Juvenile pilocytic astrocytomas:
A search for prognostic markers

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Declaration

I, Dr Naeem Osman, hereby declare that the work on which this dissertation is based is my original work (except where acknowledgements indicate otherwise) and that neither the whole work nor any part of it has been, is being, or is to be submitted for another degree in this or any other university.

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Date: 2nd January 2022

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List of abbreviations

3-bp	3 base pairs
BRAF	B-type rapidly accelerated fibrosarcoma kinase
CDK	Cyclin dependant kinase
CNS	Central nervous system
EDTA	Ethylenediamine tetra-acetic acid
EGF	Epidermal growth factor
EGFR	Epidermal growth factor receptor
ERK	Extracellular signal-regulated kinase (p indicates whether phosphorylated or not)
FGFR	Fibroblast growth factor receptor
FISH	Fluorescence in-situ hybridisation
GDP	Guanosine diphosphate
GTP	Guanosine triphosphate
H&E	Haematoxylin and Eosin
KRAS	Kirsten rat sarcoma viral oncogene homolog
LOH	Loss of heterozygosity
MAP/K	Mitogen-activated protein/kinase
MBP	Myelin basic protein
MDM-2	Murine Double Minute-2
MEK	A mitogen activated protein kinase
mRNA	Messenger ribonucleic acid
mTOR	Mammalian target of rapamycin
NF	Neurofibromatosis
OE	Overexpression
PA	Pilocytic astrocytoma/Juvenile pilocytic astrocytoma
PAS	Periodic acid-Schiff
PBS	Phosphate buffered saline
PDGF	Platelet derived growth factor
PDGFR	Platelet derived growth factor receptor
PIP	Phosphatidyl inositol phosphate

PI3K	Phosphoinositol-3 kinase
PTEN	Phosphotase tensin homolog
PTPN	Protein tyrosine phosphatase, non-receptor type
RAS	Rat sarcoma oncogene
RB	Retinoblastoma
SOS-1	Son of sevenless homolog-1
SRGAP-3	Slit-robo GTPase activating protein 3
TAC	Transit amplifying cell
TGF-β	Transforming growth factor β
TKD	Tyrosine kinase domain
WHO	World Health Organisation
WT	Wild-type

Abstract

Introduction: Juvenile pilocytic astrocytomas are one of the most frequent central nervous system tumours occurring in children. While they are classified as WHO Grade I tumours, their natural progression is difficult to predict with some patients suffering significant morbidity and mortality despite showing similar light microscopic features. Activation of the MAPK pathway of cell proliferation is a consistent finding in these tumours. Studies of these tumours are largely aimed at components of this pathway in an effort to establish reliable prognostic and predictive markers.

Aims and objectives: Our study was aimed at reviewing the light microscopic features and also evaluating the BRAF, p16 and protein kinase ERK components of the MAPK pathway. The findings thereof were correlated with the clinical picture to establish if these markers have any prognostic or predictive value.

Materials and methods: The total number of cases retrieved was 62. The light microscopic findings were evaluated. The cases were analysed for overexpression of BRAF by fluorescence in-situ hybridisation and p16 and pERK by immunohistochemistry. Our findings were considered statistically significant if $P < 0.05$.

Results: There were no specific light microscopic findings present in those cases associated with disease progression and recurrence. BRAF overexpression was associated with better clinical outcomes ($P=0.03$). There was no statistically significant correlation between p16 and pERK expression and patient outcomes.

Conclusion: Overexpression of BRAF in juvenile pilocytic astrocytomas is associated with better clinical outcomes. BRAF may still serve as a therapeutic target and reduce risks associated with surgery, especially in tumours that are not surgically accessible. Further evaluation of p16, pERK and other components of the MAPK pathway of cellular proliferation will undoubtedly be useful in identifying therapeutic targets for those patients who experience disease recurrence and progression.

1 Introduction and Literature review

1.1 Introduction

Astrocytomas are the most frequent central nervous system tumours occurring in children, the most common subtype being juvenile pilocytic astrocytomas. The World Health Organisation classifies these tumours as grade I lesions. The mainstay of treatment is complete surgical resection, however, some tumours arise in surgically inaccessible sites. Approximately 20% of patients experience tumour recurrence or progression irrespective of whether resection was complete or incomplete. To date there is no consistent panel of markers known to identify tumours that pursue an aggressive course or those that will be refractory to treatment. This may be attributed to the genetic heterogeneity of the tumours.

The RAS/MAPK/ERK pathway of cell growth and proliferation is upregulated in a variety of tumours including astrocytomas. Our study aims to evaluate the expression of BRAF, p16 and pERK components of the RAS/MAPK/PRK pathway via fluorescent in-situ hybridization (FISH) and immunohistochemical stains on archived paraffin blocks. The findings will be correlated with tumour location and patient outcome in an attempt to further understand the mechanisms responsible for the differing behaviours of these tumours despite their identical morphology. We also aim to use our findings to establish a panel of prognostic markers which could be incorporated into daily practice to differentiate early on between cases that are likely to be cured and those that are likely to recur or progress.

1.2 Epidemiology

1.2.1 Incidence

The incidence of paediatric CNS tumours varies worldwide, ranging from 1.15 to 5.14 cases per 100 000 children. The highest incidence is seen in the United States[1]. The prognosis is dependant on a number of factors that includes subtype of tumour and tumour location.

Amongst paediatric patients (age 0-19 years) JPA is the most prevalent CNS tumour, representing approximately 19.7% of all cases of paediatric CNS tumours[2]. Most cases occur sporadically. Few cases are seen in patients older than 50 years. The sex distribution is fairly equal with some studies reporting a slight male predominance [3].

1.2.2. Genetic predisposition

Patients with type 1 neurofibromatosis (NF-1) show a predisposition to developing juvenile pilocytic astrocytomas. Approximately 15% of NF-1 patients develop juvenile pilocytic astrocytomas, with the optic nerve being the most common site [2]. These patients may also develop bilateral pilocytic astrocytomas. Conversely, one-third of patients who have pilocytic astrocytomas of the optic nerve have NF-1 [2,49]. Juvenile pilocytic astrocytomas have also been described in patients with Noonan syndrome, a neuro-cardio-facial-cutaneous syndrome characterised by germline mutations in PTPN, RAS and SOS1 genes which are also components of the MAPK pathway.

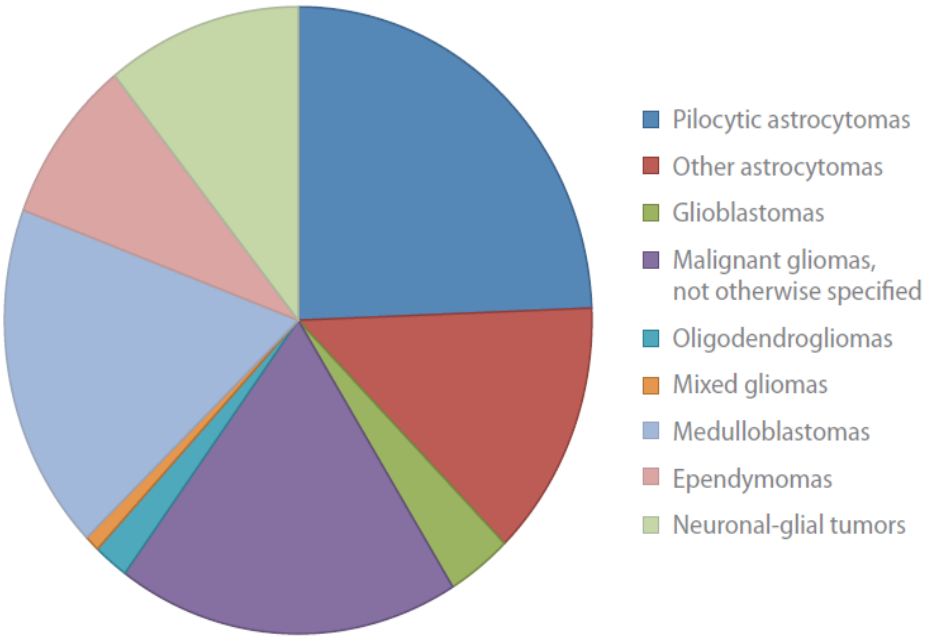

Figure 1: Primary CNS tumour distribution in children aged 0-14 years.
(Central Brain Tumour Registry of the United States)

1.3 Localisation

JPAs may arise anywhere along the neuraxis, however, in the paediatric population most tumours arise in the cerebellum. Other less common sites include the optic nerve, thalamus, hypothalamus, basal ganglia and cerebral hemispheres. JPAs of the spinal cord are not

uncommon and in children represent up to 11% of spinal tumours[4]. Primary diffuse leptomeningeal pilocytic astrocytomas are extremely rare.

1.4 Clinical presentation

JPAs are slow growing tumours with no metastatic potential. Symptoms arise based on tumour location and a consequence of raised intracranial pressure. Symptoms are generally longstanding and have usually been present for more than 6 months preceding diagnosis[5]. The most common presenting symptom is headache, seen in more than 90% of cases. Vomiting and lethargy are also common presenting features. Symptoms gradually worsen as hydrocephalus increases in severity. JPAs of the optic pathway tend to result in visual loss. Tumours arising in the hypothalamus may result in hypothalamic/pituitary dysfunction e.g diabetes insipidus. Tumours in the region of the thalamus may result in hemiparesis as a result of internal capsule compression. JPAs arising in the spinal cord produce non-specific symptoms and signs of an expansile mass. Seizures are an unusual presentation as these tumours do not commonly arise in the cerebral cortex.

1.5 Radiology

On neuroimaging JPAs are well-delineated tumours and demonstrate variable enhancement with contrast. The lesions may be solid or cystic. Cyst formation is an important diagnostic clue and these may be solitary and large with a mural nodule or multiple small intratumoural cysts.

1.6 Pathology

1.6.1 Macroscopic features

On macroscopic examination, JPAs are well-circumscribed tumours which may be solid or cystic. The cerebellar pilocytic astrocytomas classically show a cyst with a mural nodule. Those involving the spinal cord tend to show syrinx formation which extends over several segments. The cystic component, if present, usually contains serous fluid and the solid nodule appears rubbery/gelatinous and pink-grey in colour.

1.6.2 Microscopic features

On light microscopic examination, JPAs are tumours of low to moderate cellularity. Four main histologic patterns have been described, i) the classic biphasic pattern with alternating loose and dense areas, ii) a microcystic pattern with cystic areas containing proteinaceous

material, iii) cellular, piloid areas that often contain numerous Rosenthal fibres, iv) a diffuse, patternless growth which may at times can be difficult to distinguish from higher-grade astrocytomas.

The classic biphasic pattern comprises hypercellular areas of bipolar and multipolar cells that display long “hair-like” cell processes alternating with microcystic areas (figure 2). The cell nuclei are typically round to spindle-shaped and are cytologically bland.

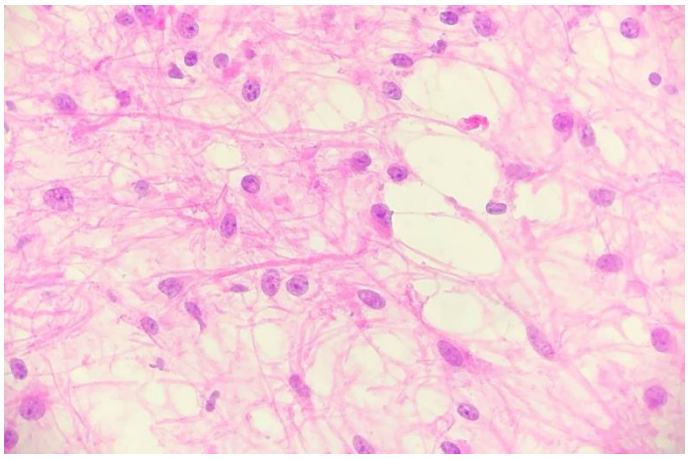

Figure 2: Tumour cells with “hair-like” cell processes and background microcystic spaces

JPAs are highly vascular tumours as evidenced by their contrast enhancement on radiologic imaging. This is characterised by the presence of glomeruloid-type blood vessels. There is, however, no endothelial cell multilayering as is seen in higher-grade astrocytomas. The presence of these type of vessels should thus not be a reason for “up-staging” of the tumour.

Rosenthal fibres are corkscrew shaped eosinophilic masses which may also be variably present (figure 3). Ultrastructurally, they are composed of αβ crystalline surrounded by intermediate glial filaments. Their presence can be helpful in making a diagnosis especially in cellular tumours. Their presence is, however, not a prerequisite for a diagnosis of JPA. Rosenthal fibres may also be seen in reactive gliosis.

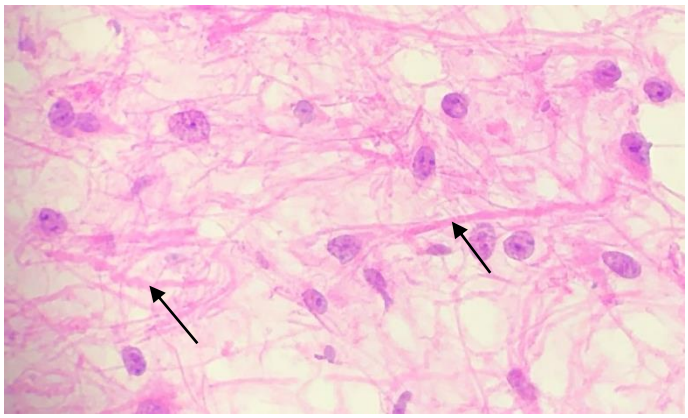

Figure 3:Rosenthal fibres

Eosinophilic granular bodies are eosinophilic PAS-positive globules present within the cell processes (figure 4). Their presence again is helpful but not a prerequisite when making a diagnosis of JPA. Eosinophilic granular bodies may also be seen in pleomorphic xanthoastrocytomas.

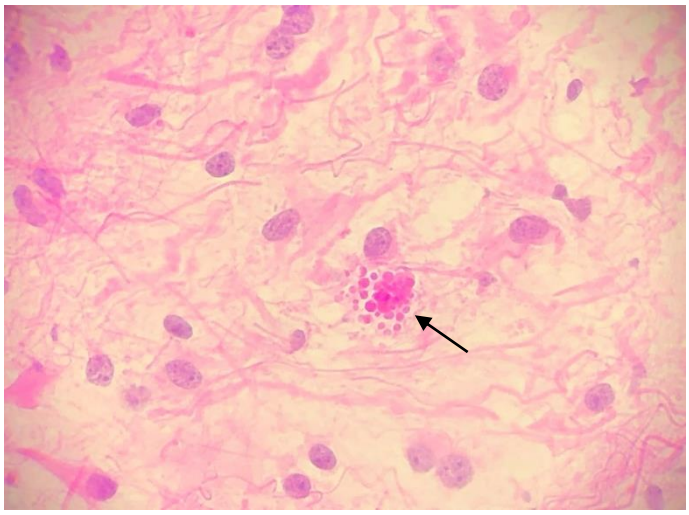

Figure 4: Eosinophilic granular bodies

Occasional lymphocytes can be seen in a perivascular distribution. Cells resembling those of a diffuse type astrocytoma in place of the usual compact and microcystic pattern can be seen. This may be more pronounced along the periphery of the tumour. Nuclear pleomorphism, isolated mitotic figures and necrosis are rarely seen, however, their presence is not a reliable indicator of progression to a higher-grade lesion. Necrosis, if present, is usually infarct-like

and not palisaded as one would see in a glioblastoma, a higher grade glioma with a worse prognosis.

Leptomeningeal invasion may be seen, but is not indicative of a more aggressive clinical course, and is more commonly associated with JPAs of the cerebellum and optic nerve.

Tibbetts et al.[2] evaluated 107 cases of pilocytic astrocytoma for histologic features that might stratify pilocytic astrocytomas into clinically relevant subgroups. They concluded that tumours with a high mitotic index and infiltrative growth patterns, usually considered worrisome in tumours at other sites, showed no clear association with clinical behaviour. They were able to identify 4 histopathologic features that correlated significantly with decreased disease-free survival. These include necrosis, oligodendroglioma-like features, vascular hyalinisation and calcification. With regards to the necrosis, it was always infarct-like. Oligodendroglioma-like morphology was also evaluated by Wong et al.[6] who examined 21 resected JPAs and found low expression of an oligodendrocyte associated protein, MBP, which was associated with a greater tendency for disease progression. A study by Horbinski et al.[7] also showed worse outcomes in tumours with oligodendroglial-like morphology. In contrast, a study by Sharmae et al.[8] expression of MBP did not have any prognostic value. There are still tumours which show none of the above adverse morphologic features and still tend towards worse outcomes, making the natural progression of these tumours difficult to predict on light microscopic grounds alone.

1.6.3 Proliferation index

An elevated Ki67 proliferation index is generally considered worrisome in most tumours, whether intracranial or extracranial. JPAs have a mean Ki67 proliferation index of 1.1% (range: 0-3.9%)[4]. In most studies to date, the Ki67 proliferation index was found to have no significant effect on clinical outcomes [4,5,9,10].

Some studies have, however, described worse outcomes in pilocytic astrocytomas with high proliferation indices. A study by Tu et al.[11] examining the proliferation indices of JPA's at different sites showed no differences in anatomic site, however, those tumours with raised proliferation indices (>4%) showed a shorter time to tumour recurrence and disease progression. The variation in Ki67 indices may possibly be affected by tissue-block storage, tissue fixation, different staining protocols and interobserver variability[12].

1.6.4 Malignant transformation/Anaplastic pilocytic astrocytoma

JPAs generally remain stable as WHO grade I lesions over the disease course and any histologic changes (mild nuclear atypia, necrosis or mitoses) are generally of a regressive nature. For those tumours showing higher-grade features, the WHO recommends the use of the term anaplastic pilocytic astrocytoma. It has been reported that 1.7% of JPAs show anaplastic features[13]. The histologic changes of anaplasia include hypercellularity, cytologic atypia, brisk mitotic activity (>4 per 10 high powered fields) and oligodendroglioma-like changes. The tumour cells may also show small cell and epithelioid morphology.

Parsa et al[14] concluded that anaplasia did not occur spontaneously but only after treatment with radiation. This was supported by Ellis et al[15] in a study of 20 JPAs, 3 of which showed anaplastic morphology. All 3 of these patients had received radiation therapy.

JPAs with anaplasia are associated with poor median overall survival compared with typical JPAs but better survival than higher-grade gliomas[10].
Anaplastic PAs have not received a WHO grade in the most recent WHO classification scheme.

1.7 Molecular and cell biology

1.7.1 Overview of molecular pathways implicated in gliomagenesis

The past decade has seen advances in molecular technologies that have yielded insights into oncogenesis. Several new genetic aberrations have been described in gliomas, many of which have therapeutic and prognostic significance. These have impacted the classification of gliomas. As a consequence, histopathologic criteria alone are no longer adequate in classifying gliomas[16].

The molecular abnormalities seen in gliomas can be broadly placed into 3 categories. A single glioma may show abnormalities involving all three categories and different tumours may show derangements at different levels along these pathways. This justifies the need for targeted therapies for the successful treatment of these tumours.

The 3 categories are;

1. Abnormalities of growth factors and/or their receptors

This category comprises aberrations of extracellular growth factors or their receptors. These factors are also known as mitogens and function to initiate cell division. An abnormal increase in production of growth factors and/or abnormal function and/or abnormal number of growth factor receptors may result in uninhibited cell growth. The most commonly involved growth factors are epidermal growth factor and/or platelet derived growth factor or their respective receptors, epidermal growth factor receptor and platelet derived growth receptor. These receptors are known as tyrosine kinase receptors as binding of the growth factors to their respective receptors results in phosphorylation of the intracellular tyrosine kinase domain. EGFR overexpression is seen more commonly in glioblastomas which results in constitutive activation of the receptor. EGFR abnormalities have thus far not been described in JPAs.

The second and third categories below have been implicated in JPAs and are thus discussed in more detail. These are signal transduction abnormalities and cell cycle control abnormalities.

2. Signal transduction abnormalities

The second category comprises abnormalities of signal transduction cascades, the pathways which link the abovementioned growth signals to intracellular processes such as gene transcription/translation. The RAS-RAF signal transduction cascade is most commonly implicated in gliomas.

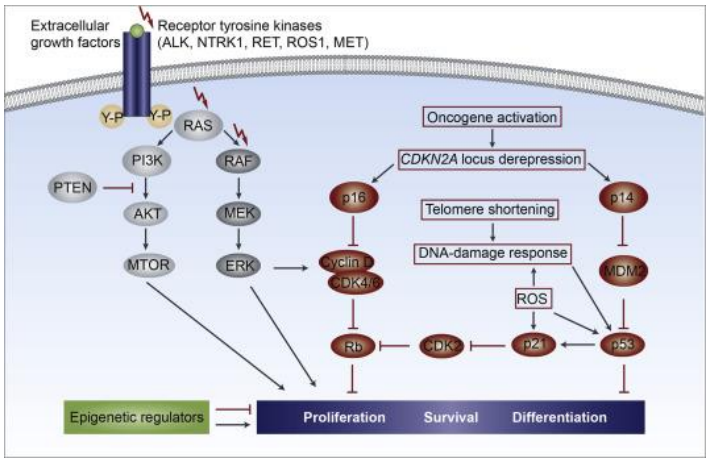

Figure 5: Signal transduction cascade

Following receptor tyrosine kinase activation, either by binding of ligands or mutation-induced constitutive activation, activation of signal transduction cascades (AKT and RAS) follows which function to transmit the growth signal to the proliferation centre of the cell. These signal transduction cascades may be activated even in the absence of receptor activation i.e. activating mutations in RAF,PI3K and AKT. As shown in the figure above, an activated RTK activates the enzyme PI3K, which in turn activates downstream enzymes, ultimately resulting in activation of AKT. Activated AKT plays a role in a number of functions which include mRNA translation and inhibition of apoptosis.
Under normal conditions PTEN, a tumour suppressor, inhibits AKT activation by dephosphorylating cofactors PIP2 and PIP3. Loss of PTEN function is one of the mechanisms of AKT activation in gliomas.
In the parallel pathway in the diagram above (RAS-MEK-ERK), activated RTK results in activation of RAS which subsequently results in activation of another kinase called RAF (RAS,MEK,ERK cascade, also known as the MAP kinase pathway). RAF kinases are direct effectors of RAS and lie at the apex of the MAPK pathway. They include A-RAF, B-RAF and RAF-1. B-RAF is of great interest as it is found to be mutated in a number of malignancies including malignant melanoma, colon carcinoma and papillary thyroid carcinoma. Mutations in BRAF result in elevated BRAF kinase activity thus enhancing MEK-ERK signalling. In contrast to BRAF mutations, A-RAF and RAF-1 mutations are rare.

Activated RAF in turn activates MEK-1 and MEK-2 by phosphorylation. The 3 Raf isoforms differ in their ability to activate MEK; BRAF is the strongest MEK kinase followed by RAF-1. ARAF is a weak MEK activator. Once activated, ERK phosphorylates numerous cytoplasmic and nuclear targets including kinases, phosphatases, transcription factors and cytoskeletal proteins. ERK signalling can, depending on the cell type, regulate processes such as proliferation, differentiation, angiogenesis and migration. ERK signalling promotes accumulation of genes required for cell cycle activation, such as cyclin D1, but can also suppress the expression of genes which inhibit proliferation. To continue proliferation certain tumour cells utilise mechanisms such as constitutively activating AKT to counteract the ERK-mediated induction of CDK-inhibitor proteins. ERK signalling also plays a role in disrupting the anti-proliferative effects of ligands such as TGF-β. It seems that the balance between ERK activity and negative feedback is more important than the absolute level of ERK activation.

Changes in cancers that lead to constitutive activation of the MAPK/ERK pathway occur at the early steps of the pathway viz. overexpression or activating mutations of receptor tyrosine kinases, RAS or BRAF mutations. The high frequency of activating mutations around the RAS-RAF axis suggests that this is the regulatory hotspot of the pathway.

3. Cell cycle control abnormalities

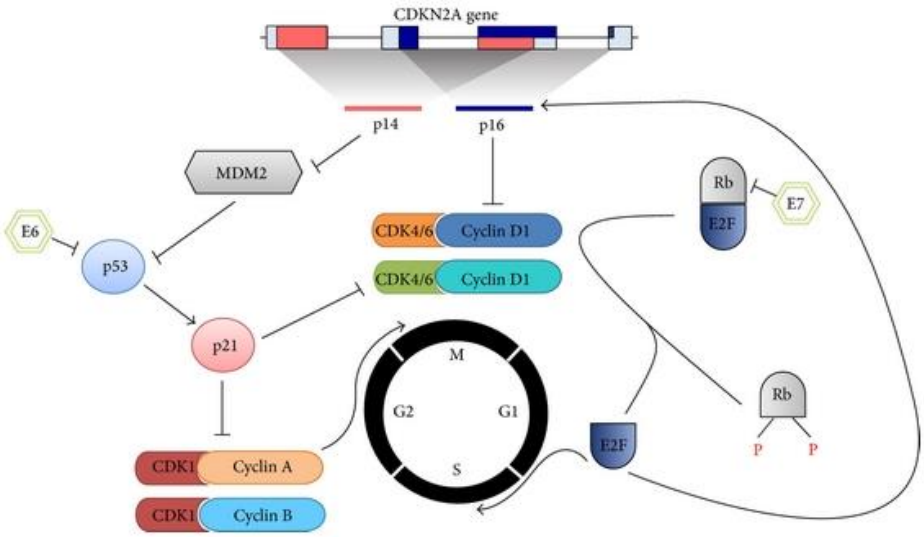

Figure 6: Cell cycle

The third category comprises molecules that are involved in control of the cell cycle, namely the p16-CDK-RB and ARF-MDM2-p53 pathways which normally function to prevent or suppress tumour growth.

Two tumour suppressors, inhibitor of CDK4 ($p16^{INK4a}$) and ARF, inhibit proliferation. In non-dividing cells, $p16^{INK4a}$ inhibits the action of cyclin dependent kinases (CDK's). Without CDK's the retinoblastoma (RB) protein forms an inactivating complex with E2F. Bound E2F is then unable to stimulate cell proliferation. Inhibition of $p16^{INK4a}$ results in activation of cyclin-CDK complexes which subsequently phosphorylate RB, releasing E2F. Therefore, $p16^{INK4a}$ loss, CDK amplification, or RB loss disrupts cell cycle control and results in uncontrolled cellular proliferation[18].

Similarly, in quiescent cells, ARF forms an inactivating complex with MDM2, preventing MDM2 from binding and inhibiting p53. Thus, free p53 is phosphorylated and protected from degradation and it inhibits cell cycling. One of the mechanisms by which p53 reduces proliferation is by activating transcription of p21CIP1 (CDK inhibitor protein), which forms

inhibitory complexes with cyclin-CDK's. Thus $P16^{INK4a}$ loss, MDM2 amplification or p53 loss releases inhibition of cell division.

1.7.2 Molecular changes in Pilocytic astrocytomas and their prognostic significance.

Of the abovementioned 3 groups, the group which involves signal transduction abnormalities has been more extensively studied in pilocytic astrocytomas. Under non-pathological conditions MAPK/ERK signalling components are expressed in most areas of the brain. This pathway has been implicated in various neurological functions like memory formation, pain perception and development of the midbrain and cerebellum[17.]

Studies focusing on the molecular biology of JPAs have shown that these tumours are distinct from higher grade gliomas and the genetic alterations seen in high-grade gliomas such as P53 mutations, MGMT methylation, EGFR amplification, PTEN loss and loss of heterozygosity (LOH) on 1p or 19q are not seen in JPAs[18].

Activation of the MAPK pathway is well described in other malignancies, e.g thyroid and colorectal carcinomas and malignant melanomas. Most of these neoplasms involve activating point mutations in BRAF, with the presence of such alterations being associated with worse outcomes[7,19].

Studies have shown that a majority of JPAs have alterations in this signaling pathway in the form of BRAF duplications[7,19]. Not all JPAs appear to have specific alterations of BRAF but still show MAPK pathway activation, which may occur by BRAF-independent mechanisms.

BRAF is frequently activated by gene fusion or point mutation. The most common alteration described is a duplication at chromosome 7q34 whereby the regulatory domain of BRAF is lost and the catalytic domain is fused to KIAA1549 resulting in constitutive activation of downstream signalling in the MAPK pathway. The frequency of this fusion varies from 50% to 100% in studies[17]. A total of 5 different fusion combinations of the 2 genes have been described i.e. BRAF exon 9 with KIAA1549 exon 16 (comprising 60% of fusion events), BRAF exon 11 with KIAA1549 exon 16, BRAF exon 9 with KIAA1549 exon 15, BRAF exon 10 with KIAA1549 exon 18 and BRAF exon 9 with KIAA1549 exon 19[17]. Some tumours have also been reported as showing multiple of these fusions within a single

tumour[20]. Hawkins et al[42] showed that midline supratentorial tumours had a higher frequency of BRAF-KIAA1549 fusions than lobar tumours.
Other studies show that patients with infratentorial/posterior fossa tumours tend to display a higher frequency of the KIAA1549 fusion[22,23]. Tumours showing this BRAF-KIAA1549 fusion are associated with a better prognosis [21,22,23,24,25]. Other studies, however, showed no significant difference in outcome compared to those without rearrangement [7,10,12,26,27,28,29,30]. This BRAF fusion has not been described in other gliomas.

An additional BRAF fusion at 7q34 has also been described in a small number of pilocytic astrocytomas involving BRAF and FAM131B[31], however, the clinical implications thereof are uncertain.

The second most common change described is a BRAFV600E mutation characterised by a valine to glutamate substitution at position 600 that also results in constitutive activation of BRAF. This mutation has been found in approximately 10% of cases of JPA and are described more frequently in extracerebellar locations [16,10,32,33]. Only 2% of cerebellar JPAs show a BRAFV600E mutation[33]. This mutation is, however, seen more frequently in other gliomas such as pleomorphic xanthoastrocytomas and gangliogliomas (70% and 20% respectively)[16].

As BRAFV600E mutations are seen more commonly in other brain tumours as mentioned above, testing for this mutation would not be as specific in confirming a diagnosis of pilocytic astrocytoma as would BRAF fusion testing. There have been no histological differences identifiable between cases showing BRAF fusion and those showing BRAF V600E mutations. There are also not many studies addressing the impact of a BRAFV600E mutation on patient prognosis. Hawkins et al.[24], however, describes an increased risk for progressive disease in those tumours showing BRAFV600E mutations. If this finding can be duplicated it would raise the question as to why tumours with BRAF fusion and those with BRAF V600E mutations behave differently when they both produce constitutive activation of BRAF. For this reason it has been suggested that JPAs in different sites may have a different cell of origin[19]. This has, however, not been extensively studied.

Other, less common alterations of the MAPK pathway have also been described. These include RAF1-SRGAP3 fusion, $BRAF^{insT}$, KRAS mutations, PTPN 11 mutations and FGFR mutations and fusions[10,34,35,36,37,38].
Fusion of RAF1 with SRGAP3 on chromosome 3p25 results in deletion of the N-terminal Ras-binding regulatory domain, producing constitutive BRAF activity[36].

A 3-bp (TAC) insertion that encodes an extra threonine residue adjacent to the V600 codon has been described in some JPAs [36]. This alteration has been referred to as $BRAF^{insT}$ and shows kinase activity similar to the BRAF V600E mutation.

KRAS mutations involving codons 12, 13 and 61 are seen in less than 5% of JPAs [34,35,39].
Duplications of a portion of the tyrosine kinase domain of FGFR1 have been described (approximately 24% of cases in one study)[37]. These alterations are thought to constitutively activate the receptor independent of ligand activation. Jones et al[38] reported mutations affecting the TKD of *FGFR1 i*n 14/141 JPAs. These tumours were negative for BRAF alterations. These *FGFR1*-mutant JPAs were seen in extracerebellar and midline locations[38].

These latter, less common, MAPK pathway alterations result in constitutive activation of the pathway but appear to be mutually exclusive of each other. The clinical impact of these less common mutations remains to be elucidated.

The activation of BRAF in melanomas has been associated with a process called oncogene-induced senescence. In this process an inciting oncogenic stimulus also limits neoplastic growth via the induction of cellular senescence. The often indolent disease course of most JPAs suggests that oncogene-induced senescence may play a role[34]. The study by Raabe et al[34], showed that BRAF V600E expressing cells stopped proliferating and induced markers of oncogene-induced senescence namely acidic B-galactosidase, PAI-1 and p16. In their study, Raabe et al.[34] also found that immunohistochemical expression of p16 was seen in majority (86%) of the 66 JPAs evaluated. Those tumours that were negative for p16 showed significantly shorter overall survival. This finding was supported by Horbinski et al[7] who also concluded that loss of p16 was associated with shorter overall survival, regardless of BRAF fusion status. There was, however, no statistically significant relation identified between shorter survival and tumour location or the extent of previous surgical resection.

In contrast to patients with neurofibromatosis, only 4% of sporadic JPAs show loss of NF-1 allelles[18].

Phosphorylation of ERK1/2 has been observed in all investigated JPAs, in keeping with constitutive MAPK pathway activation in these tumors[40]. pERK staining correlates with the presence of MAPK pathway activation and is often associated with the BRAF duplication described above[10,40].

Cytogenetic analyses of JPAs have shown either a normal karyotype or a variable number of aberrations. Gains of chromosomes 9q, 19 and 22 and losses of chromosome 18 have been described[1,7,18]. A study by Belirgen et.al[41] showed copy number alterations of 2p11.2 and 9p11.2 which correlated with a better prognosis. Their study also showed alterations of 1p36.2 which were associated with worse outcomes. LOH of chromosome 17p13 has also been described, associated with a worse outcome[26].
Also, in JPAs of patients under 15 years, 50% had only one chromosome affected whereas those older than 15 years showed multiple chromosomal gains[1,26].

Studies in this area of molecular prognostic markers are still ongoing and larger cohorts will undoubtedly provide newer insights.

1.9 Management

The mainstay of treatment for JPA is surgical resection without causing unacceptable neurologic deficit. Reported rates of gross total resection range from 50-89% in cerebellar tumours[42]. The reported resection rate for tumours of the optic tract and hypothalamus is only 3.2% and these tumours have an overall recurrence rate of 19%[20]. Subtotal resection may occur both intentionally to avoid morbidity and, unintentionally. When unexpected residual tumour is identified and can be safely resected, immediate reoperation is recommended[42]. However, when the tumour is inaccessible or has recurred after more than one surgical intervention, chemotherapy and radiotherapy are considered. For residual tumours that are deemed unsafe for repeat operation, treatment is deferred until the patient becomes symptomatic or the lesion progresses on imaging.

1.10 Morbidity and Mortality

Overall mortality is low with rates between 0% and 4% in large series[43,44].

Studies have reported 5-year progression free survival of 45-65% for residual tumour of any size and long-term stability or regression of residual tumour in 33-65%[42]. Possible mechanisms for spontaneous regression include ischaemia secondary to disruption of blood supply at first intervention, and inhibition of angiogenesis.

Approximately one-fifth of patients recur after complete surgical resection[35]. Higher rates of tumour recurrence are seen in children younger than 36 months, supratentorial tumours and following subtotal resection[19]. Lesions in inaccessible sits, such as the hypothalamus and brainstem, can rarely be removed completely. These tumours are also at higher risk for recurrence and significant morbidity.

1.11 Future directions

Although surgical resection remains the mainstay of treatment of JPAs, the identification of constitutive MAPK activation has opened the door for molecularly targeted therapies to be used in surgically inaccessible tumours. In view of the increased morbidity associated with surgery and standard chemo-radiation regimens, pharmacological blockade is an attractive approach and kinase inhibitors currently on trial include MEK inhibitors, RAF inhibitors such as Sorafenib and mTOR inhibitors.

2. Study Design

2.1 Study Objectives

The aims of the study were:

- To evaluate the expression of BRAF using fluorescence in-situ hybridisation (FISH)
- To evaluate the immunohistochemical expression of the tumour suppressor p16
- To evaluate the immunohistochemical expression of the protein kinase ERK
- To compare the immunohistochemistry findings in each cohort
- To correlate the findings of the immunohistochemistry and FISH tests with tumour location and clinical outcome (i.e. disease progression, recurrence or cure) and determine if these markers have any significant prognostic or predictive value.

2.2Materials and Methods

2.2.1 Ethics approval

Ethics approval for our study was obtained from the Human Research Ethics Committee (Reference number HREC 133/2012), Faculty of Health Sciences, University of Cape Town.

The study proposal was approved by the Department of Clinical Laboratory Sciences Research Committee and the Faculty of Health Sciences Postgraduate Committee at UCT. The funding for this study was received from the National Health Laboratory Service (NHLS) Research Trust (Grant number 004_94355) and the Department of Neurosurgery at Red Cross Childrens Hospital.

2.2.2 Sample selection

An electronic search of the laboratory information system database (DISA) at the Division of Anatomical Pathology, National Health Laboratory Service was performed. The search included all cases of JPAs between January 1990 and December 2012.

Stained slides of the cases were retrieved from the Departmental archives at Groote Schuur Hospital and Red Cross Childrens Hospital and reviewed. The diagnosis in each case was confirmed, the morphologic data recorded and any additional information from the reports, also recorded. A suitable block of tumour tissue was selected for FISH testing and immunohistochemical staining. Cases where minimal residual tumour tissue were present in the tissue block were excluded from the study.

2.2.3 Fluorescence in-situ hybridisation

FISH testing was done using the Vysis BRAF Spectrum Gold FISH probe kit. This approximate 510kb probe contains the entire BRAF gene found on chromosome 7. The probe was supplied ready-to-use.

The procedure was performed using the Kreatech KBI-60007 kit as follows;

Pretreatment:

- 4um paraffin sections were mounted on positively charged slides
- Mounted slides were incubated at 56°C for 16 hours
- Slides were then placed in xylene for 20 minutes
- This was followed by 3 minutes each in 100%, 85% and 70% ethanol respectively
- Slides were then placed in distilled water for 3 minutes at room temperature
- Sections were then covered with pepsin solution and left for 20 minutes at room temperature
- Slides were then left for 1minute in distilled water at room temperature

- This was followed by 5 minutes at room temperature in 2x SSC
- Slides were then placed for 1 minute each in 70%, 85% and 100% ethanol
- This was followed by air-drying at room temperature

Co-denaturation

- 10ul of probe was applied to the tissue section
- Slide was then covered with a glass slip and sealed with Fixogum
- Slides were then incubated for 5 minutes at 80°C in *Thermobrite*

Hybridisation

- Slides were incubated overnight at 37°C in *Thermobrite*

Post-hybridisation

- Wash buffer I (0.4 x SSC/0.3% Igepal) was pre-warmed to 72°C
- Fixogum was removed from the slides
- Slides were then placed for 2 minutes in Wash Buffer II at room temperature
- This was followed by 2 minutes at 72°C in the pre-warmed Wash Buffer I
- The slides were then placed for 2 minutes in fresh Wash Buffer II at room temperature
- This was followed by 1 minute each in 70%, 85% and 100% ethanol at room temperature
- Slides were then left to air-dry
- This was followed by application of DAPI counterstain
- Glass cover slips were applied and microscopy was commenced

Assessment of FISH slides:

The microscopy was performed using an Olympus BX40 fluorescent microscope at the Division of Anatomical Pathology Red Cross Children Hospital. The slides were viewed under oil immersion at 100x magnification.

Cells that showed 3 or more gold signals were interpreted as having BRAF overexpression. The number of cells out of 100 that showed 3 or more signals were counted and this count was repeated in 3 different areas of the tumour. The percentage of positive cells was then determined as the average number of positive cells in the 3 fields. A minimum of 300 non-overlapping nuclei were therefore assessed in each case.

Tumours which showed greater than 10% positivity were considered as having BRAF gene overexpression.

2.2.4 Immunohistochemical staining

Table 1**:** Antibodies used for P16 and pERK immunohistochemistry

Primary antibody (Mouse/Rabbit) (mono/poly)	Supplier	Positive control
Erk1/2 [Thr202/Tyr204] (R)(m)	Cell signalling technology	Colon adenocarcinoma
P16 (M)()	Roche	Cervix

(Key: M – mouse, R-rabbit, m – monoclonal and p- polyclonal)

Immunohistochemistry Method

- Three micron tissue sections were cut from formalin fixed paraffin embedded blocks and picked up onto Histobond slides.
- The slides were incubated at 60°C overnight
- Sections were deparaffinised in xylene, rehydrated in graded ethanol and washed in water.
- Endogenous peroxidase activity was blocked by treating the slides with a 3% hydrogen peroxide (H2O2) solution for 10 minutes.
- Slides were washed well in water.
- After washing heat-mediated antigen retrieval was performed using EDTA
- Slides were then rinsed in PBS
- Slides were then blocked for non-specific binding using a 5% goat serum solution at room temperature for 10 minutes (DAKO: X0907)
- The goat serum was then drained off and the slides were incubated with the primary antibodies (table 1) for 45 minutes at room temperature
- After incubation the slides were washed in PBS
- Slides were then treated with goat antimouse immunoglobulins labelled with horse radish peroxidase (DAKO: Envision K4001) at room temperature for 30 minutes
- Slides were then washed well in PBS
- Positivity was developed by applying 3.3 diaminobenzidine (DAKO: K3466) at room temperature for 5-10 minutes

- Slides were washed well in water
- Slides were then immersed in a 1% $CuSO_4$ solution for 5 minutes
- Slides were then washed well in running water
- Slides were counterstained in haematoxylin for 30 seconds and blued in Scotts
- Slides were washed in water, followed by dehydration using graded ethanols, cleared with xylene then mounted with Entellan

Assessment of immunohistochemical stains

The intensity of staining was scored as 1+, 2+ or 3+. Positive staining was only considered in tumours that showed staining in greater than 10% of cells. Tumour cells without staining or staining in less than 10% of tumour cells were scored as 0.

Score	**Intensity of staining**
0	No staining in tumour cells or staining in less than 10% of tumour cells
1+	Mild nuclear staining in >10% of tumour cells
2+	Moderate nuclear staining in >10% of tumour cells
3+	Strong nuclear staining in > 10% of tumour cells

2.3 Clinical data and pathological features

The clinical data recorded were age, gender, site of tumour and whether the patient was known to have recurrent/progressive disease post-surgery.

2.4 Statistical analysis

Data analysis was performed using the statistical programme STATA (Stata Corp. LP, College Station, TX 77845, United States of America). Descriptive statistics were employed for basic characterization of both predictor and outcome variables. Bivariate comparisons (using Student's t-test, chi-squared and Fisher exact tests as appropriate) were used to identify basic associations. A *P*-value of **< 0.05** was considered statistically significant for analyses.

3. Results

3.1. Clinical and pathologic characteristics.

A total of 62 cases was used in our study population. Cases in which there was no residual tissue available for further testing were excluded. Cases in which clinical data was incomplete were still included. On review of the H&E sections of the tumour, all the tumours showed classical features of juvenile pilocytic astrocytoma with variable cellularity. Only one of the cases in our study sample showed "infarct-type" necrosis and oligodendroglioma-like features. This tumour was sited in the parietal lobe and was not associated with disease progression or recurrence. There was no cytologic atypia or mitotic activity identified in any of the tumours. There were also no calcific foci identified within the tumours.

The highest frequency of JPA was seen in the 5-10 year age group (52% of cases) (Tables 2 and 4). The most common site of involvement was the cerebellum (68% of cases) (Table 2). The least common sites were the hypothalamus and midbrain with only one case being reported at each of these sites (Table 2). The tumour showed a slight predominance in males in our study group (53% of cases -Table 2).

Of the patients who underwent surgery, 76% were considered cured, 5% experienced progressive disease and 18% had recurrence of disease (timeline uncertain) (Tables 3 and 4). Disease recurrence and progression were not limited to a particular site.

Table 2: Clinical parameters

Characteristic	**n=62 (%)**
AGE (years)	
<5	20 (32)
5-10	32 (52)
11-15	10 (16)
GENDER	
Male	33 (53)
Female	29 (47)
SITE	
Cerebellum	42 (68)
Parietal lobe	5 (8)
Temporal lobe	4 (6)
Optic tract	3 (5)
Pineal region	2 (3)
Hypothalamus	1 (2)
Midbrain	1 (2)
Brain, NOS	4 (6)

Table 3: Patient outcomes

Clinical	Frequency	Percentage (%)
Cure	47	76.92
Progression	3	4.62
Recurrence	12	18.46
Total	**62**	**100.00**

Table 4: Patient outcomes in relation to site of tumour

Site	Cured	Progression	Recurrence	Total
Brain, NOS	1	0	3	4
Cerebellum	34	1	7	42
Hypothalamus	0	1	0	1
Midbrain	1	0	0	1
Optic tract	3	0	0	3
Parietal lobe	4	0	1	5
Pineal region	1	0	1	2
Temporal lobe	3	1	0	4
TOTAL	47	3	12	62

3.2. BRAF-FISH

Of the 62 cases in our study group, 17 (26%) showed BRAF overexpression (Table 5). Tumour cells showing more than two BRAF signals were interpreted as having BRAF overexpression (figure 7) . Most cases were in the 5-10 year age group. Nine of the cases were seen in males and eight in females. Sixteen of the cases showing overexpression were tumours of the cerebellum whereas 1 of the cases was sited along the optic tract (Table 6). Tumours which showed BRAF overexpression were not associated with tumour progression or recurrence (p value = 0.032), which is statistically significant and in keeping with the literature (Table 7).

Table 5: BRAF expression

BRAF-FISH	Frequency	Percentage (%)
Wild-type	45	73.85
Overexpression	17	26.15
Total	**62**	**100.00**

Table 6: BRAF expression in relation to tumour site

Site	BRAF	BRAF (OE)	Total
Brain, NOS	4	0	4
Cerebellum	26	16	42
Hypothalamus	1	0	1
Midbrain	1	0	1
Optic tract	2	1	3
Parietal lobe	5	0	5
Pineal region	2	0	2
Temporal lobe	4	0	4
TOTAL	45	17	62

Pr=0.174

Table 7: BRAF expression vs tumour outcome.

Disease course	BRAF (WT)	BRAF (OE)	Total
Cured	30	17	47
Progression	3	0	3
Recurrence	12	0	12
TOTAL	45	17	62

Pr= 0.032

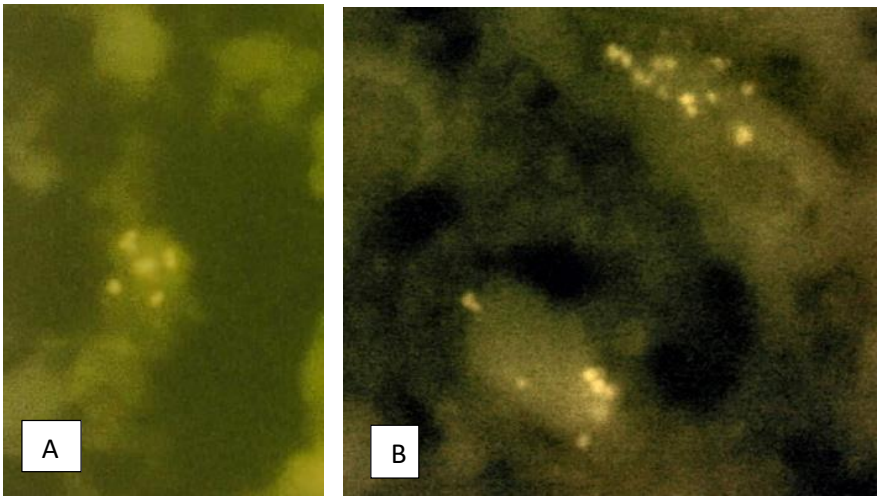

Figure 7: (A,B): BRAF-FISH: Tumour cells showing more than 2 BRAF signals (100x)

3.3. P16

Nuclear staining in more than 10% of tumour cells was considered positive. The staining pattern was graded out of 3, according to the intensity of staining (fig. 8). Those with complete absence of staining, weak cytoplasmic blush and staining of less than 10% of tumour cells were considered negative. Only 4 cases of our study sample showed negative staining for p16 (table 8). The negative staining was seen in one tumour at each of the following sites; the cerebellum, parietal lobe, pineal region and hypothalamus (table 10). Two of the negative cases showed no disease progression. One of the negative cases had disease progression and the remaining case which was negative for p16 showed tumour recurrence after surgery (table 9). All 4 negative cases showed wild-type BRAF on FISH testing. Forty-four of the p16 positive cases also showed wild-type BRAF on FISH testing. Seventeen of the positive cases were associated with BRAF overexpression on FISH testing (table 11). Two of the p16 negative cases were also negative for pERK immunohistochemical staining, whereas the remaining 2 negative cases showed positive pERK staining. There was no statistically significant correlation between p16 staining and disease outcomes. There was no statistically significant correlation between p16 staining, pERK staining and BRAF-FISH.

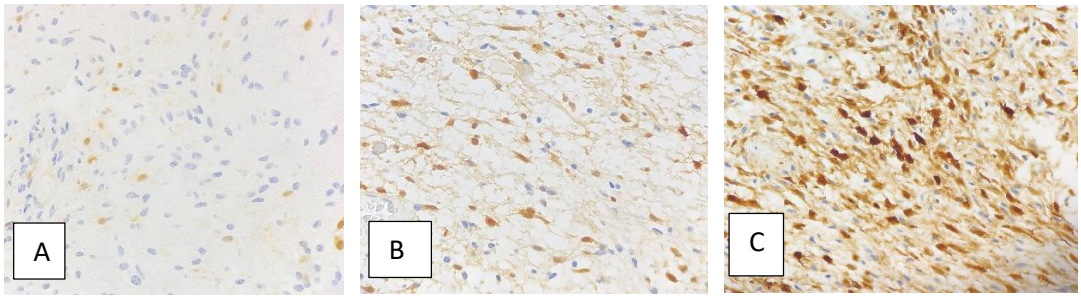

Figure 8: P16 staining. (A) 1+, (B) 2+, (C) 3+

Table 8: p16 expression

p16	Frequency	Percentage (%)
Negative	4	6.15
1+	22	38.46
2+	23	35.38
3+	13	20.00
Total	**62**	**100.00**

Table 9: p16 and tumour outcome

Disease course	p16 Neg	p16 1+	p16 2+	p16 3+	Total
CURED	2	18	18	9	47
PROGRESSION	1	1	1	0	3
RECURRENCE	1	3	4	4	12
TOTAL	4	22	23	13	62

Pr=0.358

Table 10: p16 in relation to tumour site.

Site	p16 negative	p16 1+	p16 2+	p16 3+	Total
Brain, NOS	0	3	1	0	4
Cerebellum	1	14	18	9	42
Hypothalamus	1	0	0	0	1
Midbrain	0	1	0	0	1
Optic tract	0	0	2	1	3
Parietal lobe	1	1	0	3	5
Pineal region	1	1	0	0	2
Temporal lobe	0	2	2	0	4
TOTAL	4	22	23	13	62

Pr=0.002

Table 11: BRAF and p16 expression.

BRAF-FISH	p16 Neg	p16 1+	p16 2+	p16 3+	Total
Wild-type	4	18	14	10	46
Overexpression	0	4	9	3	16
Total	4	22	23	13	62

Pr=0.264

3.4. pERK

Nuclear staining in more than 10% of tumour cells was considered positive and the intensity of staining was graded out of 3 (fig. 9). Three cases showed negative staining for pERK (table 12). Two of the negative cases were tumours of the parietal lobe and one was of the pineal region (table 15). Two of the negative cases were associated with tumours that showed no disease progression or recurrence. The 3rd case which showed negative pERK staining was associated with recurrent disease (table 13). All 3 negative cases were associated with wild-type BRAF on FISH (table 14). Two of the negative cases were also negative for p16 immunohistochemistry. The 3rd negative case showed diffuse p16 positivity. pERK staining had no statistically significant correlation with disease outcomes (table 16). There was no statistically significant correlation between pERK, BRAF and p16.

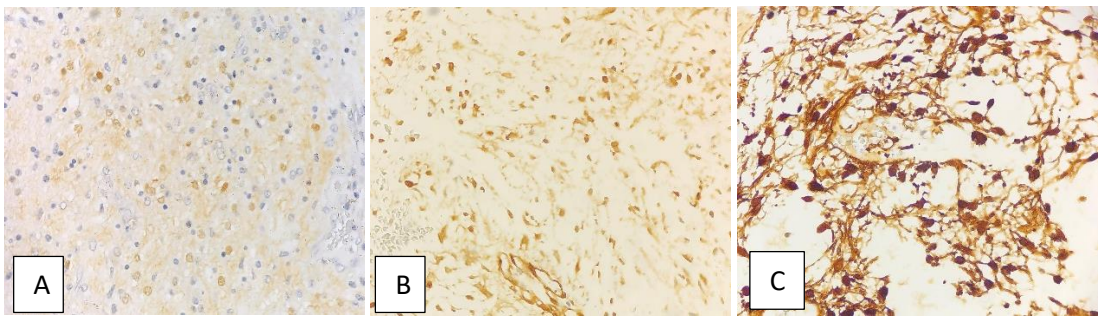

Figure 9: pERK staining. (A) 1+, (B) 2+, (C) 3+

Table 12: pERK staining

pERK	Frequency	Percentage
Negative	3	4.62
1+	10	15.38
2+	14	26.15
3+	35	53.85
Total	**62**	**100.00**

Table 13: pERK and patient outcomes

Disease course	pERK Neg	pERK 1+	pERK 2+	pERK 3+	Total
Cured	2	8	11	26	47
Progression	0	1	0	2	3
Recurrence	1	1	3	7	12
Total	3	10	14	35	62

Table 14: pERK and BRAF staining

BRAF-FISH	pERK Neg	pERK 1+	pERK 2+	pERK 3+	Total
Wild-type	3	9	11	24	47
Overexpression	0	1	3	11	15
Total	3	10	14	35	62

. Pr=0.390

Table 15: pERK in relation to tumour site

Site	pERK negative	pERK 1+	pERK 2+	pERK 3+	Total
Brain, NOS	2	2	0	0	4
Cerebellum	0	5	11	26	42
Hypothalamus	0	0	0	1	1
Midbrain	0	0	0	1	1
Optic tract	0	2	1	0	3
Parietal lobe	2	1	2	0	5
Pineal region	1	0	0	1	2
Temporal lobe	0	0	2	2	4
Total	5	10	16	31	62

Table 16: pERK vs P16 staining

P16	pERK neg	pERK 1+	pERK 2+	pERK 3+	Total
Negative	2	0	1	1	4
1+	0	5	5	14	24
2+	0	4	6	11	21
3+	1	1	2	9	13
Total	3	10	14	35	62

Pr=0.43

4. Discussion

Juvenile pilocytic astrocytoma is the most common intracranial tumour occuring in the paediatric population. These tumours are considered low-grade and are classified as WHO Grade I tumours. The outcome of affected patients, however, varies significantly, ranging from complete cure with surgical resection to multiple recurrences/progressive disease despite surgery. There are no reliable morphologic or molecular parameters that predict tumour behavior and patient outcomes. There are also few studies that document the long term outcome of patients with JPAs. This may be due to small study cohorts and loss of patient follow-up. With the advent of molecular medicine and the increasing availability of next generation sequencing, studies are now focused on the evaluation of the molecular pathways, notably the RAS/MAPK/PERK pathway, involved in juvenile JPAs to better

understand their natural progression. Some of these studies have shown conflicting results. Better understanding of the molecular pathways of this tumour would undoubtedly pave the way for development of pharmacological blockade of these tumours thus mitigating the risks associated with surgery and chemoradiation and possibly overtreatment.

The aim of our study was to evaluate components of the MAPK pathway viz. p16 and pERK (by immunohistochemistry) and overexpression of BRAF (by FISH analysis) and determine if there was any significant association with disease outcomes.

Our study population consisted of 62 patients. JPAs were seen most commonly in the 5-10 year age group with an almost equal male to female ratio. The cerebellum was the most common site of the tumour, accounting for 68% of cases.

On review of the H&E-stained sections, all tumours showed similar, classic morphologic features of JPAs. Tibbets et al.[2], Wong et al.[6] and Horbinski et al.[7] described the presence of nuclear atypia, mitoses, necrosis and oligodendroglioma-like morphology as being associated with worse clinical outcomes. Only one of the cases in our study sample showed "infarct-type" necrosis and oligodendroglioma-like features. This case was, however, not associated with disease progression or recurrence. Of the 15 cases in our study sample which showed disease recurrence/progression, none of the cases showed the high-grade features described by the above-mentioned authors. Although the presence of the morphologic findings of nuclear atypia, mitoses, necrosis and oligodendroglioma-like areas described by these authors may indeed be markers of early disease recurrence and progression, their absence may not necessary imply a better outcome.

JPAs in our cohort that showed BRAF overexpression by FISH analysis were associated with favourable clinical outcomes (p value = 0.032). The underlying BRAF abnormality leading to overexpression was, however, not investigated (mutation vs fusion vs other). This association with a favourable outcome is congruent with the findings described by a number of studies [21,22,23,24,25]. Sixteen of the 17 cases in our cohort that showed BRAF overexpression were located in the cerebellum. The one remaining tumour was located along the optic tract. This would be in keeping with the findings of Jakob et al.[22] and Hasselblatt et al.[23] of tumours in a posterior fossa location more commonly showing BRAF abnormalities. Hawkins et al.[24] also reported that BRAF overexpression is more common in cerebellar tumours and optic tract

tumours with overall better survival. Other studies have, however, shown that BRAF overexpression has no association with disease progression or recurrence.[2,7,12,26,27,28]. These studies were performed on similar sample sizes (between 70 and 167) and also by a similar combination of rt-PCR and FISH and possible reasons for the discrepancy in findings are not obvious.

With regards to p16 immunohistochemical staining, there was variable positive staining in the majority of the tumour specimens. Only 4 of the 62 specimens showed negative staining. There was no statistically significant correlation between p16 and BRAF overexpression. Raabe et. al.[34] investigated p16 immunohistochemical expression in juvenile pilocytic astrocytomas and also demonstrated expression of p16 in majority of the pilocytic astrocytomas evaluated. Those tumours that were negative for p16 showed significantly shorter overall survival in their study. This finding was supported by Horbinski et al.[7] who also concluded that loss of p16 was associated with shorter progression free survival, regardless of BRAF fusion status. Our results do not support this finding as none of the negative cases in our study were associated with disease progression or recurrence. A larger study population over a longer follow-up period would undoubtedly aid in better assessment of p16 in these tumours.

With regards to pERK staining, all but 3 of our cases showed positive staining with pERK which would be in keeping with constitutive activation of the MAP kinase pathway described in the pathogenesis of these tumours[40]. As activation of the MAP kinase pathway is described in all pilocytic astrocytomas, the negative pERK staining in 3 of our cases may be a consequence of poor tissue fixation or block storage. An alternative possibility is that these cases may represent tumours which have arisen along a pathway other than the MAP kinase pathway as none of them showed BRAF alterations either.

In conclusion, the molecular abnormalities in JPAs are not as well understood as those of higher grade glioblastomas. Our study shows that BRAF overexpression may be associated with better disease outcomes. The underlying abnormality resulting in overexpression was, however, not evaluated (i.e. fusion vs. point mutation). Although this is congruent with some studies there are, however, studies concluding that BRAF gene abnormalities, although present, have no clinically prognostic significance. pERK staining in pilocytic astrocytoma is indicative of MAP kinase pathway activation in gliomagenesis. There are not many studies

evaluating p16 expression in pilocytic astrocytomas but further studies would undoubtedly prove useful. A better understanding of these pathways would pave the way for development of pharmacologic targets, thus mitigating the risks associated with surgery (for inaccessible lesions) and chemoradiation.

5. Study Limitations

As with the majority of studies, the current study is subject to limitations. Our study sample was of a small size. There was no clear indication as to whether surgery performed was complete resection or debulking. The patients in our sample were considered cured when there was no documentation of a return to hospital. This however does not exclude the possibility of them presenting with recurrences at hospitals outside of our drainage area. Further studies using a larger sample size and over a longer period with adequate follow-up would undoubtedly yield more statistically significant results.

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6. Appendix

6.1 Original ethics approval letter

UNIVERSITY OF CAPE TOWN

Health Sciences Faculty
Human Research Ethics Committee
Room E52-24 Groote Schuur Hospital Old Main Building
Observatory 7925
Telephone [021] 406 6338 • **Facsimile** [021] 406 6411
e-mail: shureta.thomas@uct.ac.za

02 April 2012

HREC REF: 133/2012

Dr N Osman
c/o Dr K Pillay
Anatomical Pathology
D7
NGSH

Dear Dr Osman

PROJECT TITLE: JUVENILE PILOCYTIC ASTROCYTOMA: THE SEARCH FOR PROGNOSTIC MARKERS.

Thank you for submitting your study to Faculty of Health Sciences Human Research Ethics Committee for review.

It is a pleasure to inform you that the HREC has **formally approved** the above-mentioned study.

Approval is granted for one year till the 15th April 2013.

Please submit a progress form, using the standardised Annual Report Form (FHS016), if the study continues beyond the approval period. Please submit a Standard Closure form (FHS010) if the study is completed within the approval period.

Please note that the ongoing ethical conduct of the study remains the responsibility of the principal investigator.

Please quote the HREC. REF in all your correspondence.

Yours sincerely

PROFESSOR M BLOCKMAN
CHAIRPERSON, HSF HUMAN ETHICS
Federal Wide Assurance Number: FWA00001637.
Institutional Review Board (IRB) number: IRB00001938

This serves to confirm that the University of Cape Town Human Research Ethics Committee complies to the Ethics Standards for Clinical Research with a new drug in patients, based on the Medical Research Council (MRC-SA), Food and Drug Administration (FDA-USA), International Convention on Harmonisation Good Clinical Practice (ICH GCP) and Declaration of Helsink guidelines.
The Human Research Ethics Committee granting this approval is in compliance with the ICH Harmonised Tripartite Guidelines E6: Note for Guidance on Good Clinical Practice (CPMP/ICH/135/95) and FDA Code Federal Regulation Part 50, 56 and 312.

s/thomas

6.2. Latest ethics progress report/renewal

UNIVERSITY OF CAPE TOWN

FACULTY OF HEALTH SCIENCES
Human Research Ethics Committee

FHS016: Annual Progress Report / Renewal

HREC office use only (FWA00001637; IRB00001938) This serves as notification of annual approval, including any documentation described below.			
☑ Approved	Annual progress report	Approved until/next renewal date	30.6.22
☐ Not approved	See attached comments		
Signature Chairperson of the HREC/ Designee		Date Signed	9/6/21

Note: Please email this form and supporting documents (if applicable) in a combined pdf-file to hrec-enquiries@uct.ac.za.
Please clarify your plan for research-related activities during COVID-19 lockdown.
Please use the latest form found on our website:
http://www.health.uct.ac.za/fhs/research/humanethics/forms

Comments to PI from the HREC
Thank you for the deviation document

Principal Investigator to complete the following:

1. Protocol information

Date (when submitting this form)	3 JUNE 2021		
HREC REF Number	133/2012	Current Ethics Approval was granted until	30.6.2017 LAPSED
Protocol title	PILOCYTIC ASTROCYTOMAS:A SEARCH FOR PROGNOSTIC MARKERS		
Protocol number (if applicable)			
Are there any sub-studies linked to this study?		Yes	
If yes, could you please provide the HREC Reference number for all sub-studies? Note: A separate FHS016 must be submitted for each sub-study.		Pending	
Principal Investigator	Prof Komala Pillay		

(Note: Please complete the Closure form (FHS010) if the study is completed within the approval period)

6.3 Data collection summary

BRAF-FISH	P16-IHC	pERK-IHC	age (yrs)	site	recurrence/progression
8%	3	2	9	n/a	Recurrence
18%	2	3	10	cerebellum	
13%	1	2	2	cerebellum	
14%	2	3	2	cerebellum	
7%	1	3	7	pineal region	
3%	3	3	5	right eye	
11%	1	3	6	suprasellar	
25%	3	1	6	right eye	
11%	2	3	7	cerebellum	
7%	3	3	12		recurrence
8%	3	3	6	cerebellum	
4%	2	2	8	cerebellum	
3%	1	1	4 months	cerebellum	
11%	1	2	7	cerebelum	
13%	0	0	7	pineal region	recurrence
8%	2	2	5	cerebellum	
7%	3	3	3	cerebellum	
12%	1	1	12	cerebellum	
2%	1	2	1	temporal lobe	
3%	2	1	7	cerebellum	
6%	1	2	4	cerebellum	
8%	2	1	13		recurrence
10%	1	3	8	cerebellum	
12%	2	3	8	cerebellum	
1%	1	1	3	cerebellum	
1%	2	1	7	left eye	
2%	3	0	12	parietal lobe	
6%	2	3	5	cerebellum	
5%	0	2	7	occipital lobe	
3%	2	1	5	cerebellum	
20%	3	3	6	cerebellum	
2%	1	3	9		recurrence
6%	1	2	no clinical data		
19%	1	3	3	cerebellum	recurrence
4%	1	2	10	cerebellum	
5%	1	1	11	left parietal lobe	
20%	1	3	9	cerebellum	
17%	3	3	9	cerebellum	
20%	2	2	11	cerebrum	
5%	1	3	11	cerebellum	
2%	1	3	5		
3%	2	2	2	cerebellum	
19%	2	3	4	cerebellum	
3%	2	2	2	optic tract	
10%	3	3	3	cerebellum	
3%	2	3	1	cerebellum	
2%	3	3	2	cerebellum	
18%	2	2	11	cerebellum	
negative	2	3	3	cerebellum	
<1%	1	3	6	cerebellum	
2%	1	3	6	midbrain	
negative	3	2	7	left parietal	
negative	2	2	1	temporal lobe	
negative	0	0	10	parieto-occipital	
8%	3	3	13	cerebellum	
18%	2	3	4	cerebellum	
negative	2	3	1	temporal	
4%	1	3	1	cerebellum	
3%	0	3	3	hypothalamus	
1%	1	3	13	right insular region	
11%	2	3	10	cerebellum (E cape)	
30%	2	2	8	cerebellum	