in whom CPC was diagnosed. All tumor specimens were reviewed by a neuropathologist (W.H.), and immunohistochemical staining for INI-1 (BAF47) was performed in all tumors to exclude atypical rhabdoid teratoid tumors. Regarding surgery, the degree of resection was based on the surgical report and postoperative imaging. Gross-total resection was defined as no identifiable residual tumor. Near-total resection referred to a greater than 95% resection, STR to a resection greater than 50% but less than 95%, partial resection to resection greater than 10% but less than 50%, and biopsy to a resection of less than 10% of the tumor mass.

The ICE protocol combined 3 g/m^2 ifosfamide (on Days 1 and 2), 150 mg/m^2 etoposide (on Days 1 and 2), and 500 mg/m^2 carboplatin (on Day 3). An incremental increase of the carboplatin dose by 50 mg/m^2 was performed every cycle to reach a maximum dose of 600 mg/m^2 according to clinical tolerance. Each course of ICE was delivered every 21–28 days, when the neutrophil count was greater than 1000/mm^3 and the platelet count was greater than 100,000/mm^3. Neuroimaging studies (MR imaging or CT scanning) were usually performed every 2 cycles.

Assessment of tumor response was based on the radiological report and the review of imaging. The response was classified as follows: a complete response was defined as no evidence of detectable disease; a partial response was defined as a 50% or more reduction in the product of perpendicular diameter; and an objective effect was characterized by a greater than 25% but less than 50% decrease in tumor size. Progressive disease was defined as an increase in tumor size greater than 25%. Stable disease related to all others situations.

Statistical Analysis

Estimation of PFS and overall survival was performed using the Kaplan-Meier analysis. Overall survival was calculated from the date of diagnosis to the date of last follow-up or date of death from any cause. The PFS were calculated from the date of initial diagnosis to the date of earliest radiological disease progression. Statistical analyses were performed using SPSS version 15.0 (SPSS, Inc.).

Results

Patients Characteristics

Of the 38 patients in whom choroid plexus tumors were diagnosed between 1985 and 2006, 17 had a diagnosis of CPC. Three patients were excluded. One patient diagnosed with secondary CPC following radiation and chemotherapy for ependymoma was excluded due to insufficient data. Another was treated with a different chemotherapy regimen and whole-brain radiation therapy after incomplete resection. The third patient was excluded from the analysis after being found to have an atypical teratoid rhabdoid tumor on central pathology review. Therefore, 14 patients (7 boys and 7 girls) were included in this review. The median age at diagnosis was 18.6 months (range 1.1–65.3 months). Six patients (43%) were younger than 12 months old at diagnosis. The vast majority of the tumors were located in the lateral ventricles (8 in the left and 3 in the right lateral ventricles, and 3 in the fourth ventricle). Appropriate initial staging, with imaging of the entire neuraxis and CSF examination, was available in 8 patients (57.1%). The CSF examination was reported to be positive or suspicious in 2 of the 6 tested patients. Four patients were considered to have metastatic disease at diagnosis (2 with Stage M1, 1 with Stage M2, and 1 with Stage M3).[3]

Initial Resection

All children underwent surgery at diagnosis. One patient underwent embolization prior to initial surgery. Two patients achieved an initial GTR but one of them died of intraoperative hemorrhage. One patient underwent an NTR, 5 an STR, 1 a partial resection, and 5 underwent a biopsy. The latest patient in this series developed an intratumoral hemorrhage 24 hours after initial biopsy and required urgent decompressive craniectomy and debulking surgery that led to a partial resection. One patient who had a poor postsurgical neurological outcome proceeded to undergo palliative therapy. Four patients with signs of chronic ICP and significant persistent cerebral edema after initial surgery required high doses of steroids until a second resection was performed. Overall, 12 patients received postoperative ICE chemotherapy. After neoadjuvant chemotherapy, second surgery was performed in 10 of the 12 remaining patients, leading overall to 9 GTRs, 2 NTRs, and 1 STR of the primary tumor site (Table 1). Two of these second procedures were preceded by elective embolization.

TABLE 1: Summary of treatment and outcome*

Case No.	Age at Dx (mos), Sex	Met Stage (MRI/CSF cytology)†	Initial Op	Adjuvant Chemo?	Chemo Prior to 2nd Op	Tumor Response to Chemo Prior to 2nd Op	2nd Op of Primary Tumor	Total No. of Cycles of Chemo	RT Indication (vol dose)	Relapse	Salvage Therapy	Current Status, FU (mos)
1	9, M	–/UK	STR	no								DOD, 2.5
2	24.2, F	–/UK	STR	yes	ICE × 5	partial	GTR	ICE × 8	relapse CSI 3600/5400 cGy	1	CSI + chemo	DOD, 25.4
3	1.1, M	–/UK	STR	yes	ICE × 3	OE	GTR	ICE × 6	0	?	chemo	alive, ≥234.9
4	7.5, F	–/UK	PR	yes	ICE × 4	SD	STR	ICE × 16	local residual disease CSI 3600/4860 cGy	1	0	DOD, 60.2
5	28.3, M	M2/UK	STR	yes	ICE × 5	partial	GTR	ICE × 7	residual met disease CSI 3600/4500 cGy	1	0	DOD, 17.0
6	6.7, F	–/UK	GTR	no								intraop death
7	5, F	–/M1	STR	yes	ICE × 4	OE	GTR	ICE × 7	0	?	0	alive, ≥151.4
8	40.3, M	–/M0	NTR	yes		SD‡	no op	ICE × 10	0	0		alive, ≥139.7
9	63.3, F	–/M1	biopsy	yes	ICE × 3	SD	GTR	ICE × 6	0	0		alive, ≥113.9
10	2.8, M	M3/UK	biopsy	yes	ICE × 4; HDC × 2 (Cb & thiotepa)	SD	GTR	ICE × 4; HDC × 3 (Cb & thiotepa)	0	0		alive, ≥52
11	52.6, M	–/M0	GTR	yes		NA	no op	ICE × 8	0	1	0	DOD, 10.6
12	65.3, M	–/M0	biopsy	yes	ICE × 2	SD	GTR	ICE × 8	0	0		alive, ≥52
13	13, F	–/M0	biopsy	yes	ICE × 5	OE	GTR	ICE × 7	0	1	HDC × 3 (Bu & thiotepa)	alive, ≥40.7
14	56.4, F	–/UK	biopsy/PR§	yes	ICE × 4	SD	NTR	ICE × 5; HDC × 3 (Cb & thiotepa)	0	0		alive, ≥22.4

* Bu = busulfan; Cb = carboplatin; Chemo = chemotherapy; CSI = craniospinal irradiation; DOD = died of disease; FU = follow-up; HDC = high-dose chemotherapy; Met = metastatic; NA = not applicable; OE = objective effect; PR = partial resection; RT = radiotherapy; SD = stable disease; UK = unknown; ? = questionable relapse in retrospect; – = negative.

† Tumor staging is according to the staging system by Chang et al.

‡ This patient did not undergo a second surgery. The tumor response was assessed after 4 cycles of ICE.

§ Partial resection was performed 24 hours after the initial biopsy.

A comparison of the initial histology with subsequent specimens showed a similar macroscopic appearance in all patients. However, the most striking finding was the evidence of fibrosis and collagenization within the tumor following chemotherapy, observed in 5 of 10 specimens. Collagenization was particularly apparent around large blood vessels and tumor nests in post-ICE chemotherapy specimens (Fig. 1). Comparison of first and second surgical specimens also showed evidence of tumor necrosis in 2 cases and trabecular maturation in 2 other cases, while a significant decrease in the number of mitotic figures and/or the Ki 67 proliferation index was observed in 7 cases.

After repeated surgical procedures, 2 patients had local GTR but persistent residual abnormalities at the metastatic sites, one in the left frontal horn and the other in the thoracic spine (Cases 5 and 10).

Postoperative Therapy

The ICE chemotherapy was administered in 12 patients after initial surgery. The total number of cycles of ICE delivered ranged from 4 to 16 (median 7 cycles). Ten patients who underwent second-look surgery received a median number of 4 cycles of ICE (range 2–5) prior to the second resection. Most recently, 2 patients with extensive disease received consolidation chemotherapy with 3 courses of sequential high-dose carboplatin and thiotepa with stem cell rescue following 4 cycles of ICE. Eleven patients with measurable disease after initial surgery were assessable for tumor response prior to second surgery: 2 patients had a partial response, 3 had an objective effect, and 6 had stable disease. Two patients received craniospinal irradiation for persistent disease (local in one and disseminated in the other) at 28 and 36 months of age, respectively. They both died of disease progression 39 and 9 months after radiation therapy.

Postoperative Outcome

Of 12 patients who underwent postsurgical treatment, 5 (41.7%) experienced progression or relapse at a median time of 12 months (range 5.2–58.8 months) from diagnosis. One patient developed an isolated single distant lesion, and 4 had disseminated metastatic relapse. In addition, 2 patients had questionable relapses in retrospect. One patient (Case 3) with a right lateral ventricular tumor presented with an isolated nodule in the left occipital horn 5.2 months after diagnosis. He received additional chemotherapy for 7 months (bleomycin/vinblastine/cisplatin, ICE, and carboplatin/vinblastine/etoposide). Repeated surgery after additional chemotherapy disclosed normal choroid plexus tissue. The patient is alive 17 years after diagnosis. Another patient (Case 7) showed diffuse thickening along the spinal cord on the end-of-treatment MR image, and CSF cytology was reported to be suspicious for malignant cells. She was offered palliative care and remains alive and well 12 years later without further therapy.

Two patients received active treatment at the time of recurrence. One underwent craniospinal irradiation with transient improvement but eventually died of disease progression. Another patient with isolated distant relapse 15 months after diagnosis (Case 13) underwent repeated surgery (GTR) followed by high-dose chemotherapy (busulfan and thiotepa) with stem cell rescue and remains in complete remission 26 months after relapse. All other patients with disseminated relapse died of disease progression.

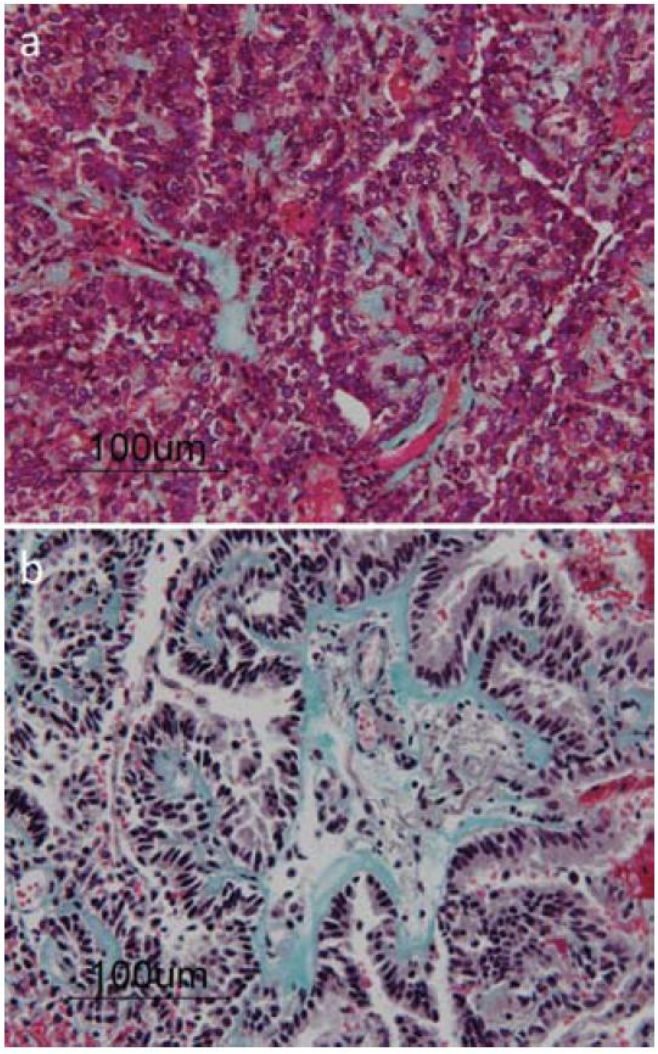


Fig. 1. Trichrome staining at time of initial resection **(a)** and second surgery following chemotherapy **(b)**. The second specimen (post-ICE) shows the fibrovascular core of the papillary tumor to be more fibrotic than was evident on the original biopsy.

At a mean follow-up time of 6.9 years (range 2.2–19.7 years), 8 (66.7%) of the 12 patients who received treatment with curative intent were alive. The PFS and overall survival for this group at 5 years were 53.3 ± 16.1% and 74.1 ± 12.9%, respectively. All the survivors had a resection greater than 95% (GTR and NTR), and none of the survivors received radiation therapy (Fig. 2).

Neurocognitive and Functional Outcome

Of the 8 survivors, 6 patients have significant neurocognitive impairment. Six patients underwent formal neuropsychological testing and 2 underwent developmental assessment (Table 2). One patient had normal academic performance 8 years after diagnosis (Case 8), and 1 patient diagnosed at the age of 13 months showed normal

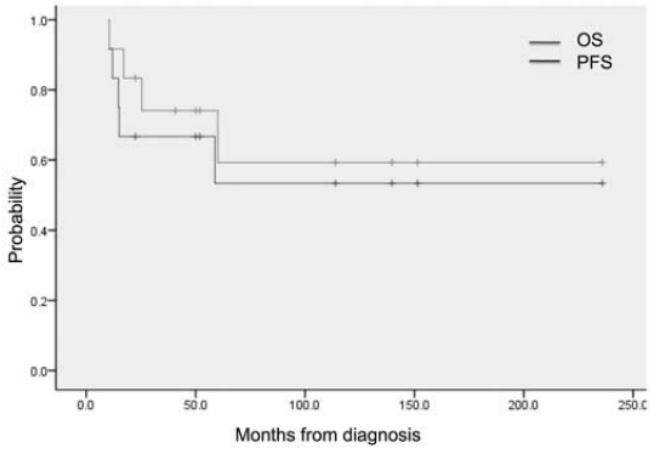


Fig. 2. Kaplan-Meier survival curves showing overall survival (OS) and PFS rates of the 12 patients who received treatment with intent to treat.

development at 2 years of age (Case 13). Details of neurocognitive assessments are described in Table 3.

Two patients are legally blind due to chronic increased ICP. Two other patients had residual dense right homonymous hemianopia. The 2 patients with visual impairment also suffer from severe hearing loss requiring hearing aids and another patient shows high frequencies hearing loss. Finally, 2 of the 8 survivors are being treated for epilepsy.

Discussion

We report here a large institutional series of consecutive patients who were diagnosed with CPC and were treated with a consistent approach combining neoadjuvant ICE chemotherapy and delayed second-look surgery in case of incomplete initial resection. Following this strategy, an NTR was achieved in 11 of 12 patients, and 8 of these patients are alive. Complete resection is a well-recognized prognostic factor in CPC.[2,6,8] However, given the high vascular nature of these tumors, patients with CPC may not always be safely amenable to complete resection at the time of diagnosis. Previous reports have advocated for initial limited surgical diagnostic procedure to reduce the risk of profuse intraoperative bleeding and to allow delayed resection during a second surgical procedure.[23] Our experience confirms that second-look surgery can safely achieve a complete resection in most patients.[27] However, this 2-stage approach may have some limitations. In this series, 1 patient with very large tumor experienced a life-threatening intratumoral hemorrhage following a limited biopsy. This patient required emergency debulking surgery, which was complicated by an extensive hemispheric stroke. Three other patients required high doses of steroids to control severe increased ICP, and 2 of these patients are legally blind as a result of chronic increased ICP.

The precise role of adjuvant therapy (chemotherapy and/or radiotherapy) in CPC remains controversial, especially in the setting of complete resection. In a review of literature, Wrede et al.[28] found a survival advantage for patients receiving chemotherapy with or without radiation therapy, regardless of the extent of resection. In another literature review of 75 patients in whom CPC was diagnosed between 1985 and 2000, Fitzpatrick et al.[7] found a significant benefit of adjuvant treatment (radiation with or without chemotherapy) only for patients with less than GTR. Chemotherapy in CPC may contribute to decreasing the size but more importantly the vascularity of the tumor, and therefore may facilitate second-look surgery.[21] In our experience, the use of the ICE regimen did not result in dramatic tumor shrinkage. However, from the surgeon's perspective, the role of chemotherapy was significant in reducing intraoperative blood loss and therefore in improving resection rates and outcomes. In a series overlapping ours, Kamaly-Asl et al.[14] described a significant reduction in blood loss from 111 to 22% of the estimated blood volume in the group who received neoadjuvant chemotherapy prior to second-look surgery

TABLE 2: Neurocognitive and functional outcomes*

			Formal Neuropsychological or Developmental Evaluation		Outcome			
Case No.	Age at Dx (mos), Sex	Age at Last FU (yrs)	Type	Diagnostic Outcome	Vision	Hearing	Seizure	Lansky Score†
3	1.1, M	18.6	neuropsychological	developmental disability	normal	normal	yes	90
7	5, F	13	neuropsychological	learning disability	normal	high-frequency HL	no	100
8	40.3, M	15	neuropsychological	normal development	normal	normal	no	100
9	63.3, F	14.8	neuropsychological	developmental disability	blind	hearing aids	yes	90
10	2.8, M	4.6	developmental	developmental delay	blind	hearing aids	no	90
12	65.3, M	9.6	neuropsychological	intellectual disability	rt dense homonymous hemianopia	normal	no	100
13	13, F	4.5	developmental	normal development	normal	normal	no	100
14	56.4, F	6.6	neuropsychological	intellectual disability	rt dense homonymous hemianopia	normal	no	80

* HL = hearing loss.

† Scores are based on the performance measurement scale of Lansky et al.

TABLE 3: Neurocognitive test data*

Case No.	Age at Assessment (yrs)	VIQ	PIQ	FSIQ	Receptive Language	Reading	Spelling	Mathematics
3†	9.42	—	—	—	40	—	—	—
7	11.42	79	100	81	95	83	74	66
8	10.67	112	93	103	—	104	115	101
9†	8.42	50	—	—	—	—	—	—
12	7.92	79	61	60	57	60	70	57
14	5.67	78	77	74	—	—	—	—

* Standard scores with a mean of 100 and standard deviation of 15. Abbreviations: FSIQ = full-scale IQ; PIQ = performance IQ; VIQ = verbal IQ; — = area was not assessed.

† For these patients, only limited assessment was possible due to their very low functioning.

($p = 0.01$). In an attempt to reduce tumoral vascularity, other groups have reported the potential benefit of preoperative embolization. However, with only 3 patients who underwent this procedure (1 at the time of diagnosis and 2 prior to second-look surgery), no meaningful conclusion can be drawn from our series.[19] The surgical findings of reduced blood loss following adjuvant ICE chemotherapy are corroborated by the comparison of pathology specimens from the same patients with an obvious increase in perivascular collagen within the fibrovascular cores of the tumor papillae between the first and the second operation as illustrated in Fig. 1.

The median number of courses of ICE prior to second-look surgery was 4. The interval between initial and second-look surgery was not predetermined in our experience. Such interval should take into account the potential toxicity of chemotherapy and the ability to medically control cerebral edema when using chemotherapy and hyperhydration in children at risk for increased ICP due to persistent mass effect. Other factors may influence this interval, such as the age of the child, particularly in neonates and infants in whom significant increase in the size of the brain over a 3–6-month period may be critical in facilitating a safer resection of the tumor. The risk of infection associated with the use of high-dose steroids and the potential need for shunt revisions must also be taken into account. In the setting of a retrospective study, it would be perilous to establish a correlation between the duration of adjuvant chemotherapy and functional outcome. Ideally, response to chemotherapy should help decide the optimal timing of second surgery. However, since chemotherapy after a median of 4 cycles (about 4 months) did not lead to major shrinkage, our experience does not provide sufficient evidence that a shorter duration of chemotherapy would be as successful in reducing intraoperative blood loss. The optimal balance between the benefit of neoadjuvant chemotherapy and the risks of delayed surgery remains to be determined.

With a median follow-up of 6.9 years, the 5-year overall survival of 74.1% for intent-to-treat patients favorably compares with that in previous reports.[2,4,6,8,18] Whether adjuvant chemotherapy delivered after second surgery contributes to improved survival remains unknown. However, although 90% of our patients achieved at least an NTR, 3 patients experienced relapse after GTR. The fact that 1 patient with distant relapse underwent successful salvage surgery followed by high-dose chemotherapy is also encouraging. This clearly suggests a role for adjuvant chemotherapy in CPC. However, several questions remain unanswered concerning the respective benefits and risks of adjuvant chemotherapy in this population, particularly in the context of germline *p53* mutation where the risk of secondary malignancy is not negligible. The use of nonalkylating agents in that context should be explored.

Our approach also allowed avoidance of radiation therapy. The use of adjuvant radiation in CPC is still unclear and raises ethical issues, particularly because of the large size of these tumors and the very young age of most patients at diagnosis.[1,20,25,26] Since only 3 patients received radiation therapy in the present series (1 at the time of recurrence and 2 for persistent disease), we cannot conclusively comment on any potential effects of such therapy. However, despite avoidance of radiation therapy, the majority of the survivors have neurocognitive impairment. Severe ICP at diagnosis, overproduction of CSF by the tumor leading to persistent hydrocephalus[5,7,13,19] (sometimes even after complete resection), and vascular complications may negatively impact intellectual outcome. Frequently reported complications such as chronic subdural collections, repeated shunt obstructions, and/or infections may additionally affect neurocognitive outcome.[10,12,19] In light of the neurocognitive profile of the survivors, the use of radiation should carefully be weighed since its benefit has not been clearly established.

In our series, 4 patients (28.6%) had metastatic disease at diagnosis. The prognostic value of the metastatic status at diagnosis has not been demonstrated in CPC.[17] Appropriate staging was only performed in 8 of our patients, and this limits our interpretation of the results and their generalization. In the St. Jude experience, 7 of the 10 patients with CPC had evidence of metastatic disease at diagnosis, and 5 of them were long-term survivors.[5] Similarly, among the 8 long-term survivors in the present series, 3 had evidence of metastatic disease at diagnosis. However, the radiological findings of the patient with Stage M3 disease in our experience did not change over time. This finding and the "pseudorecurrence" observed in the patient in Case 7 question the reliability of the radiological and cytological staging of this tumor, especially when atypical cells are present in the CSF or when the spinal MR imaging shows evidence of clumping of the roots or subtle leptomeningeal enhancement.[9] Some

of the abnormalities observed on spinal MR imaging in patients with CPC might be related to disorders of CSF flow and CSF resorption rather than to metastatic disease, and the diagnosis of metastatic disease may be overestimated in choroid plexus tumors, particularly in CPCs. Whether confirmation of the metastatic stage may require histological authentication is a matter of debate. However, implications in terms of treatment are significant, particularly with regard to radiation doses and volumes, when the decision to proceed to focal radiation is balanced against craniospinal radiation in these young patients.

Conclusions

In our series, second surgery following neoadjuvant ICE chemotherapy led to a high rate of complete resection. The ICE chemotherapy facilitated second surgery by reducing tumor vascularity. Following this strategy, the survival rate was 66.7% in the intent-to-treat patients. Despite avoidance of radiation therapy, the majority of long-term survivors experienced significant neurocognitive impairment. Future studies should further explore the safety of such staged approaches that might in addition benefit from the introduction of new antiangiogenic molecules.

Disclosure

This work was supported by a grant from B.R.A.I.N child foundation.

Author contributions to the study and manuscript preparation include the following. Conception and design: L Lafay-Cousin. Acquisition of data: all authors. Analysis and interpretation of data: L Lafay-Cousin, DJ Mabbott, U Tabori, U Bartels, E Bouffet. Drafting the article: L Lafay-Cousin. Critically revising the article: L Lafay-Cousin, DJ Mabbott, W Halliday, U Tabori, U Bartels, M Greenberg, E Bouffet. Reviewed final version of the manuscript and approved it for submission: all authors. Statistical analysis: DJ Mabbott. Study supervision: L Lafay-Cousin, E Bouffet.

References

1. Allen J, Wisoff J, Helson L, Pearce J, Arenson E: Choroid plexus carcinoma—responses to chemotherapy alone in newly diagnosed young children. **J Neurooncol 12:**69–74, 1992
2. Berger C, Thiesse P, Lellouch-Tubiana A, Kalifa C, Pierre-Kahn A, Bouffet E: Choroid plexus carcinomas in childhood: clinical features and prognostic factors. **Neurosurgery 42:** 470–475, 1998
3. Chang CH, Housepian EM, Herbert C Jr: An operative staging system and a megavoltage radiotherapeutic technic for cerebellar medulloblastomas. **Radiology 93:**1351–1359, 1969
4. Chow E, Reardon DA, Shah AB, Jenkins JJ, Langston J, Heideman RL, et al: Pediatric choroid plexus neoplasms. **Int J Radiat Oncol Biol Phys 44:**249–254, 1999
5. Due-Tønnessen B, Helseth E, Skullerud K, Lundar T: Choroid plexus tumors in children and young adults: report of 16 consecutive cases. **Childs Nerv Syst 17:**252–256, 2001
6. Duffner PK, Kun LE, Burger PC, Horowitz ME, Cohen ME, Sanford RA, et al: Postoperative chemotherapy and delayed radiation in infants and very young children with choroid plexus carcinomas. **Pediatr Neurosurg 22:**189–196, 1995
7. Eisenberg HM, McComb JG, Lorenzo AV: Cerebrospinal fluid overproduction and hydrocephalus associated with choroid plexus papilloma. **J Neurosurg 40:**381–385, 1974
8. Fitzpatrick LK, Aronson LJ, Cohen KJ: Is there a requirement for adjuvant therapy for choroid plexus carcinoma that has been completely resected? **J Neurooncol 57:**123–126, 2002
9. Geerts Y, Gabreëls F, Lippens R, Merx H, Wesseling P: Choroid plexus carcinoma: a report of two cases and review of the literature. **Neuropediatrics 27:**143–148, 1996
10. Greenberg ML: Chemotherapy of choroid plexus carcinoma. **Childs Nerv Syst 15:**571–577, 1999
11. Gupta N: Choroid plexus tumors in children. **Neurosurg Clin N Am 14:**621–631, 2003
12. Johnson DL: Management of choroid plexus tumors in children. **Pediatr Neurosci 15:**195–206, 1989
13. Kahn EA, Luros JT: Hydrocephalus from overproduction of cerebrospinal fluid, and experiences with other parillomas of the choroid plexus. **J Neurosurg 9:**59–67, 1952
14. Kamaly-Asl ID, Bouffet E, Halliday W, Drake JM, Rutka JT, Dirks PB, et al: Neoadjuvant chemotherapy reduces operative haemorrhage and improves resection for choroid plexus carcinomas. **Childs Nerv Syst 21:**852, 2005 (Abstract)
15. Lansky SB, List MA, Lansky LL, Ritter-Sterr C, Miller DR: The measurement of performance in childhood cancer patients. **Cancer 60:**1651–1656, 1987
16. McEvoy AW, Harding BN, Phipps KP, Ellison DW, Elsmore AJ, Thompson D, et al: Management of choroid plexus tumours in children: 20 years experience at a single neurosurgical centre. **Pediatr Neurosurg 32:**192–199, 2000
17. Meyers SP, Khademian ZP, Chuang SH, Pollack IF, Korones DN, Zimmerman RA: Choroid plexus carcinomas in children: MRI features and patient outcomes. **Neuroradiology 46:**770–780, 2004
18. Packer RJ, Perilongo G, Johnson D, Sutton LN, Vezina G, Zimmerman RA, et al: Choroid plexus carcinoma of childhood. **Cancer 69:**580–585, 1992
19. Pencalet P, Sainte-Rose C, Lellouch-Tubiana A, Kalifa C, Brunelle F, Sgouros S, et al: Papillomas and carcinomas of the choroid plexus in children. **J Neurosurg 88:**521–528, 1998
20. Pierga JY, Kalifa C, Terrier-Lacombe MJ, Habrand JL, Lemerle J: Carcinoma of the choroid plexus: a pediatric experience. **Med Pediatr Oncol 21:**480–487, 1993
21. Razzaq AA, Cohen AR: Neoadjuvant chemotherapy for hypervascular malignant brain tumors of childhood. **Pediatr Neurosurg 27:**296–303, 1997
22. Rickert CH, Paulus W: Tumors of the choroid plexus. **Microsc Res Tech 52:**104–111, 2001
23. St Clair SK, Humphreys RP, Pillay PK, Hoffman HJ, Blaser SI, Becker LE: Current management of choroid plexus carcinoma in children. **Pediatr Neurosurg 17:**225–233, 1991–1992
24. Strojan P, Popović M, Surlan K, Jereb B: Choroid plexus tumors: a review of 28-year experience. **Neoplasma 51:**306–312, 2004
25. Wolff JE, Sajedi M, Brant R, Coppes MJ, Egeler RM: Choroid plexus tumours. **Br J Cancer 87:**1086–1091, 2002
26. Wolff JE, Sajedi M, Coppes MJ, Anderson RA, Egeler RM: Radiation therapy and survival in choroid plexus carcinoma. **Lancet 353:**2126, 1999 (Letter)
27. Wrede B, Liu P, Ater J, Wolff JE: Second surgery and the prognosis of choroid plexus carcinoma—results of a meta-analysis of individual cases. **Anticancer Res 25 (6C):**4429–4433, 2005
28. Wrede B, Liu P, Wolff JE: Chemotherapy improves the survival of patients with choroid plexus carcinoma: a meta-analysis of individual cases with choroid plexus tumors. **J Neurooncol 85:**345–351, 2007

Manuscript submitted August 25, 2009.
Accepted March 9, 2010.

Address correspondence to: Lucie Lafay-Cousin, M.D., M.Sc., Department of Pediatric Oncology and Bone Marrow Transplantation, Alberta Children's Hospital, 2888 Shaganappi Trail NW, T3B 6A8, Calgary, Alberta, Canada. email: lucie.lafay-cousin@albertahealthservices.ca.

Appendix VI – Neurosurgery Focus paper

Neurosurg Focus 20 (1):E10, 2006

Genetics of choroid plexus tumors

IAN D. KAMALY-ASL, M.B., CH.B., F.R.C.S.(SN), NAVID SHAMS, AND MICHAEL D. TAYLOR, M.D., PH.D., F.R.C.S.(C)

Greater Manchester Neurosciences Centre, Hope Hospital, Manchester, United Kingdom; and Division of Neurosurgery, The Hospital for Sick Children, Toronto, Ontario, Canada

Choroid plexus tumors consist of papillomas and carcinomas. A variety of germline and somatic genetic changes have been demonstrated for each of these subtypes. In this paper, the authors summarize the current knowledge of the genetic bases of these tumors.

KEY WORDS • choroid plexus carcinoma • choroid plexus papilloma • *TP53* gene • *hSNF5/INI1* gene • genetics

Choroid plexus tumors are rare tumors of neuroectodermal origin. They represent approximately 0.5% of all brain tumors, and their annual incidence is 0.3 cases per 1 million population.[5,29] Associated with the development of these tumors are both germline and somatic abnormalities located at several genetic loci.

The TP53 *Gene*

The *TP53* gene is located on 17p13.1; this gene expresses the protein product p53, which influences tumor suppression via a variety of mechanisms including DNA repair, apoptosis, cellular differentiation, and angiogenesis.[18] A mutation of the *TP53* gene causes a loss of p53 function as well as prolongation of the half life of the protein. This means that increased immunohistochemical staining for p53 protein can be used as a surrogate marker of gene mutation.[14]

Choroid plexus carcinomas are one of the tumors found in Li-Fraumeni families with *TP53* germline mutations.[8,10,26,27] In addition, spontaneous germline and somatic p53 mutations have both been identified in patients with choroid plexus carcinomas.[28,32] Positive nuclear staining for p53 protein is evident in the majority of choroid plexus carcinomas (10 of 11), whereas it is only seen rarely in choroid plexus papillomas (one of 12).[3] Mutations of *TP53* have not been extensively studied in patients with choroid plexus papillomas; however, germline mutations have been reported.[11,16]

The hSNF5/INI1 *Gene*

The *hSNF5/INI1* gene is located on 22q11.2 and encodes a member of the SWI/SNF adenosine triphosphate-dependent chromatin-remodeling complex.[17] Germline mutations of this gene have been described as rhabdoid predisposition syndrome. In families with this mutation, researchers have identified the development of both renal and extrarenal malignant rhabdoid tumors, choroid plexus carcinomas, atypical teratoid rhabdoid tumors, and medulloblastomas.[17,23] Somatic mutations of the *hSNF5/INI1* gene have also been reported in cases of choroid plexus carcinoma.[32] Authors of several papers offer descriptions of the genotypic and phenotypic overlap between choroid plexus carcinomas and atypical teratoid rhabdoid tumors;[4,30] however, immunohistochemical studies have shown that, in the majority of cases of choroid plexus carcinomas, hSNF5/INI1 protein expression is preserved.[6,7] It therefore has been suggested that tumors believed to be choroid plexus carcinomas with *hSNF5/INI1* mutations may actually be atypical teratoid rhabdoid tumors.[6]

There is no evidence of *hSNF5/INI1* point mutations in patients with choroid plexus papilloma.[11]

Other Syndromes

Aicardi syndrome is a rare, X chromosome–linked dominant condition that is observed in female patients. When it does occur in males with the normal allotment of sex chromosomes, this condition proves lethal during the early gestational period.[1] Affected female patients have callosal agenesis, infantile seizures, and chorioretinal lacunae. These children have visual impairments and usually display severe developmental delays and problematic seizures. Several authors have reported choroid plexus papillomas in girls with Aicardi syndrome.[1,22,24,25]

Hypomelanosis of Ito is a descriptive condition caused by a variety of chromosomal abnormalities and is often associated with other neurological and skeletal abnormalities. In the setting of an X;17(q12;p13) translocation, hypome-

lanosis of Ito has been associated with the development of choroid plexus papillomas.[20,21,31]

The constitutional 9p duplication is another rare abnormality whose association with choroid plexus hyperplasia and choroid plexus papilloma has been reported.[12] Extra copies of chromosome arm 9p have also been found in patients harboring either a sporadic choroid plexus papilloma or carcinoma—a finding that implicates this locus in the formation of both of these tumors.[15]

Chromosomal Imbalances

Multiple chromosomal imbalances have been described in reports of comparative genomic hybridization of choroid plexus tumors.[15] Patients with choroid plexus papillomas have frequently displayed the following chromosomal additions and deletions: +7q (65%); +5q (62%); +7p (59%); +5p (56%); +9p (50%); +9q (41%); +12p and +12q (38%); +8q (35%); −10q (56%); −10p, and −22q (47%). Patients with choroid plexus carcinomas have primarily displayed the following additions and deletions: +12p; +12q, and +20p (60%); +1, +4q, and +20q (53%); +4p (47%); +8q and +14q (40%); +7q, +9p, and +21 (33%); −22q (73%); −5q (40%); −5p and −18q (33%). Certain imbalances are characteristic of the type of tumor and the age of the patient at presentation; from this we can infer a different genetic basis for these tumor variations. A survival analysis showed a survival advantage in patients with choroid plexus carcinomas in whom there was a gain of 9p and a loss of 10q ($p = 0.0186$, log-rank test). Nevertheless, the population group in this analysis was small (10 patients) and was not controlled for different treatments.[15]

Polyomavirus Infection

The related ubiquitous polyomaviruses SV40, JC, and BK have been implicated in the development of choroid plexus neoplasms.[2,9,10,13] Choroid plexus tumors are induced experimentally when the common viral gene product, T antigen, is transgenically expressed in mice.[19] The mechanism of action is the binding of the large T antigen with both p53 and pRb tumor suppressor proteins, complexes demonstrated in humans harboring choroid plexus tumors.[2,33]

Conclusions

A variety of genetic loci are implicated in the development of choroid plexus carcinomas and choroid plexus papillomas. The loci associated with carcinoma generally differ from those associated with papilloma, which leads us to infer a separate genetic basis for these two phenotypically related lesions. Carcinomas, in particular, can be difficult to manage in very young patients, and increased knowledge of the molecular biology of these tumors will hopefully lead to improvements in their treatments and outcomes.

References

1. Aicardi J: Aicardi syndrome. **Brain Dev 27:**164–171, 2005
2. Bergsagel DJ, Finegold MJ, Butel JS, et al: DNA sequences similar to those of simian virus 40 in ependymomas and choroid plexus tumors of childhood. **N Engl J Med 326:**988–993, 1992
3. Carlotti CG Jr, Salhia B, Weitzman S, et al: Evaluation of proliferative index and cell cycle protein expression in choroid plexus tumors in children. **Acta Neuropathol (Berl) 103:**1–10, 2002
4. Gessi M, Giangaspero F, Pietsch T: Atypical teratoid/rhabdoid tumors and choroid plexus tumors: when genetics "surprise" pathology. **Brain Pathol 13:**409–414, 2003
5. Gupta N: Choroid plexus tumors in children. **Neurosurg Clin N Am 14:**621–631, 2003
6. Judkins AR, Burger PC, Hamilton RL, et al: INI1 protein expression distinguishes atypical teratoid/rhabdoid tumor from choroid plexus carcinoma. **J Neuropathol Exp Neurol 64:** 391–397, 2005
7. Judkins AR, Mauger J, Ht A, et al: Immunohistochemical analysis of hSNF5/INI1 in pediatric CNS neoplasms. **Am J Surg Pathol 28:**644–650, 2004
8. Krutilkova V, Trkova M, Fleitz J, et al: Identification of five new families strengthens the link between childhood choroid plexus carcinoma and germline *TP53* mutations. **Eur J Cancer 41:**1597–1603, 2005
9. Lednicky JA, Garcea RL, Bergsagel DJ, et al: Natural simian virus 40 strains are present in human choroid plexus and ependymoma tumors. **Virology 212:**710–717, 1995
10. Malkin D, Chilton-MacNeill S, Meister LA, et al: Tissue-specific expression of SV40 in tumors associated with the Li-Fraumeni syndrome. **Oncogene 20:**4441–4449, 2001
11. Mueller W, Eum JH, Lass U, et al: No evidence of *hSNF5/INI1* point mutations in choroid plexus papilloma. **Neuropathol Appl Neurobiol 30:**304–307, 2004
12. Norman MG, Harrison KJ, Poskitt KJ, et al: Duplication of 9P and hyperplasia of the choroid plexus: a pathologic, radiologic, and molecular cytogenetics study. **Pediatr Pathol Lab Med 15:** 109–120, 1995
13. Okamoto H, Mineta T, Ueda S, et al: Detection of JC virus DNA sequences in brain tumors in pediatric patients. **J Neurosurg 102:**294–298, 2005
14. Pollack IF, Finkelstein SD, Woods J, et al: Expression of p53 and prognosis in children with malignant gliomas. **N Engl J Med 346:**420–427, 2002
15. Rickert CH, Wiestler OD, Paulus W: Chromosomal imbalances in choroid plexus tumors. **Am J Pathol 160:**1105–1113, 2002
16. Rutherford J, Chu CE, Duddy PM, et al: Investigations on a clinically and functionally unusual and novel germline p53 mutation. **Br J Cancer 86:**1592–1596, 2002
17. Sevenet N, Sheridan E, Amram D, et al: Constitutional mutations of the *hSNF5/INI1* gene predispose to a variety of cancers. **Am J Hum Genet 65:**1342–1348, 1999
18. Sherr CJ: Principles of tumor suppression. **Cell 116:**235–246, 2004
19. Small JA, Blair DG, Showalter SD, et al: Analysis of a transgenic mouse containing simian virus 40 and v-myc sequences. **Mol Cell Biol 5:**642–648, 1985
20. Steichen-Gersdorf E, Trawoger R, Duba HC, et al: Hypomelanosis of Ito in a girl with plexus papilloma and translocation (X;17). **Hum Genet 90:**611–613, 1993
21. Sybert VP, Pagon RA: Hypomelanosis of Ito in a girl with plexus papilloma and translocation (X;17). **Hum Genet 93:**227, 1994
22. Taggard DA, Menezes AH: Three choroid plexus papillomas in a patient with Aicardi syndrome. A case report. **Pediatr Neurosurg 33:**219–223, 2000
23. Taylor MD, Gokgoz N, Andrulis IL, et al: Familial posterior fossa brain tumors of infancy secondary to germline mutation of the *hSNF5* gene. **Am J Hum Genet 66:**1403–1406, 2000
24. Trifiletti RR, Incorpora G, Polizzi A, et al: Aicardi syndrome with multiple tumors: a case report with literature review. **Brain Dev 17:**283–285, 1995
25. Uchiyama CM, Carey CM, Cherny WB, et al: Choroid plexus papilloma and cysts in the Aicardi syndrome: case reports. **Pediatr Neurosurg 27:**100–104, 1997
26. Varley JM, Evans DG, Birch JM: Li-Fraumeni syndrome–a molecular and clinical review. **Br J Cancer 76:**1–14, 1997

27. Vital A, Bringuier PP, Huang H, et al: Astrocytomas and choroid plexus tumors in two families with identical p53 germline mutations. **J Neuropathol Exp Neurol 57:**1061–1069, 1998
28. Wang L, Cornford ME: Coincident choroid plexus carcinoma and adrenocortical carcinoma with elevated p53 expression: a case report of an 18-month-old boy with no family history of cancer. **Arch Pathol Lab Med 126:**70–72, 2002
29. Wolff JE, Sajedi M, Brant R, et al: Choroid plexus tumors. **Br J Cancer 87:**1086–1091, 2002
30. Wyatt-Ashmead J, Kleinschmidt-DeMasters B, Mierau GW, et al: Choroid plexus carcinomas and rhabdoid tumors: phenotypic and genotypic overlap. **Pediatr Dev Pathol 4:**545–549, 2001
31. Zajac V, Kirchhoff T, Levy ER, et al: Characterisation of X;17(q12;p13) translocation breakpoints in a female patient with hypomelanosis of Ito and choroid plexus papilloma. **Eur J Hum Genet 5:**61–68, 1997
32. Zakrzewska M, Wojcik I, Zakrzewski K, et al: Mutational analysis of *hSNF5/INI1* and *TP53* genes in choroid plexus carcinomas. **Cancer Genet Cytogenet 156:**179–182, 2005
33. Zhen HN, Zhang X, Bu XY, et al: Expression of the simian virus 40 large tumor antigen (Tag) and formation of Tag-p53 and Tag-pRb complexes in human brain tumors. **Cancer 86:** 2124–2132, 1999

Manuscript received September 15, 2005.
Accepted in final form November 18, 2005.

Address reprint requests to: Ian D. Kamaly-Asl, M.B., Ch.B., F.R.C.S.(SN), Greater Manchester Neurosciences Centre, Hope Hospital, Manchester, M6 8HD, United Kingdom. email: ian.kamaly@srht.nhs.uk.