THE REPUBLIC OF TURKEY
BAHÇEŞEHİR UNIVERSITY

ANALYSIS OF TREATMENT MODIFYING DIAGNOSTIC AND PROGNOSTIC MUTATIONS IN MENINGIOMA TUMORS

Master's Thesis

RASHID MOHIY-UD-DIN

İSTANBUL, 2019

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This is to certify that we have read this thesis and we find it fully adequate in scope, quality and content, as a thesis for degree of Master of Arts.

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PREFACE

First of all , ALHAMDULILLAH that i had been able to successfuly complete my masters research. Cordial & Humble Thanks to my Mom, Dad , Brother (Hamid) and Sister (Ameema) for their continuous support and encouragement.

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ABSTRACT

ANALYSIS OF TREATMENT MODIFYING DIAGNOSTIC AND PROGNOSTIC MUTATIONS IN MENINGIOMA TUMORS

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Master's Program Neuroscience

Thesis Supervisor: Assist. Prof. Dr. Timuçin AVŞAR

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Meningioma is known to be the second most common among the tumors of the central nervous system. Much of the efforts has been placed recently by researchers in exploring their etiology on genetic basis inorder to devise a way for better therapeutic outcome. The grading of meningioma tumors has a direct effect upon its prognosis and therapeutic plan by clinicians. Many patients are mis-graded due to limitation with the above mentioned subjective sort of parameters. The current grading for Meningioma provided by WHO provides classification that relies mainly upon histopathological, cytological and anatomical characteristics and does not have any inclusion of genetic basis. Including the genetic

screening parameter and corelating it with the histopathological characteristics, together may provide a better insight to the classification of menignioma grades that woyld be more objective in nature as compared to the previous subjective ones. For the above mentioned purpose, we tried to perform a retrospective study on genetic basis that involved isolation of 96 meningioma samples, extracting the DNA and via subsequent PCR amplification and sequencing via NGS technique, the data for SNP variations was collected. The obtained data on genetic and molecular grounds was then further corelated with the histopathological data of the patients that was collected from the “Medical Park Hospital”, Istanbul (Turkey). For statsitical analysis of the aquired data, Chi Square test and Fisher Exact tests were performed. Based on the results of statistical analysis and certain significant findings in our study, as indicated by the p-values, it implies that the future grading and classification of meningioma shall be designed in such a way that it considers to include the genetic variation paramters taken together in consideration along with the cytological, anatomical and histopathological characteristics.

Keywords: PCR, NGS, Odds ratio, Fisher Exact Test, Chi Square Test.

ÖZET

ANALYSIS OF TREATMENT MODIFYING DIAGNOSTIC AND PROGNOSTIC MUTATIONS IN MENINGIOMA TUMORS

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Meningioma, merkezi sinir sistemi tümörleri arasında en yaygın ikinci olarak bilinmektedir. Son zamanlarda araştırmacılar tarafından etiyolojilerini genetik temelde araştırmak için çabaların çoğu daha iyi bir terapötik sonuç elde etmek için bir yöntem geliştirmek amacıyla yapılmıştır. Meningioma tümörlerinin derecelendirilmesinin prognozu ve klinisyenler tarafından tedavi planı üzerinde doğrudan etkisi vardır. WHO'nün sağladığı Meningioma için mevcut sınıflandırma, histopatolojik özelliklerin birleştirilmesi üzerine sınıflandırma sağlar ve herhangi bir genetik temel içermez. Genetik tarama parametresinin dahil edilmesi

ve bunun histopatolojik özelliklerle ilişkilendirilmesi, menignioma derecelerinin sınıflandırılmasında daha iyi bir görüş sağlayabilir. Yukarıda belirtilen amaç için, 96 meningioma numunesinin izolasyonu, DNA'nın ekstrakte edilmesi ve ardından PCR amplifikasyonu ve NGS tekniği ile SNP varyasyonları için veriler toplanan genetik bazda retrospektif bir çalışma yapmaya çalıştık. Genetik ve moleküler zeminde elde edilen veriler daha sonra "Medical Park Hospital", İstanbul'dan (Türkiye) toplanan hastaların histopatolojik verileri ile daha da ilişkilendirildi. Elde edilen verilerin istatistik analizleri için, Odds oranı hesaplandı ve Chi Square testı ve Fisher Exact testı. İstatistiksel analizin sonuçları ve önemi, p-değeri ile belirtildiği gibi, menenjiyomun gelecekteki derecelendirmesinin ve sınıflandırılmasının, birlikte göz önünde tutulan varyasyonların genetik temelini içerecek şekilde tasarlanacağını ima eder. histopatolojik özellikleri ve anatomik yerleri ile.

Anahtar Kelimeler: PCR, NGS, Oran Oranı, Fisher Exact Testi, Chi Square Testi.

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ABBREVIATIONS

ANOVA	:	Analysis Of Variance
CSF	:	Cerebrospinal fluid
DNA	:	Deoxyribonucleicacid
FISH	:	Fluorescence in situ hybridization
FN	:	False negative
FP	:	False positive
IHC	:	Immunohistochemistry
MTHFR	:	Methylene tetrahydrofolate reductase
MTRR	:	Methyl tetrahydrofolate Homocysteine Methyl Transferase Reductase
NGS	:	Next Generation Sequencing
PCR	:	Polymerase Chain Reaction
PIK3CA	:	Phosphatıdylınosıtol 3-kınase, catalytıc, alpha
PTEN	:	Phosphatase and tensin homolog
RAD54L	:	DNA repair and recombination RAD54-like
RNA	:	Ribonucleicacid
SNPs	:	Single nucleotide polymorphisms
TM	:	Meltıng Temperature
WHO	:	World Health Organization

1. INTRODUCTION

Intense genetic and bilogical interest has been prevailed in the subject of Meningiomas. Infact the Meningiomas are known to be among the very first studied solid tumors and neoplasms that were tried to be explored by using various cytogenic techniques. Most surprisingly, for instance, is the event of the monosomy of Chromosome 22, which is attributed as the most frequent possible genetic etilogical cause now a days, was reported as early as 1972. And this is the reason that Meningioma, in this timespan became one of the most widely Cytogenetically studied neoplasm in humans.

The screening and assessment of the genotypic alterations/aberrations at the molecular and genetic level by utilization of several advanced techniques that includes fluorescence in situ hybridization (FISH), or comparative genomic hybridization (CGH), microsatellite analysis and automated sequencing etc has thus now provided a further rapid facilitation, recently in the past decade.

In the recent days, in case of Glioma, the genetic alterations that corresponds to a specific pathoogical discourse are also related to the clinical outcome. This follows from the case where the deletions on the arms of chromosome 1p and 19q are now clearly known to be associated with responsiveness to chemotherapeutic outcome.

However in case of Meningioma, there hasn’t been found any association typically between the genetic aberrations and clinical relevance. And just a theoretical model had been constructed so far which is based on the comprehensive analysis between histological grade and clinicopathological features that was up to date for a certain span as well. This model basically distinguishes the early alterations from the later alterations found in Meningioma.

The early alterations are presumably associated with formation of meningioma tumors while the later one involved in the Meningioma tumor progression.

Apart from the genetic alterations, much efforts have been put in the investigation of other biological features as well. After late 1970s, the hormone receptors have been focused upto huge extent but even inspite of this great attention to this particular aspect the expression profiles of such hormonal receptors are not that well characterized, indicating that the progress has been relatively stagnated. It is therefore that the results showing the therapeutic effects being obtained by targetting these receptors are mainly disappointing.

Here, we also need to enlighten the aspect of edema formation and its association with meningioma angiogenesis. The mechanisms of Edema formation had been a major field of interest since 1990s, that has been driving the attention of researchers, specifically focusing on the imaging techniques and their advancements. As it is evident that the "Angioarchitecture" of any tumor related to Meningioma or others, is of key importance, therefore much effort was put in identifying a variety of growth factors that were further analyzed inorder to find out their extent of contribution to Meningioma angiogenesis and edema formation.

Further to this, in the same context, our retrospective study shall also suggest certain genetic aspects related to classification and WHO grading of meningioma, SNP transcript variants and chromosomal alterations with the common genes involved.

2. LITERATURE REVIEW

2.1 CENTRAL NERVOUS SYSTEM AND MENINGES

Meninges refers to the three layers or membranes of tissues (called the Dura mater, Arachnoid and Pia mater) that lines the skull and vertebral canal and enclose and surround the brain and spinal cord and surrounding the neuraxis as well. These layers of tissues that surround the brain and spinal cord and are protective in nature. From outermost to the innermost layer, one can classify these layers in order form as: Dura matter, Arachnoid, Sub-Arachnoid space and Pia matter respectively.

Meninges surrounding the brain and spinal cord are in continuous fashion and are also linked together via Foramen Magnum that functions as a passage of CNS and connects the brain with spinal cord through the skull. The three meningeal layers with brief details are as follows: (Decimo et al, 2012)

2.1.1. Dura Mater

The dura mater is known as the most superior layer among the meninges. The term Dura mater is a Latin word which means "hard mother" as it is tough and inflexible. This tissue basically functions to protect the brain from any sort of displacement by forming several structures that function to separate the cranial cavity into certain compartments and hindering the brain displacement.

2.1.2. Arachnoid

The arachnoid or arachnoid mater is the middle one among the meningeal layers and it is also protective in nature. However, there are some sinuses formed by the dura mater and the arachnoid projects into those sinuses as well. Such projections of the arachnoid are known as arachnoid granulation/arachnoid villi. The main function of these Arachnoid Villi is to transfer the CSF from ventricles back into the blood stream. There is also a space found between the arachnoid and pia mater, known as sub-arachnoid space. This subarachnoid space is filled with CSF. The cranial nerves and all of the blood vessels that enters the brain, pass through this space.

The terminology of "Arachnoid" refers to a spider-web like appearance of the blood vessels in this subarachnoid space, via which they pass. The arachnoid layer of the meninges consist of the arachnoid mater or membrane, which is a thin and transparent that closely resembles a loosely fitting sac that covers the brain and spinal cord.The tumor cells of Meningiomas consists of neoplastic meningothelial (arachnoidal cap) cells.

2.1.3. Pia Mater

The innermost meningeal layer is the Pia mater. Unlike the Dura mater and Arachnoid, the Pia mater is closely adhere to the brain and continues to run down into the sulci and fissures of the cortex.

The Pia mater fuses with the ependyma, which is a thin membrane of the glial cells lining the ventricles to form structures called the choroid plexes.All of the ventricles contain the choroid plexuses that produce CSF. (Decimo et al, 2012)

2.2. MENINGIOMA

Meningiomas are the most common ones among the benign intracranial tumors, found to be arise from the arachnoid cap cells. The arachnoid cap sort of cells are thin in nature and are noted to have a spider web like appearance which tends to cover the brain and spinal cord. The arachnoid comprises one of the three most important layers that additionally also includes the dura mater and pia mater which are collectively referred to as meninges. Meningiomas, in majority are found to be benign however slow growth has been noted among them also, unless they become extensive to be discovered and incase if left untreated, then depending upon their exact locations of presence, they can pose severe disability and chronic life-threatening condition to the suffering subject. Many patients have been noted to have a single meningioma while on the other hand, patients can be seen with a tendency to develop secondary meningioma tumors that specifies that such tumors are originated from one point initially but their growth has led them to expand to other parts also in the brain and spinal cord. (Buerki et al, 2018)

A meningiomal tumor that arise from the meninges (membranes that surround your brain and spinal cord) tends to compress and squeeze the adjacent brain nerves and vessels. Meningiomas are also considered to be the most common non-glial primary tumors of the central nervous system and the most common extra-axial neoplasms, accounting for approximately 15 percent of all intracranial tumors. (Zenkl et al, 1972)

2.2.1. Historical Discovery Of Meningioma

Felix Plater, a Swiss physician and professor is a well-known name in history regarding his efforts for the initial classification of the intracranial tumors. He, in 1614 for the very first time reported a lesion that was quite comparable with a meningioma.

Further in 1774, the same ‘tumor like meningioma’ was discovered by Antoine Louis, who was a French surgeon and he named it as, ‘fungus durae matris’. (Bhat et al, 2014)

Later on, in 1915, an American scientist, Harvey Cushing who was a neurosurgeon and a pathologist noticed the same meningeal tumors to arise mainly from the arachnoid cap cells between the dura and pia mater and Harvey then for the first time coined the term of Meningioma for them, that was accepted globally.

Harvey was keen to work further upon the exploration of these tumors, along with his student Percival Bailey in a hospital setting of Peter Bent Brigham, where they tried to design a classification system for these Meningioma tumors that was purely based on the histopathological characteristics and respective analyzed data. They were successful to describe four of the meningioma variants that includes meningothelial, fibroblastic, angioblastic, and osteoblastic.

The quest for the more refined form of classification of meningioma tumors didn't stopped here and two American neurosurgeons and pathologists, Bailey and Bucy tried to provide a more modified version of the histopathology-based classification that was mostly similar to the currently used WHO 2007 classification system for meningiomas. (Bhat et al, 2014)

2.2.2. Meningioma Classification On Basis Of Tumor Location

As location wise, the meningioma tumors vary widely and even some meningiomas are noted to have presence adjacent to the dural lining specifically in the location of the venous sinuses of brain and skull (this is the area where the arachnoid cap cells have a presence in a quite plentiful manner), such tumors required their classification on afore mentioned basis. The typical classification of Meningioma including a variety of subtypes and specifically based on the location of the tumor is now presented as follows:

i. Cavernous Sinus Meningioma: This is one of the specific types of meningioma that tends to occur near to the area of **coronary** sinuses; the area which is responsible for draining the deoxygenated blood to the heart from the brain.

ii. Cerebellopontine Angle Meningioma: This type of meningiomal tumor is positioned right near to the margin of the cerebellum, along with the region associated with pons; and hence this is one of the reasons, apart from this menineal tumor, the acoustic neuromas are also noted to appear in this area typically.

iii. Cerebral Convexity Meningioma: The location of this meningioma is found to be at the cerebral convexity of the upper surface of the brain, as the name of this tumor already indicates.

iv. Foramen Magnum Meningioma: The large oval or opening (which is also referred to as **foramen**) lies at the base of the skull specifically in the occipital bone in humans. It is also the region from where the connections of the lower portion of the brain stem also derive a passage. The tumor located at such a region is referred to as foramen magnum meningioma.

v. Intraorbital Meningioma: The intra orbital meningioma appears to be located in regions around the eye-sockets mainly.

vi. Intraventricular Meningioma: The ventricles of the brain that are basically the chambers through which the CSF makes a passage to the brain and get circulated over keeps a room for developing the intraventricular type of meningioma.

vii. Olfactory Groove Meningioma: The location of these tumors is typically found to be at the region of the nerves that are connecting the nose to the brain. These tumors are noted to grow right alongside of midline floor of the anterior cranial fossa.

viii. Parasagittal/Falx Meningioma: Its location is typically found at Falx cerebri, where a layer of dura mater descending down vertically in a parasagittal way and seperates the brain into two hemispheres.

ix. Petrous Ridge Meningioma: The temporal skull bone has a petrous part that is not only in pyramid shape but also wedged at the skull base. This peculiar portion of the temporal bone is also known to support certain section of the organ that facilitate hearing. Meningioma arising at this point refers to the petrous ridge.

x. Posterior Fossa Meningioma: This sort of tumor is found to occur near the back of the brain where posterior fossa, a small space in the skull is present, mainly near to the brainstem and cerebellum.

xi. Sphenoid Meningioma: The large sphenoid sinuses, two in number are located behind the nose and between the eyes. The notable location of this tumor is near the sphenoid bone behind the eyes.

xii. Spinal Meningioma: The spinal meningioma, as the name is indicating is found to be located in spine, and in some cases towards or facing the spinal cord.

xiii. Suprasellar Meningioma: Suprasellar is usually referred to a saddle-shape depression in sphenoid bone, that is located close to the area of the skull where pituitary gland is present and hence the tumor is classified as suprasellar meningioma.

xiv. Tentorium Meningioma: Tentorium cerebelli are located close to the region of brain considered to be connecting to the brainstem. These are rare types of tumors usually found adjacent to the surface of the tentorium cerebella in the brain. (Kresak J, 2014)

2.2.3. Types Of Meningioma

It is known that most of the Meningioma tumors that are classified as “WHO grade-I” are benign in nature, the WHO grade-II classified Meningiomas are referred to as intermediate or atypical while a more aggressive biological behavior is shown by the WHO grade III Meningiomas , referred to as anaplastic meningiomas, that may tend to invade the brain and also infiltrate and affect the bone, muscle, dura or venous sinuses. (Brassesco et al, 2009)

There are certain histological parameters like cellular architecture, nuclear pleomorphism, excessive mitotic activity, necrosis and invasion of neighboring tissue that distinguishes the malignant meningioma from the benign counterparts, while the histological characteristic features that are associated with the Atypical meningiomas are intermediate of the above two and hence they show increased tendency to recur again. (Müller et al, 1999) Previous irradiation therapy to childhood cancer and also Neurofibromatosis are widely known to play a role in development of Pediatric meningiomas.

However, complex numerical and structural aberrations have been shown by Atypical and malignant meningiomas, as indicated by several other authors. (Pelz et al, 2007) The radiation induced counterparts also show some distinct structural aberrations, although quite rare but classified separately. (Brassesco et al, 2009)

2.2.3.1. Typical meningioma

Typical meningioma is referred to as a tumor that is slow-growing in nature and is usually considered as benign, typically found to be originating from the meninges which are membranous layers (protective in nature) encompassing the brain and spinal cord.

The typical meningiomas usually seem to have a uniform, an analogous appearance that appears almost like a hemisphere that have notably an extra-axial mass, mostly found upon the regions of cerebral convexity, parasagittal or otherwise from the sphenoid wing.

Such kind of meningioma tumors, likewise can also arise in quite unanticipated locations that may include the orbits, paranasal sinuses, ventricles or uniquely intraosseous or within the region of the calvaria. (Buerki et al, 2018)

2.2.3.2. Atypical meningioma

Atypical meningioma refers to a higher aggressive and dynamic form of meningioma. It signifies a WHO grade II tumor (accompanied by the two histological variants that comprise of the clear cell meningioma, and choroid meningioma).

These types of tumors are generally noted to grow faster and appears to have more of a complex, heterogeneous and aggressive nature. It is also noted to have a tendency to recur. (Buerki et al, 2018)

2.2.4. Grading Classification By WHO For Meningiomas

The meningiomas as mentioned are divided into several categories by WHO and continuously mdified at regular intervals after 1990, 2000 and 2007. Here in the follwoing passage we shall tend to elaborate the recently used latest classification system provided by WHO for meningioma grading. (Kresak J, 2014)

2.2.4.1. WHO grade I meningioma

Based on the criterias defined by WHO, there exists three grades of Meningioma, out of which several variants of each type exists i-e:

i. Most of the Meningioma tumors are WHO grade I (benign)
ii. As an estimated view, WHO grade II are noted to be equivalent to 6 percent and they have an increased likelihood to recur again.
iii. The WHO grade III are found to be quite rare, having a malignant nature with a considerable potential for metastasis. (Kresak J, 2014)

2.2.4.2. WHO grade I variants

The WHO Grade-I meningioma has several variants and some of them are as follows:

i. Angiomatous meningioma is the first one among the WHO Grade-I variants. The salient features of this variant are as follows:
    a. About 2 percent of all meningiomas are noted to be Angiomatous
    b. The vascular component in this type of meningioma usually exceeds 50 percent of total tumor area
    c. The meningothelial cells in this particular variant are observed to encompass the small blood vessels.

d. This meningioma variant is found to have large vessels
e. The Ki67 index in this variant type is noted to be 2 percent at Mean
f. In a case of its complete resection, it doesn't appear to recur. (Am J Surg Pathol 2004;28:390)
g. Hemangioblastoma can be considered as differential diagnosis, that is found to be stained positive in this case with NSE and inhibin.

ii. Fibroblastic is the second one among the Grade I variants:
    a. They appear as firm sort of tumors that are composed of cells having a spindle like appearance and having certain boundaries around the cells which are quite indistinct
    b. Their architecture is in the sheet pattern like that usually lacks any lobules or typical meningothelial whorls as well
    c. Their resemblance is more noted towards the schwannoma or solitary fibrous tumor while they are also found to be EMA+ focally and noted to consist of thick collagen bundles most often.

iii. Lymphocyte rich is the third type of variant that is considered to have association with other disorders that includes Castleman disease and some other sort of hematopoietic neoplasms.

iv. Meningothelial type of variant is considered to be the most common variant. Some of the peculiar features it possess are as follows
    a. The tumor cells shows an appearance of Syncytial type of cells and epithelial cells, while the cell boundaries of these cells are still indistinct upon observation and presence of classic whorls is evident.
    b. The presence of psammoma bodies, although sparse in nature may laso be found.

v. Metaplastic variant of Grade I meningioma can also be found to have bone foci along with the presence of fats and cartilage in its structural mioety.

vi. Microcystic is a rare variant and have extensive microcystic formation. The other features are following
    a. Cells have elongated processes and loose myxoid background
    b. Overall resembles microcysts

c. Has focal "classic" features
d. Variable pleomorphism
e. Absence of the trabeculae and cords is noted and there are no remains of infiltrates from inflammation
f. Microcysts that are extracellular in this case are also observed by EM.
g. Psammomatous variant, which is most commonly noted to appear in area located near spine is found to posses several psammoma bodies as well

vii. Secretory grade I variant tend to appear as Eosinophilic secretions and
   a. The cytologic sort of atypia is noted in this variant type
   b. Secretion of CEA is also noted

viii. Transitional variant have characteristics similar to Meningothelial and Fibroblastic types. They appearance of the cells of this variant type is noted to be in whorls, quite prominent in nature and the syncytial cells being in the cluster form along with the presence of psammoma bodies. (Kresak J, 2014)

2.2.4.3. WHO grade II meningioma

The WHO Grade II Meningiomas are commonly referred to as Anaplastic meningiomas as well.

i. WHO grade II
ii. Comprising 5 – 15 percent of meningiomas
iii. Diagnostic criteria is either:
   a. The mitotic figures are noted between 4 - 19 mitotic figures/10 HPF OR
   b. There is a chance of brain invasion as well OR
   c. Have most of these histologic features:
      i. Cellularity is found to be increased
      ii. Cells are noted mostly to be small but with a high N/C ratio
      iii. The nucleoli are huge as well as easily noticeable.

iv. The growth of these tumor cells appears to have no such definite pattern and is also in the form of sheet (most probably, this is also associated with the loss of lobular architecture)
v. Apart from the "spontaneous" type of Foci, a geographic necrosis is also noted
vi. The grading pattern of these cells doesn't take that much effect even if the invasiveness is noted there, either in dura mater or soft tissues and bones.
vii. The grade of cells of such a variant is also not affected by the nuclei that are specifically of atypical type or the pleomorphic ones.
viii. Ki67 is not a true diagnostic criterion, however it is usually greater than 4 percent and up to 20 percent.
ix. May be associated with prior irradiation
x. 29 percent of them usually recur (as compared to 9 percent of classic meningiomas and 50 percent of anaplastic meningiomas)
xi. 10-year survival is 79 percent but 26 percent will assume a malignant phenotype. (Kresak J, 2014)

2.2.4.4. Criteria for WHO grade II

The criterion by WHO for classifying the meningioma as Grade II, is based upon the following histological parameters:

i. Mitotic figures are noted between 4 - 19 mitotic figures/10 HPF OR
ii. There is a chance of brain invasion as well OR
iii. Three of the histologic features from the following:
    a) Cellularity is found to be increased
    b) Cells are noted mostly to be small but with a high N/C ratio
    c) The nucleoli are huge as well as easily noticeable

d) The growth of these tumor cells appears to have no such definite pattern and is also in the form of sheet (most probably, this is also associated with the loss of lobular architecture)
e) Apart from the "spontaneous" type of Foci, a geographic necrosis is also noted (Kresak J, 2014)

2.2.4.5. WHO grade II variants:

Clear cell meningioma is one of the WHO Grade II variant apart from the Choroid and Anaplastic and the salient features of this type are following

a) Rare variant, 0.2 - 0.8 percent of all meningiomas
b) WHO grade II due to aggressiveness
c) 20 to 60 percent chance of recurrence even with gross total resection
d) More common in younger patients (average age noted was 29 years)
e) Estimation of cauda equina and cerebellopontine areas
f) The site at which Clear cell meningioma is found to be is predicted for Cerebellopontine, cp angle and cauda equina. (Kresak J, 2014)

Chordoid meningioma is another WHO Grade II variant apart from the Clearcell and Anaplastic and have the following features

a) WHO grade II is known to have a high potential for its recurrence
b) They comprise less than 1 percent among all meningioma types.
c) The type is seen to affect the adults most often (average age being about 47 years) and apparently does not involves any symptoms that are related to its complex cascade system.
d) Cases in young adults /pediatric population may be associated with hematologic conditions, such as Castleman disease and certain disorders related to hematology

are noted to be involved in cases in which the young adults as well as the pediatric age groups specifically are subjected to WHO Grade II tumors.

e) The specific locations at which Choroid meningioma is found to be is supratentorial but in regions of spinal cord and the optic nerve, it is also found to be infratentorial or either intraventricular. (Kresak J, 2014)

2.2.4.6. WHO grade III meningioma

The Anaplastic type of meningioma is an uncommon and quite a rare subtype among all of the meningiomas. It holds a grade of III according to the criterions defined by the World Health Organization (WHO) and its morphological features are not only harmful but malignant as well. The salient features of this type are following

a) According to the terms of WHO 2016, the definition of such type of meningioma is that it possess cytological characteristics that are harmful and malignant (and also being quite similar to that of that of a carcinoma, melanoma or high-grade sarcoma). It may also appear to show a notable elevation in the mitotic activity and is regarded as Grade III meningioma.
b) Grade III meningiomas due to their malignant behavior are also referred to as malignant meningioma
c) They comprise 1 – 3 percent of all the meningiomas.
d) Apart from denovo, it may most probably be associated with tumors of recurrence or the ones affected by the former radiation transmissions.
e) Associated with aberrant CpG island hypermethylation profile
f) Occasional metastases beyond the CNS
g) These tumors appear to recur with a rate of about 50 to 94 percent and therefore the survival rate at the average is just about 2-5 years. (Kresak J, 2014)

2.2.4.7. Criteria for WHO grade III meningioma

The criterion by WHO for classifying the meningioma as Grade II, is based upon the following histological parameters

a) The mitotic figures observed in its case are usually 20 or even more than that per 10 HPF
b) The histologic features they usually present is sarcomatous in a frank pattern or a carcinomatous type
c) The grading pattern of these cells doesn't take that much effect even if the invasiveness is noted there, either in dura mater or soft tissues and bones.
d) The grade of cells of such a variant is also not affected by the nuclei that are specifically of atypical type or the pleomorphic ones.
e) Ki67 is not a true diagnostic criterion, however it is usually greater than 4 percent and up to 20 percent (Kresak J, 2014)

2.2.4.8. WHO grade III variants:

Papillary meningiomas are the first one among the WHO Grade III variants. Papillary meningioma has the follwoing salient features

a) They are classified as WHO grade III
b) Aggressive, high rate of recurrence, may metastasize
c) Often occurs in younger patients
d) May be difficult to recognize as meningothelial
e) Helps to refer to imaging or surgeon to verify a dural based lesion
f) The site at which Papillary meningioma is found to be is most often the supratentorial. However, the regions like spinal cord and the posterior fossa are also considered to be the prime locations of its development.

Rhabdoid meningioma is the second variant type among WHO Grade III and it has the follwoing salient features

a) They are also regarded as WHO grade III because of their destructive and harmful mechanism of action and their metastatic potential
b) They have a higher rate of recurrence
c) Rhabdoid morphology may increase with recurrences
d) The site at which Rhabdoid meningioma is most probably found to be is supratentorial, infratentorial or spinal. (Kresak J, 2014)

2.2.5. Meningioma Incidence And General Etiology

Meningioma or the Meningeal tumors that targets and affects the coverings of the brain are known to basically arise from Meningiothelial cells, and roughly 20 percent of human intracranial and intraspinal neoplasms are attributed to be associated with these tumors. (Sadetzki et al, 2000)

Female population of the middle age, among the general population is much frequently affected by the disease of Meningioma, (especially occurring at the 5^{th} decade of their life). (Sadetzki et al, 2000)

Unlike the above incidence ratio of meningioma in middle age women, the incidence of such intracranial meningiomas as per adult and pediatric age groups are known to be quite uncommon, i-e the incidence of which ranges between 0.4 percent to 4.6 percent of the entire primary brain tumors in that particular age group. (Krampla et al, 2004)

The Meningiomal tumors are known to be approximately contributing to 20 percent of all of the primary intra cranial neoplasms. The annual incidence approximately was about 6 per 10,000 cases for Meningioma. (Krampla et al, 2004). The sixth and seventh decade of life is noted to be of the peak incidence for Meningioma. Regarding the patients of middle age, the meningiomas are found to be significantly more widely prevalent in females as compared to males and specifically among this age group the frequency of Meningioma from men to women is more than 2:1. Majority of the Meningiomas tend to develop from the inside of the intracranial cavity, the spinal canal or rarely the orbit and are found to be

linked with the Dura mater. Some meningiomasprimarily, may also originate as intraosseous tumors, , especially those that arise from the sphenoid wing.

Seizures is the most common complication tht the Meningioma patients suffer from. It is approximated that about 40 percent of the Meningioma patients experience Seizuer as their primary and initial clinical symptom. Other clinical smptoms like Hearing loss or ringign in ears (Tinnitis), loss of smell, memory loss are also observed in the Meningioma patients but they are mostly dependent upon the location of tumor.The developmet and progression of Meningioma is predisposed by certain factors that may be exogenous or endogenous. (Vernooij et al, 2007)

To date, it is widely known that the patients suffering from Meningioma have most commonly their neurofibromatosis type 2 (NF2) gene affected. The preponderance of patients who tend to acquire meningiomas basically are suffering from neurofibromatosis type 2 (NF2). On the other hand, families have been reported with increased susceptibility to meningiomas but having no link or association with NF2 genetic aspect. (Vernooij et al, 2007)

Ionizing radiation is one of the most common factor that clearly reflects to take part and associated as exogenous factor in the development of meningiomas, having an average latency period of several decades (precisely after 2-3 decades). The Sex hormones, in addition to the above ionizing radiation factor, have also remained involved as a crucial determinant because of the aforesaid more representation of females among the Meningioma patients. (Wiemels et al, 2010)

2.2.6. Meningioma Prevelance And Epidemiologic Research

Most of the Meningiomas are quite slowly growing and often take several years to expand without showing any symptoms. However, in some of the cases, the drastic effects they

tend to produce on the adjacent brain tissue, nerves or vessels can cause serious complications.

According to the cases that were reported in US between 2002 and 2006 for all primary brain brain tumors and CNS tumors, approximately 33.8 percent of them attributed to Meningiomas as the most frequently diagnosed primary brain tumors.

There is no age limit specifically for meningioma as they can occur at any stage of life however mostly the meningiomas are discovered at older ages and affecting the women most commonly. (Vernooij et al, 2007)

The signs and symptoms of Meningioma can be very subtle at the beginning and doesn't show much pace at first, however at the later stags of life the symptoms gets worsen. Dependent on te locaton of the tumor in the brain or spine, the signs and symptoms also vary accordingly upto some extent and most commonly they may include Vision changes (such as double seeing or blurriness), Headaches (that usually get worsen with time), Ringing in the ears (tinnitis) or complete hearing loss, general body weakness along with Memory loss and loss of smell.

As we just mentioned, it may take a considerable time for Meningioma to show its evolved signs and symptoms, however in certain instances, the Meningioma patients may experience a sudden onset of seizers and abrupt changes in memory and vision. In such cases, meningioma requires emergency care as well. (Wiemels et al, 2010)

In regrd of the etiological factors, seems the Meningiomas are less explored and unterstudied as in relevance to the malignant glial tumors. And this is because of a number of various challenges that has been posed to the researches in Meningioma. Some of the challenges includes (i). as Meningioma being a relatively rare diseasetherefore sufficient numbers aren't available that are required to conduct elaborative , comprehensive and large multicentre studies ; (ii) the latency period of Meningioma is quite long i-e 20-30 years or

more after the initial treatetment for tumors by established and accepted doses of ionizing radiation, hence making the acertainment of exposure affected subjects challenging and hard because of the recall preference; (iii) as autopsy studies have suggested the prevalence of subclinical disease upto 2.8 percent of the population , indicating that the susceptible persons make a larger pool than those who are diagnosed clinically with confirmed disease and (iv) The difficulty in the evaluation preference and inclination, many of the meningiomas are detected unexpectedly through MRIs concerning the disorders such as head trauma or sinus diseases. (Wiemels et al, 2010)

Conservative management and treatment is usually provided to such unexpectedly detected meningiomas and also to a notable number of fundamentally determined and identified meningioma cases, meaning that they don't need to involve any surgical intervention apparently. There is another way that epidemiologists can use inorder to remove or minimize the detection biasness and that is solely via ascertaining the cases for which the surgical treatment or removal has been done and this will ensure the only cases having clinically significant meningioma.

Up to date, among the cases of intracranial tumors, only a few of the epidemiologic studies have been adequately emphasized and thoroughly focused inorder to study and asess the risk factors of Meningioma seperately. The two main Epidemiologic studies consists of certain large European cohorts like Interphone[4], and the Million Women Study that was conducted in the United Kingdom [5].
From the ''Interphone'' study conducted upon large European cohort, several region specific case-control - orEuropeanindividual country based studies were also spawned. (Wiemels et al, 2010)

2.2.7. Population Statistics

Pathologically confirmed meningiomas are estimated to have shown Prevelance of approximately 97.5/100,000 in the United States, among which 170,000 individuals are

known to be at current are diagnosed with aforementioned neoplasm. (Hinsdale, 2010) Since the surgical management of Meningioma is relatively less practised, therefore these estimates are low. According to the data from imaging studies and autopsy, the subclinical Meningioma rates are estimated upto 2.8 percent in women.

As per data from the Central Brain Tumor Registry of the United States (CBTRUS), a several fold hgher incdence has been demostrated among the females as compared to males i-e the aas per adjustment according to the age group and the rate of incidence (per 100,000 person years) is noted to be 8.36 and 3.61 for women and men likewise respectively. (Hinsdale, 2010) For the rare pre-pubertal meningiomas, such female:male ratio can be inverted as 2:1 approximately. (Menon, Le and Xiao et al, 2009)
In case of Atypical Meningiomas and the malignant ones, the fraction is comprising of a total 5 percent with a slight male predominance.

The Black NonHispanics are reported to have incidence rates slightly more than those of the White NonHispanics, the ratio being 5.90 and 5.94, respectively. (Hinsdale, 2010) Age-specific rates of occurrence shows a rising risk with life time and duration of age in both male and female. According to the CBTRUS data and over the past decade, the increasing risk of Meningioma can also be considered as an artifact of frequently rise in the proper recording of this disorder. (Wiemels et al, 2010)

2.2.8. Meningioma And Family History

The relation among the family tree, genetic background and the risk for meningioma has also been studied only in few studies. The fact that Meningioma risk poses a several fold increase to the first degree or the priamry relatives was first reported in Sweden by Malmer et al. who started to examine cancer risk in spouses and first degree relatives of brain tumor patients in Sweden. Malmar and the team found that inspite that the risk factor for the first degree relatives increased upto teo fold (standardized incidence ratio [SIR] 2.2), however,

interestingly they couldn't found any evidence for the spouses of the affected individuals to be at risk.

The research till date has shown that the adult age group of the population is much more susceptible and one to three percent of adult population is known to harbor the meningioma But interestingly the number of families whose famiy members are diagnosed with meningioma are relatively much rare than this. This indicates that the clinial import of meningioma as a disorder is critically based on the phenotypic expression (in wide spectrum) of mutated genome. Most of the cases of such families are attributed to inherited NF2 mutations.
Currently the data in regard of segregation analysis or family based linkage studies of meningioma have not been reported yet. (Wiemels et al, 2010)

2.2.9. Current Treatment Protocols

The treatment protocol for meningioma is designed very carefully by the practitioner that depends on many factors. The most important factors that contribute to predict the respective treatment for meningioma includes the precise or appropriate location of the tumor and secondly to get aware of the nature of the tumor whether it is benign or malignant and thirdly it also depends upon the feasibility of potential treatment options and health preferences by the clinician. (Ref: Johns Hopkins Medicine, The Johns Hopkins University, Brain Tumor Centre)

In general, currently the treatment protocol for meningioma usually consists of:
(i). Surgical Treatment (based on parameters)
(ii). Radiation Therapy (Adjuvant Chemotherapy needed in some cases)
(iii). Rehabilitation Therapy

2.2.10. Genetic Landscape And Etiological Background Of Oncogenes

Meningiomas, since a long period of time remained a focus of oncoresearch and considerable biological and genetic interest prevailed in them. Meningiomas are considered to be among the first solid neoplasms that were investigated and studied by employing cytogenetic techniques.

Family history and candidate geneare both the key important factors that determine the inherited susceptibility to meningioma in case of DNA repair genes.

People who have some sort of mustations in the neurofibromatosis gene (NF2) are at much increased risk for meningioma.

The ionizing radiation especially of a high dose remains an undeniable and established risk for Meningioma, however the low doses o radiation may also pose a certain risk and the question regarding the types and doses that contribute to the meningioma even at the low doses is still understudied or much remains contraversial about them.

Hormones whether endogenous or exogenous are hypothesized to play a pivotal and an etilogical role as the hormone receptors are the best place for these tumors to be harbored and also from the fact that women are more prone to meningiomas i-e the chances to develop the meningiomas being double in relevance to men. (Yuzawa et al, 2016)

Much of the emphasis being put on the research in brain tumors and especially blending it with the advent of the innovative genetic and molecular epidemiologic mechanisms and tools, is obviously quite promising in the etiological exploration of the intracranial meningioma.

A number of researchers have examined the relationship between specific genetic variants and meningioma risk, focusing on genes involved in DNA repair, cell cycle regulation, detoxification, and hormone metabolic pathways.

Majority of meningioma neoplasms are known to be sporadic in nature and interestingly such patients with sporadic lesions doesn't appear to show a family history of any kind of brain tumor.The germline mutations in the NF2 gene especially on the 22q12 chromosome are considered to be the most common possible factor taking part in Meningioma formation and development. Neurofibromatosis 2 (NF2) is a rare autosomal dominant disorder that is primarily characterized by mutations in the NF2 gene.

However, many genes other apart from NF2 are also implicated in familial meningioma as plausible factors.Many of the studies have also been conducted in which focus has been made upon GST and Cyp450 genes that are involved in detoxification and metabolism of exogenous and endogenous carcinogens. (Yuzawa et al, 2016)
Few significant associations with variants in these genes caused an increased risk of meningioma are described in the following manner.

The most common and frequent gene alterations in Meningiomas are found to be located in the chromosomal region 22q12.2 and these are represented by mutations in the NF2 tumor suppressor gene.From the previous cytogenetic examinations and several studies, the monosomy of Chromosome 22 had been observed in about 70 percent of the cases of Meningioma. (Collins et al, 1990).

Such an observation was highly supported with further data and consequent molecular investigations as the loss of heterozygosity (LOH) was identified by the genetic studies at the polymorphic markers on 22q and widely found to be the most prevalent genetic and molecular alteration in about 40 to 70 percent cases of all meningiomas. (Seizinger et al, 1987) Sporadic meningiomas were discovered to have 60 percent of the mutations in the NF2 gene that were typically found to be linked with LOH at 22q.(12–14).The truncating effect of NF2 mutations have shown us quite clearly that the NF2 gene product merlin (also referred to as schwannomin) is critically significant in the development of Meningioma pathogenesis. (Dumanski et al, 1987)

In predominant number of Meningiomas, Merlin is found to have diminished immunoreactivity and well correlated with LOH 22q.(15–17)Merlin is considered to belong to the family of structural proteins that serve to link the cytoskeleton to proteins of the cytoplasmic membrane.

However yet it is not precisely understood about the tumor suppressive mechanism of Merlin.

The factors considered to be significant and crucial to the neoplasm formation includes the signaling cascade disruption that in turn leads to rearrangement and reorganization of the cytoskeletal moiety. (Evans et al, 2001)

Significant inhibition of proliferation of meningioma cells with the Over-expression of Merlin has been observed at in-vitro studies in NF2negative and NF2-positive both lines and it has been noted that Merlin plays acts as a negative regulator for progression and growth of tumor. (Ikeda et al, 1999)

Among all of the benign meningioma variants, the frequency of the NF2 aberrations/Merin alterations varies upto certain degree and it is different for all of them. However the NF2 mutations are known to be more predominantly harbored by the transitional and fibroblastic meningiomas i-e upto 70 percent to 80 percent of cases approx while such type of mutatuons are carried in the lowest ratio i-e in about 25 percent of the cases of meningothelial meningiomas.(Wellenreuther et al, 1995)

The fibroblastic variant and LOH 22q were also found to be closely coorrelated. (Ruttledge et al, 1994)

Correspondingly, the expression of Merlin was found to be quite low in most cases of the transitionalandfibroblasticmeningiomas while this was rarely noticed in case of meningothelial tumors. (Hitotsumatsu et al, 1997) The low frequency of NF2 alterations found in meningothelial meningiomas clearly indicates that the genetic etiological background and origin of these meningiomas is different from that of the other two variants and largely does not depend upon the factor of NF2 gene alteration.

Furthermore it is also noted that the freqency of NF2 mutations observed in atypical and anaplastic as well as in benign fibroblastic and transitionalmeningiomas appears almost to

be the same inferring the lack of any involvement and association of NF2 alterations with/and progression to higher-grade meningiomas.Rather, the development, as well as the formation of a large number of benign meningiomas, is associated with the NF2 alterations and therefore NF2 mutations are regarded as representative factors that depict these early alterations.

In certain cases of Meningiomas, however, an unusual phenomenon has been noted i-e even in the absence of Merlin, NF2 mutation or LOH 22q could not be detected. Degradation of Merlin via Protease M-Calpain was proposed in this regard that provided the answer to this phenomena as an alternative sort of mechanism. (Kimura et al, 1998). In among more than 50 percent of Meningioma cases, the mechanism of activation of M-Calpain has been illustrated and thoroughly explained by different studies conducted, however the link between state of Merlin and that of M-Caplain activation couldn't be confirmed yet. (Ueki et al, 1999) On the other hand, the relationship between the loss of Merlin and LOH22q proposed several other mechanisms like methylation, homozygous deletions and undetected NF2 mutations that are now considered to take part in the loss of Merlin.

Most commonly, the sufferers of NF2 are perceivedto develop meningiomas.The variation between the sporadic and NF2 associated meningiomas is noted in numerous aspects that includes for example the appearance and detection of NF2 meningiomas in quite earlier period of life, they are often complicated and as well as multiple and usually belong to the fibroblastic variant as compared to the sporadic ones. (McLendon et al, 1998) The deletions on Chromosome arm 22q that refers to a typical feature of associated with NF2-meningiomas are noticed to be present in nearly 100 percent of the cases, indicating much higher frequency/incidence in relevance to sporadic meningiomas. (Lamszus et al, 2000). While some of the alterations that occur on the genetic and molecular level like the deletions on 1p, 6q, 9p, 10q, 14q, and 18q set of chromosomes are found to have the same frequency as the sporadic meningioma cases. (Lamszus et al, 2000) With NF2 associated tumors, several of the studies and investigations revealed the frequency of atypical or malignant meningiomas to be uniform mostly and no such increase was noted. (Antinheimo et al, 1997)

(The MIB-1 labeling index was defined as the percentage of immunoreactive tumor cells in the evaluated area. It must be in the range from 0.0 to 100.0. An MIB-1 labeling index value of <0.0 or >100.0 is an unacceptable error. (Source : Nature.com Articles)).)It's quite interesting that the immunoreactive tumor cells percentage or simply the MIB-1 labeling index in meningiomas that are related to NF2 alterations revealed to be higher but this was supported by only a single study (Antinheimo et al, 1997) while on the other hand, a more thorough investigation, backed by further established studies shown that a large number of samples were lacking such an increase and the MIB-1 labeling index was same in comparison to sporadic meningiomas.(Lamszus et al, 2000). A study conducted recently shows that the meningiomas associated with NF2 mutations collectively with the pediatric meningioma cases have indicated a greater percentage for the presence of WHO grade II and grade III tumors as compared to the sporadic cases. (Perry et al, 2001).

The characteristic of brain invasiveness is however contrarily a benign type of tumor that was considered comparable to the grade II (WHO). In light of the above scenario, this was noted that weak or rare association exists between the development of malignant meningiomas and NF2 patients and also to mention that different other and additional reasons are behind the mortality of these patients that needs to be investigated.

The presence of various other tumor suppressor genes have also been proposed and indicated by a number of studies and although they may lie outside the NF2 region but have been implicated in meningioma cases with a quite significance. Some of them are now as follows:

The number of LOH22 mutations, quite interestingly surpasses the frequency of NF2 mutations and the data obtained from the mapping of deletions on the Chromosome 22q also shows that such type of mutations doesn't involve the locus of NF2 in some tumors. Moreover, a case of multiple meningiomas has been presented from a single patient that depicted the monosomy for chromosome 22 but were lacking NF2 mutations. (Perry et al, 1999)

Gene cloning has been tremendously performed in recent years in Oncology and especially for those tumor suppressor genes that are located on Chromosome 22q and are widely considered as candidate genes for tumor suppression. Many of such genes have been screened to look either for mutations or to observe their altered levels of expression in meningiomas.

ADTB1 (b-adaptin, BAM22), RRP22, and GAR22 are those three of the genes that lie next to the vicinity of NF2 gene and their locations apparently map to the 22q12.2 region. Among the above genes, extensive cloning was performed for the ADTB1 gene and this was done on the basis of 140-kb homozygous deletion in sporadic meningioma. Data examination from the expression analysis revealed only 12 percent of the sporadic meningioma cases to be lacking in ADTB1 transcripts.

In 110 of the cases of the sporadic meningiomas however, no mutations were found to be detected that proposes the involvement of certain other epigenetic mechanisms behind such gene inactivation. (Peyrard et al, 1996) Likewise, 12 cases of such meningiomas have shown to lack any RRP22 or GAR22 mutations and interestingly the LOH involvement was found to be there in region of 22q12-q22 in half of those cases while none of them exhibited NF2 mutations.

The number of other candidate genes that are located outside the 22q12.2 region is also not rare. It is also the same 22q12.1 segment in which the MN1 gene is located. Initially, the disruption of MN1 was attributed to translocation in a meningioma (Lekanne et al, 1995), yet the followup by the subsequent studies illustrated its role more profoundly as an oncogenic transcription coactivator as compared to a tumor suppressor.

In case of the atypical tumors of the teratoid/rhabdoid nature, a frequent mutation is noted in the gene of hSNF5/INI1 (SMARCB1) whose regional mappiing is found to be at Chromosome 22q11.23. Reports from a single study that was conducted for analyzing 126 meningiomas, only 4 out of them have found to be shown an identical missense mutations (Schmitz et al, 2001). The interestng point that was noticed among this study was the appearance of NF2 mutations that was shown by 4 tumors. This indicates clearly that the

loss of hSNF5/ INI1 functionality does not mean that this is going to be considered as an alternative mechanism to the NF2 inactivation in the same regard of meningiomal pathogenesis, however it also remains evident that the silencing of the gene function of hSNF5/INI1 may have a link with NF2 disfunctioning, that co-operates with the impairment of NF2 function as well.

The CLTCL1/CLH-22 gene also maps to the region of Chromosome 22q11.21 and analysis have shown that in a study comprising of 46 meningioma cases, 37 of them have noted to be lacking the expression of CLTCL1/CLH-22 gene. The above ratio accounts for 80 percent of the cases analyzed during that study and it clearly points out the relevance between this gene and the consequent tumor development.

Tumors having initiaiton or progression due to absence of gene expression may or may not have their location traced to Chromosome 22, But still the mutations in the gene CLTCL1/CLH-22 haven't been still reported and in addition to that , the picture about the mechanism of the loss of expression of this gene is still not clear.

The location of DAL-1 protein maps to the chromosomal region 18p, 11.3. It is found to have tumor suppressor properties and while relating itself to the 4.1 family proteins that are membrane-associated proteins, the notable point about this protein is that it shows an important homology with a specific protein i-e Merlin.

In anaplastic meningiomas, the proportion of which accounts for 87 percent of meningioma cases, the lack of DAL-1 protein is only insignificantly noted as compared to atypical meningioma cases (the proportion of which accounts for 7 percent to 76 percent). This clearly implies that Lack of DAL-1 protein serves as a factor in early events of meningioma tumorigenesis.

Interestingly, an important point to be noted in this regard is the presence of these immunohistological changes is found to be predominantly reflected at the molecular mRNA expression level. Although the detection of LOH that maps to the Chromosomal region of 18p11.3 is found in almost 71percent of the meningioma cases investigated, however the possible mechanism that accounts for the inactivation of DAL-1 is still not

revealed.Here, its well to be added that on one hand the exposure to mutations was not detected but on the other hand the screening also did not covered the full gene. Considering the numerous frequent LOH at the prominent locations of DAL-1 gene, homozygous deletions are not likely to be found as a certain way of mutation and hence, therefore, the epigenetic alterations persist to be the more definite and likely possibility of such mutation. (Gutmann et al, 2000)

58 percent of the well-studied cases have revealed the integrated or coupled loss of DAL-1 and merlin together and this clearly shows that both of them do not apparently belong to a single growth regulatory pathway, where the inactivation of one member causes the same effect to the other.While upon keenly observing about 70percent of the anaplastic meningioma cases and almost 50 percent among the benign ones, the loss of DAL-1 and merlin was noted to be more at a more frequent drift in relevance to the 60 percent of the atypical meningioma cases studied. This clealry proposes that both of such genetic alterations are recognized and regarded as the early changes or modifications, and the concomitant loss of whom can render a selective growth advantage.

Cytogenetic and CGH analyses have failed to recognize 18p, 11.3 as a region of frequent chromosomal losses. Microsatelliteanalysis revealed that deletions in this region are centered around the DAL-1 gene locus and are too small to be detected by karyotyping or CGH.

The main genes that were noted to be involved in meningioma progression and found to be located on chromosome 18 were MADH2, MADH4, APM-1, and DCC. The aforementioned genes with known to have tumor suppressor functions located on chromosome 18 were reviewed and thoroughly studied by Bu¨schges et al performing investigation upon 37 meningiomas. While the CGH examinations has revealed that most of the genetic losses of identified genetic material were found to be from the long arm of chromosome 18. Such losses were correlated with the progress of meningioma stage and an increase in the histological grade of the meningioma.

Despite of the above findings of interest, there was also a single mutation noted in the APM-1 gene, that was basically a missense mutation, but still no other mutations were

identified as such. In regard of pathogenesis of meningiomal tumors, it seems that these findings are unable to provide the significance of MADH2, MADH4, APM-1, and DCC alterations.

Although the mutation noted in the APM1 gene was solely found to be only the mis-sense mutation which was observed in the atypical meningioma. However, there were no such other mutations associated with the other four related genes i-e MADH2, MADH4, APM-1 and the DCC (alterations). Quite interestingly, the above four genes have been found to show certain detectable transcripts in almost all of the tumors. Findings also does not reveal any role of significance for MADH2, MADH4, APM-1 and DCC alterations to be involved in Meningioma pathogenesis.

The second most common peculiar abnormality in the widespread meningiomal cases is posed by the Chromosomal deletions on the Chromosomal region 1p. Increase in the number of 1p deletions is noted to be linked with the grade of the tumorand hence also noted to be elevated with high tumor grade. Most likely to the above scenario, the analysis of certain individual cases of the "Recurrent meningiomas" also shows that tumor progression is linked with more increased 1p deletions.

It is quite evident that the above quoted findings proposes that the genetic information that has been found to lost in regard of 1p Chromosomal region, is in relevance to the tumor progression but not to the meningioma formation.

In addition to this, many other genes that shares the same chromosomal location i-e 1p were subjectd to screening for mutations specifically alterations apart from deletions. These genes include CDKN2C (p18INK4C) and ALPL. However the results that were gathered from three seperate studies and considering more than 100 meningioma cases, only a single mutation was noted to be found in one of the gene i-e CDKN2C, along with other homozygous deletion.

There was also no detection of Methylation aberration and loss for any transcripts of CDKN2C was also not found. This clearly implies that alteration of CDKN2C gene in Meningiomas is quite rare.

The ALPL is assumed to have tumor suppressor function and as the name of this gene indicates, ALPL gene encodes for the enzyme “Alkaline Phosphatase”. The location of this gene maps to Chromosomal region 1p36.1p-34. Previously, it was proposed that the loss of 1p in a variety of Meningioma cases is basically linked with the loss of alkaline phosphatase activity and this was inturn leading to a perception that there might be a tumor suppressor characteristic or function of ALPL. The alterations related to the ALPL structure wise, were not found to be well documented and much of the data in regard of the evidence for the functional mechanism of tumor suppressing property of alkaline phosphatase is also missing.

TP73 is another candidate gene regarded as a tumor suppressor. The location of this gene is noted to be 1p.36.32. Studies suggests that the inactivation of TP73 gene plays no significant part in meningioma progression. According to one study in whch 50 meningioma cases were subjected to analysis, there was a single mutation found among them, which again indicates quite insignificant role of this gene. On the other hand, the expression of TP73 is also observed to be altered and increased with the higher grade of tumors, which clearly implies that this gene might has more of the oncogenic function that literally seems to be dominant than a conventional tumor suppressor function.

Loss in whole or componential losses of Chromosome 14, according to many different cytogenetics (cell studies) and in relevance to the genetic aberrations and losses mentioned/described for chromosome 22 and chromosome 1 are regarded as third most common Meningiomal abnormality. The deletions on the same chromosome arm i-e 14q as identified and detected by LOH and FISH analysis was found upto 31percent. The increased level of 14q deletions precisely indicates the link of their association with meningiomal progression. Detection of the losses upon this chromosome arm has a great significance in identification of the patients who have higher tumor grades and who are at increased risk of “Relapse” and this s because the deletions detected on this Chromosome arm serves as an independent prognostic factor and parameter.

The deletions on chromosome 10 has received a humble focus and just like the chromosomal alterations noted upon the chromosome 14, the deletions at chromosome 10

were at the core of attention as well. The discovery of the fact that there exist a link between the progression phenomena of meningiomal tumor and the loss of certain alleles on the chromosome 10 was provided by Rampel et al.

The long arm of chromosome 10 was found to be more prone to the deletions, supported by several consecetive analyses of LOH and CGH. However considering man meningioma cases in a wide manner, there was no gene found to be inactivated or deleted on chromosome 10, that is presumed to have a specific tumor suppressor property.

The location of PTEN gene maps to the chromosomal region 10q23.3. Several of the analyses were performed on the considerable ample collection of the samples. Neverthless the detection of mutations was almost none, with the exception of two cases. Also there were no homozygous sort of deletions noted in them.

The location of DMBT1 gene is at 10q26.11-q26.12 on chromosome 10. From several of the sample studies in consideration to atypical or malignant, for each type of meningioma, there has not been found a single homozygous deletion on this gene.Many studies based on mapping examinations have also illustrated various deleted domains (regions) on the same chromosome 10. One such chromosomal region i-e 10q24-qt was originally been tried to investigated and outlined by Simon et al.

In a collective manner of consideration, all of these investigations and findings upon any kind of loss in alleles of chromosome 10, implies a certain pattern that is quite complex and needs elaboration as till now, there has been found no identification of even a single compatible and harmonious region that is confirmed for deletion.

The identification of the loss of genetic domains, most particularly on chromosome 9 is observed to be very seldom in case of a benign or atypical tumor of meningioma , however, the scenario is opposite and such losses are noted to be more in case of malignant types of meningiomas.

The genes that are located on the shorter arm of chromosome 9 includes CDKN2A and CDKN2B that are known to be associated with tumor-suppressing functions. and

interestingly these genes are also at the core of attention due to the reason of their inactivation which is seen at a greater rate in many of different human tumors.

In regard to homozygous deletions of CDKN2A and CDKN2B, an estimate of 46 percent of them among the patient pool, were observed by Bostro¨m et al. Among the novel studies, it has been also described that there exists a connection between the CDKN2A alterations and the patient having anaplastic meningioma and thereofore those having such alterations were noted to have a comparatively shorter survival, hence serving as a significant prognostic tool as well.

Another gene regarded as a tumor suppressor is TP53, which maps its location to the short arm of chromosome 17. Quite precisely it is mutated in astrocytomas at a considerable huge rate, while in most of the cancers of general nature, the TP53 mutations show an optimum frequency. On the other hand, though, the meningiomas are almost deficient or have a very limited presence of TP53 mutations. There were no or a single sporadic TP53 mutation revealed by any of the manifold studies conducted to observe their existence.

In most of the considerable cases, the mutation of TP53 leads to an enhanced level of stability of a protein, referred to as p53 protein. This p53 protein (mutant type) is further subjectable to investigation and examination by certain immunohistochemistry techniques. The analysis performed by the aforementioned immunohistochemical ways has generated different conflicted and inconsistent results that are found to be correlated with varying grades of malignancy. Complying with the reality, a large number of studies have illustrated there exists a relationship between the aggregation of p53 and the respective grade and behavior of tumor malignancy. However, the biological point of importance for the accumulation of p53 is still not unfolded.

Although less prominent to the alterations illustrated previously, there exist several additional chromosomal alterations recognized in the meningiomas. Comparatively to the ones discussed, the most common of these are losses on 3p, 6q, X, and Y, as well as gains on 1q, 9q, 12q, 15q, and 20q. For instance, certain research studies showed unusual and very seldom increase involvement in meningioma for the genes like CDK4 and MDM2 whose location maps to the arm of chromosome 12q.

Similarly, no other specific gene alterations on these less commonly altered chromosomes could be identified in a significant number of cases.

Varying chances of meningioma risk were identified by the researchers among the GSTT1 null and the positive genotype. Risk is noted to be enhanced with the GSTT1 null type upto 3.5 fold as compared to the risk associated with GSTT1 positive genotype.

The two other genes i-e Ki-RAS and ERCC2 were not only found to be associated with the meningioma but also the tendency of meningioma with variants of the two genes involved a risk increase of upto 2 folds. These findings were put forward by Sadetzki et al. There are many additional genes that were identified to be correlated with the radiation-associated mutations, hence leading to radiation-induced meningiomas. However, mutations in the genes like TP53 and PTEN were also restricted to a very few cases and none were examined inHRAS, KRAS and NRAS genes.

Meningioma, among the tumors, remains one of the pioneer and leading one that are affiliated with a genomic driver and especially upon the primary and fundamental description of the neurofibromin (NF2) which is considered as the causative gene or agent for the neurofibromatosis 2 (NF2), under the etiologic umbrella of whom almost 50–75percent of the tumor suffering patients develop one or more meningiomas. The high, as well as the low-grade meningioma cases of the sporadic nature, are perceived and recognized to provide wide shelter to mutations, inactivation in alleles and NF2 losses in 40 to 60 percent of tumors.

The novel approaches like the next-generation sequencing, also commonly referred to as NGS has provided the answer to the efficient screening and identification of a number of recurrent somatic mutations, that constitue about 40percent of the sporadic meningiomas. Some of these genes are known as the pro-apoptotic E3 ubiquitin ligase TNF receptor-associated factor 7 (TRAF7)**,** the pluripotency transcription factor Kruppel-like factor 4 (KLF4), PIK3CA and SMO.

2.2.11. Focused Oncogenes; MTHFR, MTRR, RAD54L

As altogether in general, the molecular taxonomy of meningioma is for sure going to be duly affected by further advancements in meningioma and is significantly promising, however a percentage of meningiomas still remains to be unidentifiable on the basis of mutations in the oncogenic drivers.

Apart from the increased age factor, the exposure to the ionizing radiation is considered so far as the serious risk factor for meningioma tumors and yet it remains a fact that there exist several other environmental, genetic and life style determinants that poses certain risk for meningioma development, and that has been evaluated thoroughly in several studies performed. Emphasis still remains on the point that further investigations and studies are definitely the need of time, nevertheless these upcoming studies must need to be in quite integration with the environmental exposure, changing life styles and of course in accordance with bio-informatics and genetics. Larger pool of subjects or sample sizes, regular follow-ups in long term and also efficiency in the measurements shall definitely impart more essence to it.

Beyond mutations, insertions, deletions, and duplications at the single nucleotide level, the meningiomas are known to harbor a classic constellation of chromosomal copy number alterations (CNAs) and single nucleotide polymorphisms (SNPs). In comparison to higher-grade meningiomas, the low grades represent more of such type of alterations as compared to the losses and gains. And it is also noted that in addition to these aforementioned CNAs and SNPs, the epigenetic modifications of the genetic components also provide an answer to the various biological mechanisms, beleived to involved in meningioma progression and development.

The contemporary and innovative advancements together at molecular, genomic and epigenetic level has tried to unfold a new renaissance in investigation of meningiomas from each aspect. The systematic approaches mentioned does not only provides a promising strategy that affects and moderanise the diagnosis and disease classification, rather it is also

supposed to lead to the clinical management from a very new angle. Moreover, due to the shared bilogical, physiological and pathological characteristics between the meningiomas and other cancer types of the CNS, that includes invasiveness and intratumoral heterogeneity, may ultimately open new therapeutic avenues for the increasingly disparate tumors. (Berg E, 2017)

2.2.11.1 MTHFR/MTRR gene mutation and mechanism

The MTHFR gene mutation is the gene mutation present in about 15 percent to 20 percent of the population. People with this mutation do not produce enough or any of the enzyme needed to turn folic acid into usable folate for the body.People with MTHFR are more likely to have nutrient deficient related diseases. (Cara G and Teresa E, 2015)

Signs of MTHFR gene mutation includes Mood problems, Improvement with usable forms of vitamins, Lab tests. While the Supplements for MTHFR disorder includes :Methylated B9 (Folate) and Methylated B12. (Cara G and Teresa E, 2015)

The MTHFR (Methyl TetraHydro Folate Reductase) gene encodes for the MTHFR enzyme that takes part in one carbon metabolism pathway, the pathway which involves four major cycles. The Folate pathway, Methionine/Homocysteine cycle (Activated Methyl Cycle) and Purine synthesis pathway. (Dr. Kendra-JJ Medicine, 2018)

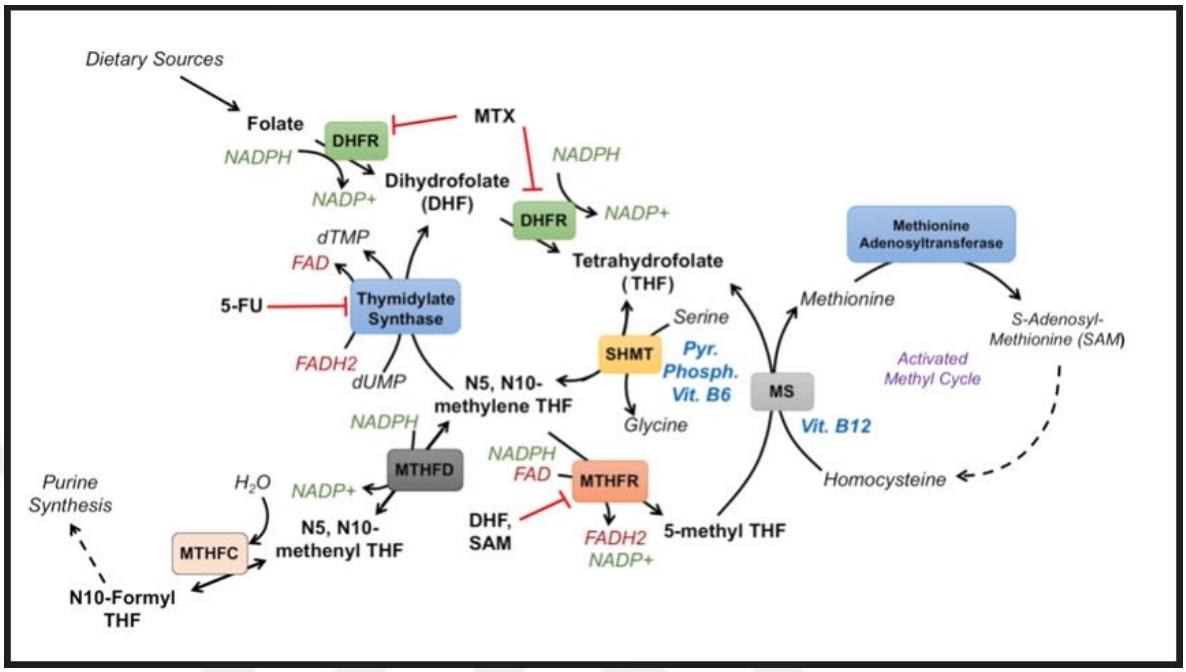
In this context, it is necessary to mention that Foalte (Vitamin B9), Pyridoxine phosphate (Vitamin B6) and Cobalmain (Vitamin B12) plays their role in these pathways at their specific positions. The Folate taken up by the body and consumed from the dietary sources is acted upon by an enzyme Dihydro Folate Reductase (DHFR) by utilizing a molecule of NADPH to convert it into Dihydro Folate (DHF). The Dihydro Folate is further acted upon by the enzyme Tetrahydro Folate Reductase (THFR) and the Dihydro Folate (DHF) gets converted to Tetrahydro Folate (THF) which is the active form of Folate and acts as a carbon donor, as a co-factor and important for several processes that includes amino acids synthesis and nucleic acid synthesis.

The Tetrahydro Folate (THF) is further acted upon by an enzyme called Serine Hydroxy Methyl Transferase (SHMT) to convert the THF into N5,N10 methylene THF. This step takes place in the presence of the vitamin B6. The N5,N10 methylene THF is further acted upon by the enzyme known as Thymidylate Synthase to recover the DHF and the reaction also converts the dioxy UMP to dioxy TMP which through a further cascade of reactions leads to DNA synthesis.

As these metabolic processes takes place in a regular balance in the body. Therefore, if the above pathway is not needed by the body temporarily to be finctional, the N5,N10 methylene THF is acted upon then by the MTHFR enzyme. This is where the MTHFR enzyme is located at such a pivotal role. This step utilizes a molecule of NADPH and FAD and the N5,N10 methylene THF is converted to 5 methyl THF. The 5 methyl THF when acted upon by the Methionis Synthase enzyme and in the presence of vitamin B12, it generates Methionine and also recovers the THF. The Methionine is then converted into SAM (S-Adenosyl Methionine) when it is acted upon by enzyme Methionine Adenosyl Transferase. The SAM is then converted further via a cascade of reactions into Homocysteine. (Dr. Kendra-JJ Medicine, 2018)

Neverthless, there is another cycle in parallel that is activated apart from the above pathway and the direction of which is quite different. In such a case, the N5,N10 methylene THF is acted upon by an enzyme MTHFD that utilizes a molecule of NADPH and converts the N5,N10 methylene THF to the N5,N10 methenyl THF, who is inturn acted by MTHFC enzyme and forming N10 Formyl THF. The N10 Formyl THF is then further processed for Purine synthesis, that are nitrogenous bases, via a cascade of reactions. (Dr. Kendra-JJ Medicine, 2018)

Fig 2.1: Description MTHFR Enzymatic Pathways



Reference: The figure was prepared by JJ Medicine, 2018 version.

2.2.11.2 Studies Related To MTHFR Gene Mutation

Several studies has been conducted in this regard in relevance to different ethnicities as the mutations of MTHFR gene has shown varying results in deifferent populations. The very first study that needed to be quoted here was performed R. Kumawat et al (2018) in India. As brain tumor is considered to be one of the main among causes of death in India, where almost half of the population is also coping in one way or another with a severe malnutrition. Hence, effort was made inorder to establish a certain relevance between the folate metabolism in this study cohort, the mutations in their foate metabolising enzymes and the screenng for any possibility of finding a SNP. The DNA methylation and the abnormal plymorphisms are the key characteristic factors that are defined to be involved in the meningioma via the MTHFR gene cascade. The individual aim of their study was the screening of an snp in the gene Methylenetetrahydrofolate reductase (MTHFR)at the position of C677T and A1298 to further evaluate its association with the meningioma in indian subjects.

A set of 288 brain tumor cases were enrolled for this study among which 76 were meningioma cases, 108 glioma and 104 healthy control subjects. According to R.Kumawat et al, the most prevalent genotype that was found for MTHFR C677T was CC (controlgroup 71.2 percent , meningioma 75 percent), followedby CT genotype (control 25 percent,meningioma 19.7percent) and TT genotype (control 3.8 percent,and meningioma 5.3 percent). While in case of MTHFR at the allelic position A1298C, AC was noted to be the most common genotype (control 50 percent , meningioma 48.7 percent followed by AA genotype (control 25 percent , meningioma 36.8 percent) and CC genotype (control 25percent , meningioma 14.5percent). The Odds ration calculated in this study was found to be 0.62 and the p-value was 0.03. The finding of this significant study clearly reveals the corealtion between the MTHFR A1298C genetic locus polymorphism of folate metabolizing enzyme with meningioma. Still large pool studies are required to be conducted. (R. Kumawat et al, 2018)

The scientific journey of R. Kumawat et al (2018) for the quest to find a relatioship between the MTRR folate metabolising genes and meningioma didn't stopped here rather they continued to conduct another study in the north indian population. During this study, an effort was made for analysis of SNPs in in MTRR gene at position A66G. The number of control subjects and the meningioma and glioma caes were kept identical to the above study.

Similarly, reduced risk of meningioma and glioma was found to be associated with the AG genotype of MTRR A66G than the AA genotype (odd ratio = 0.56, 95 percent, CI 0.32-0.97, p=0.039). Although in this study, the AG genotype showed a more prevalent proportion i-e 47 percent which is the same as documented by Rai et al., 2011 in their previous study (on subjects of south indian population), however quite interestingly, the AA genotype was found to be 44 percent which turns out to be entirely different i-e 1percent when calculated by Rai et al., 2011 for the south indian population. (R. Kumawat et al, 2018)

Upon the clinical based relevance there was no risk as such observed for SNPs in MTRR A66G to be linked with meningioma, yet contrary results have been found by Zhang et al.,

2013 and Bethke et al., 2008 who grade the AG genotype SNP of MTRR as a risk factor for adult meningioma in chinese han ethnicity and british populations respectively.

As the Folate metabolism genes having certain polymorphisms are beleived to be involved with various human cancers, and inorder to validate the findings related to it, a study was conducted by Zhang et al. in China. Again it was a population based study focusing on a specific ethinicity i-e a Chinese Han population to analyze the polymorphic variants in MTHFR (C677T allelic region and A1298C position) and MTRR (at A66G locus) folate metabolising genes.

The case control study involved 600 meningioma patients of different WHO grades (patients of the last 5 years were included as this study continued till 5 years) and 600 control subjects were also included in this study in parallel. (Zhang et al, 2013)

As a certain link of meningioma was tend to established among the previous study that explains the pivotal role of the enzyme MTHFR in regard of folate metabolism, Ruiyan et al. continued to investigate the case. Ruiyan et al. performed another case control study to look more significantly for an association between the MTHFR and meningioma in the ethnic community of a Han population in northern China. In their study, a pool of 317 meningioma patients were included along with the control group of 320 normal subjects comprising a study cohort. The genotyping for the SNPs at the two positions i-e C677T and A1298G revealed interesting results of variation. (Li, Ruiyan, et al, 2013)

The study conducted by Bethke et al. is completely focused on the same avenue i-e to screen the single nucleotide polymorphic variations at certain genetypic positions in MTHFR and MTRR folate metabolising genes in a Caucasian population at United Kingdom. The MTHFR gene at the region C677A and A1298C while the MTRR gene at A66G was screened for single nucleotide polymorphic mutations. During this study, the cohort comprises of 631 meningioma cases, 1005 glioma cases and 1101 as control subjects. The findings of their study were significant and the results of their study were going in accordance that MTHFR's, C677T and A1298C polymorphic variants were noted to have clear association with the meningioma risk. The P-value came out to be 0.02, again strongly suggesting the rejection of null hypothesis.

The relation of homozygosity of MTHFR variant with the increased risk of meningioma was an important finding in this regard, having the odds ratio (OR) of 2.1. The homozygosity of the MTRR variant A66G was also of key importance due to its link with the meningioma risk, the odds ratio (OR) calculated to be 1.41 in this case. (Bethke et al, 2008)

Similar study related to the evaluation of genetic polymorphism and MTHFR gene adn its association with the meningioma development tendency was conducted in Thailand for a cohort of Thai children. Their target allelic locations were the same as in previous studies i-e C677T and A1298C. This was again a case control study in which Sirachainan, et al (2008) tried to investigate the genetic polymorphisms in the DNA obtained from their peripheral mononuclear cells of blood. The cohort size was focused upon 73 Thai children having various CNS tumors along with a parallel cohort of 205 normal children as control subjects. The result findings were concluded around CC allele SNP of the MTHFR C1298G that was noted to be involved in pathogenesis of not only meningioma but also variety of CNS tumors including astrocytoma, oligodendroglioma and medulloblastoma etc. (Sirachainan et al, 2008)

Another study focusing mainly upon the involvement of RAD4L was conducted by Leone et al (2003) and the focused allelic region of RAD54L was C2290T. Their research was carried out in 29 meningiomas that included the allelic deletions, insearch for RAD54L mutations. However, interestingly they just found to examine only a single genetic mutation and that was the C/T transition which they located at 18th exon on a position of nucleotide 2290. They tried to obsrve a possible link between the 2290 C/T SNP swift and the meningioma tumors in this Spanish cohort. From the statisitcal data assessment they arrived at the conclusion that there is a probable association suggested between the rare T-allele and the development of meningeal tumors. (Leone et al, 2003)

2.3. THE HISTOPATHOLOGICAL SPECTRUM OF MENINGIOMAS

The Meningiomas are the tumors or neoplasms that are considered to make their route of development from the arachnoid cap cells that lies in the meningeal coverings within brain and spinal cord. They are usually beleived to be the second most common tumours among the nervous sytem neoplasm, accounting for about 34 percent. These tumors also posses an intrinsic behaviour to recur. Significant and notable advancements has been performed inorder to explain and understand the core base of these tumors from the background of genetic and molecular data. However, still the clinical diagnosis and the usual practtice follows the practice where the diagnosis is made on the basis of light microscopy technique being applied to the routinely stained haematoxylin-eosin sections and then furhter analyzing them according to the standard guidelines and criteria provided by World Health Organization (WHO). Based on the histological parameters, no doubt the modern and prevailing grading system provided by WHO is considerably useful from the prognostic point of view, though still it is hindered by such sort of assessments that are just subjective in nature and urge of a more precise and sharp determination is required that can make the clinical as well as practical applications easier.

The most recent and latest classification system provided by WHO, 2007 divides meningioma into three grades. The first grade benign tumors are directed to be distinguished from other types via their histologic subtype and the absence of anaplastic characteristics. The grade II which are usually also referred to as atypical are represented by one or more features that includes i) chordoid or clear cell type as per histology study, (ii) 4 to 19 mitoses per ten high-power field (HPFs), (iii) brain infiltration, (iv) small cell change, (v) increased cellularity, (vi) prominent nucleoli, (vii) sheet-like growth, or necrosis. (Backer et al, 2012)

Based on the histological parameters, no doubt the modern and prevailing grading system provided by WHO is considerably useful from the prognostic point of view, though still it is hindered by such sort of assessments that are just subjective in nature and urge of a more

precise and sharp determination is required that can make the clinical as well as practical applications easier.

For instance, characteristics like the small cell changes, hypercellularity, sheeting, necrosis, and mitotic count demands for a more specific descriptions and patterned evaluation based on a rationale and standardization. Therefore it is certainly advised and preferrable to revise and refine the histopathology related to meningeal tumors in a continuous manner that is shall definitely be quite promising not only for validation of histopathological data but also in proper diagnosing and appropriate grading of these tumors.

In all of the above context, Backer et al tried to design a Retrospective study at neurosurgical care centre in Mid-Norway. The study was performed at St. Olavs Hospital and University Hospital Of Trondheim. According to a certain inclusion and exclusion criteria, all the histological examinations and the medical records were obtained and assessed for 196 meningioma patients cohort.

The histological key characters considered for this assessment included the following:

(i). Apopotosis

(ii). Sheeting

(iii). Macronucleoli

(iv). Nuclear plemorphia

(v). Vesicubus nuclei

(vi). Necrosis

(vii). Hypercellularity

(viii). Small cells

(ix). Lymphocytes

(x). Lipidization

(xi). Fibrosis

(xii). Hypervascularization

(xiii). Mitotic index

(xiv). Brain infiltration

(xv). Soft tissue infiltration

Their study was aimed to examine these selected number of cases according to their histopathological parameters and to find a frequency of these subtypes and grades and also to evaluate the correlation between their distinct histopathological features. The meningiomas studied so far reveals a sort of histopathological nature that is quite heterogeneous. The study implies a confirmation for a bigger frequency of benign meningiomas for females in relevance to males. The findings also support the occurrence of meningioma at irregular sites as compared to their usual which is considered as an exceptional and rare too. Likewise, as described by some researches in the past, the atypical meningiomas are noted to be more at locations non-skull base. (Backer et al, 2012)

Due to the different set of classification systems, alterations can be seen in the frequencies of meningiomas subtypes as per their histologic malignancies. Now it is known so far that considerably up to 25 percent of even the benign meningiomas recur, however rendering the current scheme unoptimal and suggesting a continuous advancement of classification criterion strongly. (Ayerbe et al, 1999). Likewise, the classification for meningioma grading provided by WHO in 2000 was more refined and an enhancement over the previous one provided in 1993 as it carried a more objective criterion that was also readily reproducible. Further, it led to the recognition of a higher proportion of meningiomas as atypical ranging from 15 to 35 percent.

In the same context and criteria followed, 30 percent of the meningiomas were regarded as atypical. Apoptosis isn't still a part of the current WHO criteria system yet a strong corelation between the other atypical features such as necrosis, sheeting, and high mitotic count is noted. The development of collagen and increased fibrosis is another major factor

that is noticed in meningiomas without a regard to the tumor grade, linked to the meningothelial cells' proposed functions (Perry et al, 2006).

Hence, it is important to regularly con- duct quality assurance studies to improve the histopathological diagnosis and, hence, the classification systems. High mitotic count is regarded as the most significant among the criterion for defining of a meningioma as grade II. (Backer et al, 2012)

2.4. HISTOLOGICAL AND ANATOMICAL CORELATION

As till to date, not enough literature is available to suggest a link between the histological subtypes of intracranial meningeal brain tumors, called 'meningiomas' and their location of origin. And the effort for a correlation between the anatomical location of the intracranial meningiomas and the histopathological grades shall not only facilitate the specific diagnosis but also provide an accurate treatment plan to be designed by the clinicians and is quite promsing in future. Bhat. Et al tried to analyze such a corelation between the anatomical location of meningioma in human body and their histologic subtype as well.

They performed a retrospective study was conducted in a single high-patient-inflow Neurosurgical Center of Srinagar (Kashmir), under a standard and uniform medical protocol. During this study, the pathology data and surgical notes of all of the operated 729 meningiomas were analyzed from the patient files in the Medical Records Department of the mentioned hospitals. The biodata, x-rays, angiography, computed tomography (CT) scans, imaging, histopathological reports, and mortality were the tried to be sub-sequently analyzed and results oriented conclusions were drawn.

The Anatomical locations that were considered in this study in relation to the histological variants of meningiomas were the following:

(i). Supratentorial, (ii) Parasagittal Convexity, (iii). Parafalcine, (iv). Intraventricular, (v). Base of Skull (vi). Sphenoid ridge (vii). Olfactory groove, (viii). Tuberculum sellae, (ix).

Petroclival, (x). Intraorbital, (xi). Cavernous sinus, (xii). Posterior fossa, (xiii).Cerebellopontine angle, (xiv). Cerebellar convexity, (xv). Foramen magnum, (xvi). Peritorcular, (xvii). Jugular foramen, (xviii). Tentorial.

The uncommon histopathological types of meningiomas were interestingly found to have a common location of origin in the sphenoid ridge, posterior parafalcine, jugular foramen, peritorcular and intraventricular regions, cerebellopontine angle, and tentorial and petroclival areas. (Bhat et al, 2014)

The histopathological World Health Organization (WHO) Grade I (Benign Type) meningiomas were noted in 89.30 percent of cases, WHO Grade II (Atypical Type) in 5.90 percent cases and WHO Grade III (Malignant Type) in 4.80 percent of all meningiomas. Meningiomas of 64.60 of the cases as per readings of this study were found in females, while 47.32 percent were in the age group of 41-50 years, and 3.43 percent of meningiomas noticed in children as well. The overall mortality noted was 6.04 percent.

The highest mortality rate (25.71 percent) was found to be associated with WHO Grade III (malignant meningiomas). The sites that were revealed to be most commonly associated with the meningiomas of such a high mortality were: cerebellopontine angles, intraventricular region, sphenoid ridge, tuberculum sellae, and the posterior parafalcine areas. Such a correlation between the histological subtypes and the anatomical location of meningioma tumors is althought quite evident, still further research is needed inorder to establish the link more significantly. (Bhat et al, 2014)

2.5. CORELATION OF Ki67 WITH MENINGIOMA'S GRADE

Meningiomas are the slow-growing tumors with a female predominance. Meningiomas are classified into three grades according to the latest categorization of World Health Organization (WHO) 2007 (Perry et al, 2007). This grading has also severe implications on

management. Grade I tumors are treated with surgery alone whereas Grade II and III are treated with surgery and radiotherapy.

Grading system based on histopathological features has also certain limitations associated woth it while predicting the exact biological behavior of meningioma. The other factors implicated in tumor recurrence are extent of surgery, age, gender, location and brain invasion. (Jääskeläinen, 1986) Therfore it is always implied that the use of ancillary techniques is now a necessity to predict the tumor growth and recurrence, as the net growth rate of a tumor is considered to be a balance between cell proliferation and apoptosis.

Amongst the various techniques available to measure cell proliferation, Ki67 is the most widely used immunohistochemical marker. Ki-67 is a non-histone protein that is expressed in the proliferative phase of the cell cycle. It is a simple technique that can be applied on formalin-fixed-paraffin-embeded sections.

Babu et. al , in this retrospective study performed the analysis of Ki67 labeling index (Ki67 LI) in various histological subtypes and grades of meningioma and correlate it with various parameters that are known for recurrence. 429 meningiomas diagnosed during the study period were selected out of which for 300 samples according to the inclusion criteria, were subjected to immunohistochemistry with Ki67. Also this analysis was further confined to the meningiomas where Ki67 LI was available. There were 115 males and 185 females altogether and the age ranged from 2 to 75 years with mean age of 45.8 years. The statistical analysis was performed using ANOVA (Analysis of variance between groups) and unpaired t-test.

As a result a conclusive decission was made again that shos the female predominance. The age ranged from 2-75 years for the patients of this study. There were 211 Grade I, 78 Grade II and 11 Grade III meningiomas observed and recorded. The mean Ki67 LI increased from Grade I to II and from Grade II to III and these were statistically significant. Althought the Ki67 LI was noted high for the subtypes of clear cell, chordoid, papillary and rhabdoid but interestingly, there was found no statistical significance between the subtypes. Another very important point noticed was that the difference in Ki67 LI between recurrent versus non-recurrent and brain-invasive versus non-invasive meningiomas was statistically

significant. As a conclusion from the study implies that the higher Ki67 LI indicates for a high grade of meningioma, while keeping the difference in KI67 LI between recurrent and non-recurrent meningiomas was statistically significant. (Babu et al, 2011)

2.6. MTHFR ASSOCIATION WITH NEOPLASM AND MENINGIOMA

The MTHFR gene codes for the MTHFR enzyme that plays a pivotal role in folate metabolism and one carbon metabolism pathways, as mentioned in the previous context. Several studies report that MTHFR gene is linked to risk of certain types of neoplasms. A study bt Chen et al (2005) reports that MTHFR polymorphism are associated with risk of breast cancers. A study reported by Kurtin et al (2004) implies that MTHFR (C677T and A1298C) have a close link with development of colon cancer.

Similarly, study by Marchand et al (2005) also reports MTHFR (C677T) to be associated with colorectal cancer. Likewise, study by Sussana et al (2006) shows that MTHFR has been found to have some lnik with esophageal, gastric and pancreatic cancers as well.

A number of studies also suggests the association of MTHFR polymorphism to the risk of meningioma. Kumawat et al (2018) has performed two seperate studies in different time spans in the north and south of India respectively. His findings points an association between the MTHFR genentic polymorphism and the risks of adult meningioma. In the same context, a study was performed by Zhang et al (2013) in China, for northern chinese han population suggesting a close association of MTHFR (TT) polymorphism with meningioma tumors from his findings.

Functional Polymorphism in MTHFR (C677T and A1298C) as risk for meningioma was also further investigated by Bethke et al (2008) at United Kingdom). Their findings clearly supports the MTHFR association with meningioma risk. A study was also performed by Kafadar et al (2006) at Turkey to evaluate and suggest the susceptible risk for Meningioma via MTHFR Polymorphism.

2.7. MTRR ASSOCIATION WITH NEOPLASM AND MENINGIOMA

The MTRR gene codes for the MTRR enzyme that plays role in methionine and homocysteine cycle that has also a key role in processing of amino acids. Several studies report the link of MTRR gene to certain types of neoplasms. A study performed by Martha et al (2006) clearly implies the MTRR polymorphism at nucleotide (A66G) found to be involved in breast cancer.

Andrés et al (2013) in their study performed in europe has tried to describe the risk of MTRR (A66G) polymorphism associated with prostate cancer. MTRR genotype/halotype analysis have also been shown to have a keen susceptibility for colorectal cancer and this was reported by Barbara et al (2008).

A number of studies also suggests the association of MTRR polymorphism to the risk of meningioma. Kumawat et al (2018) has performed two seperate studies in different time spans in the north and south of India respectively. His findings shows a significant association between the MTRR genentic polymorphism and meningioma. In the same context, a study was performed by Zhang et al (2013) in China, for northern chinese han population suggesting a close association of MTRR (A66G) polymorphism with meningioma tumors from his findings.

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2.8. RAD54L ASSOCIATION WITH NEOPLASM AND MENINGIOMA

RAD54L, as the name indicates belongs to RAD54L helicase family and has 12 potential isoforms. The RAD54L enyme is further mainly involved in involved in DNA repair and mitotic recombination. It also have functions in the recombinational DNA repair (RAD52)

pathway and turnover of RAD51 protein-dsDNA filaments. It is also noted to play an essential role in telomere length maintenance and telomere capping in mammalian cells.

Several studies have reported the link of RAD54L gene to some types of neoplasms. It is reported as a proposed candidate oncosupressor gene in breast carcinomas by Rasio et al (1997). Leone et al (2003) performed keen study and found RAD54L polymorohic synonymous mutaution to be involved in colon carcinomas. Mastuda et al (1999) also performed studies that are suggesting the involvement of RAD54L in other tumorigenesis like lymphomas.

The risk of RAD54L with the risk of meningioma was not well investigated however Leone et al (2003) have shown the RAD54L to also be implicated in human meningiomas as a risk factor and genetic marker specifically due to its C to T SNP at locus of 2290.

3. MATERIALS AND METHODS

3.1. TUMOR SAMPLES

The grading for meningioma is based on subjective parameters which includes cytological and histopathological parameters due to which certain patients are prone to be misgraded. The correct grading has a direct impact on knowing prognosis and designing a treatment protocol for meningioma patient.

In this context, we tried to conduct a retrospective study inorder to look for any corelation between the genetic parametrs (that are objective in nature) with the above mentioned histopathological characteristics. We included three genes in our study i-e MTHFR, MTRR and RAD54L respectively.

The meningioma tumor samples for this study were obtained already from the Medical Park Oncology hospital, Istanbul (Turkey). These samples, as already collected were stored at -80*C in the nitrogen tank which were then further subjected to the procedure mentioned upon the kit meant for extraction of their DNA.

3.2. PRIMERS AND CHEMICAL KITS

The Primers for the afore mentioned genes i-e MTHFR, MTRR and RAD54L were designed by using the NCBI Primer Blast tool. The Primers utilized in this study were obtained from the company of Macrogen,(South Korea), While the Kit for PCR was imported from Bio-RAD (United States). The DNA amplification was performed by Bio-Rad PCR machine from Bio-RAD (United States).

Table 3.1: List Of Chemicals

Chemcials	Trademark
Q5 Enzyme	New England Biomedicine (United Kingdom)
NEB 2X Master Mix	New England Biomedicine (United Kingdom)
Magnesium Chloride	New England Biomedicine (United Kingdom)
Primers	MACROGEN, Seoul (South Korea)
DMSO	New England Biomedicine (United Kingdom)
PCR Apparatus	Bio-RAD, California (United States)
Image Lab Photon	Bio-RAD, California (United States)

Reference: This table was prepared by Rashid Mohiy-Ud-Din

Table 3.2: List Of Primers

Gene	Forward Primer	Reverse Primer	Tm for Q5 Protocol	Product Length	Codes
MTHFR	CCTGATTTGCTTGGCTGCTC	ACTCAGCGAACTCAGCACTC	67	529 bp	MTHFR Gene Seq 1
MTHFR	CTTGTGGTTGACCTGGGAGG	TTTGCCTCCCTAAGCCCTTC	68	478 bp	MTHFR Gene Seq 2
MTRR	AGGCTCATTTGAGATTAGTGCTGA	CTGTCATTTACAGTTAAGGAGTTGTTAC	64	559 bp	MTRR Gene Seq
RAD54L	CACTTCTCTCTGGGCGAGTT	TTCTTCCCTGCTGGGCTTAC	67	604 bp	RAD54L Gene Seq

Reference: This table was prepared by Rashid Mohiy-Ud-Din

3.3. DNA EXTRACTION FROM TUMOR SAMPLES BY ALKALINE LYSIS

As a next step in our experimentation process, the DNA samples were needed to be obtained, the process known as DNA extraction through alkaline lysis procedure. In this regard following the standard procedure of alkaline lysis (mentioned upon the kit). The tumor samples obtained from the Medical Park Oncology hospital, Istanbul (Turkey) were stored at a temperature of -80*C from where we collected a specific number (96) of them divided into several phases and days.

Afterwards when the DNA isolation from the tumor samples was completed via the alkaline lysis method, it was made sure that they are stored at a temperature of -20*C that were to be further processed for amplification via polymerase chain reactions (PCRs).

3.4. PCR PROTOCOLS

The term PCR refers to Polymerase Chain Reaction and it provides multiple copies of a small segment of DNA that we wish to amplify via a series of cycles procedured at steps with definite varying temperatures. All of the DNA samples prepared in the previous step were subjected to PCR amplification one by one, again through a protocol necessary for PCR process.

Using the correct primers, chemcial reagents of the Q5 enzyme kit and the annhealing temperatures for each of the primer that were calculated using an online tool i-e New England Biolabs TM Calculator. The machine used for performing the PCRs was obtained from the BioRad laboratories, California (United States) using a gradient of temperature for their annealing phase.

3.5. ANNHEALING TEMPERATURES FOR PCR

One of the crucial determining factor, apart from the Primers, DNA samples and PCR enzyme kit, is the appropriate calculation of a precise Annhealing temperature (TM). The term anneal refers to attachment. The annhealing temperature serves an appropriate medium for the attachment of primers to DNA strand.

The annealing step comes at second stage in PCR process i-e before the elongation step which is followed by the termination step. The annhealing temperature for all of the primers utilized in this study were calculated by using the online TM Calculator tool referred to as "New England Biolabs Tm Calculator" (NEB). Version; 1.12.0.

3.6. PCR ENZYME KIT

The multiplication of a small segment of DNA or simply the DNA amplification takes place via a Polymerase chain reaction that consists of several cycles and requires a definite set of enzymes that are meant to work together in collabortion to acheive a successful results for desired DNA segment amplification. Each of the regent and enzyme present in this kit plays a significant role in the process. The enzyme kits used for PCRs in this study were following:

i). Q5 Polymerase Enzyme Kit

ii). NEB 2X MM PCR Kit

iii). Q5® High-Fidelity PCR Kit

iv). My TAQ® 2X MM PCR Kit

3.7. GEL ELECTROPHORESIS

The amplified DNA samples obtained from PCR processing (also referred to as PCR products) were required to be analyzed further inorder to get visual representations of their results and to assure if the DNA samples has successfully been amplified.

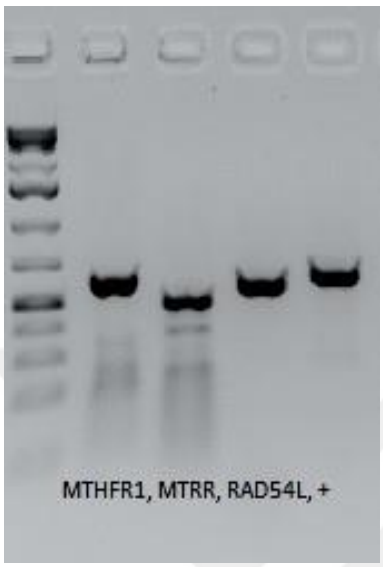
Therefore, the obtained DNA samples were then subjected to electrophoresis on a gel with the slots of fixed measurement. The gel for electrophoresis purpose was prepared using powdered Agarose and 0.5X TBE Buffer as major ingredients along with the incorporation of a certain fixed minute quantity of Ethidium Bromide.

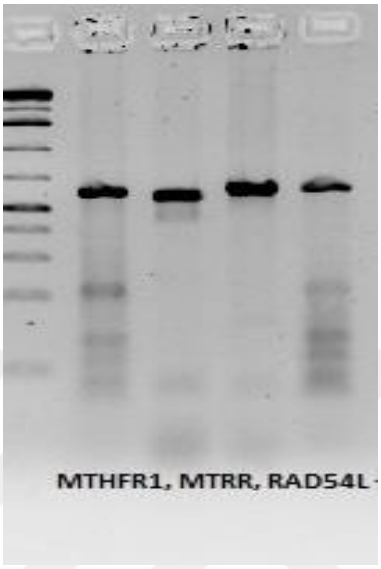
3.8. IMAGE LAB

Inorder to get the visual representations of the PCR results for the aforesaid DNA samples, the PCR products, after the gel electrophoresis were subjected to the UV detector machine by “Bio-RAD”. The results of the efficient translocation of dna from one pole of the gel to another due to electric gradient, were detected by the“Image Lab” software and the pictures were captured respectively as screenshots and dispalyed in the previous chapter as well.

The PCR results that were successful were further subjected to the Next Generation Sequencing (NGS). Some of the PCR results that describes the successful DNA amplification are preesented in the following figure:

Fig 3.1: PCR Results Forwarded To NGS

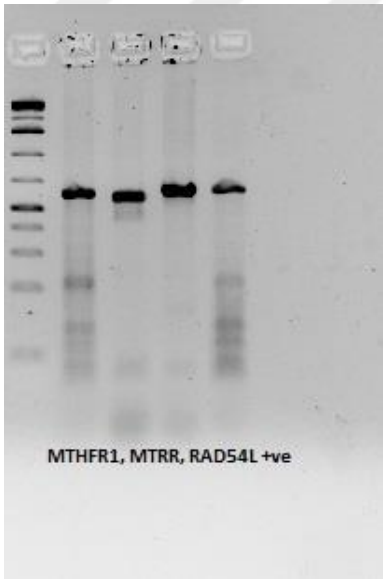


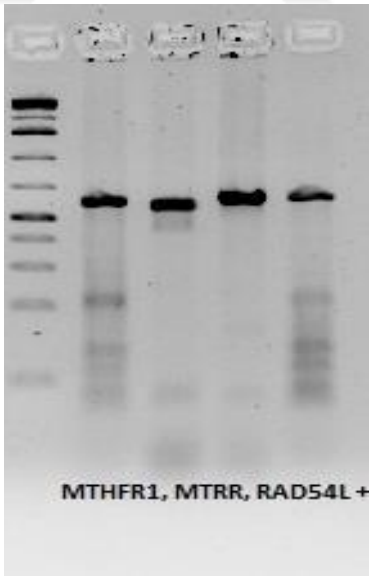


PCR Reagents:

- NEB 2X MM : 10 ul
- Primers : 1ul
- DMSO : 1.0 ul
- $MgCl^2$: 0.4 ul
- dH^2O : 5.6 ul
- DNA : 2ul

Reference: The above fiures were prepared by Rashid Mohiy-Ud-Din.





PCR Conditions

- 95c : 2 min
- 95c : 20 sec
- **50c/55c** : 20 sec
- 72c : 1 min
- 72c : 5 min
- 12c : …

Reference: The above fiures were prepared by Rashid Mohiy-Ud-Din.

3.9. CLINICAL AND HISTOPATHOLOGICAL DATA COLLECTION

As this study meant to assess if there is presence of any relationship between the genetic mutations and histopathological factors, therefore an elaborative history of the patients (the tumor samples of whom was included in the study) was tracked from the Medical Archive files.

The pathological data and clinical notes collected for each of the patient included the following information:

(i). Age of these meningioma patients

(ii). Gender of the meningioma patients

(iii).Type of meningioma they suffered

(iv). Respective subtype of meningioma

(v). The WHO Grade of their meningioma

(vi). Noted Ki67 score of these patients

(vii). Level of necrosis in these patients

(viii). Recurrence of meningioma in these patients.

3.10. NEXT GENERATION SEQUENCING

One of the last steps to be perofrmed to obtain the results was a sequencing method. The DNA or genome sequencing methods are used to screen the mutations especially the single nucleotide polymorphic (SNP) variations in different transcripts.

Among the DNA sequencing methods, the second largest and advanced method i-e NGS or Next Generation Sequencing was applied to the segmental DNA of the mentioned patient's samples inorder to observe and identify the mutations and specifically to locate the SNPs in these genes that are further evaluated for any link with meningioma tumor development and proression via the epigenetic mode or hyper/hypo methylation of DNA, causing the silencing of a tumor suppressor gene or activation of an oncogene on the other hand.

3.11. DNA SEQUENCING METHODS

Some of the key DNA sequencing methods known today are as follows:

1). Basic DNA Sequencing:

i). SANGER Sequencing (Chain termination)

ii). Maxam Gilbert Sequencing (Chemical Termination)

2). Advanced DNA Sequencing: (ShotGun Technique)

3). Next Generation DNA Sequencing :

i). Solid Sequencing

ii). Illumina Sequencing

4. RESULTS AND DISCUSSION

4.1 FREQUENCY CALCULATION OF MUTANT SNPs

As we performed a retrospective study to evaluate the association between different genetic SNP variations (objective) and meningioma's histopathological characters (subjective).Our findings in this study tried to imply a corelation between the genetic SNP variations and the hostopathologic parameters.

In this regard, we calculated the frequency of our mutant SNPs in our samples against the wild type. The frequency data that we obtained was then further compared with the ExAC frequency from the frequency data bank of ncbi.

Our results i-e the frequency of each of the mutant along with the comparison of ExAC database for frequencies are as follows:

Table 4.1: Frequency of mutant SNPs and data from ExAC databsase

Mutant SNPs with rs-ids	Our Frequency Findings	ExAC Frequencies Data
MTHFR – rs1801131	0.37	0.29
MTHFR – rs1801133	1.10	0.30
MTHFR - rs4846051	0.55	0.03
MTRR – rs1801394	1.45	0.47
RAD54L - rs1048771	0.64	-----

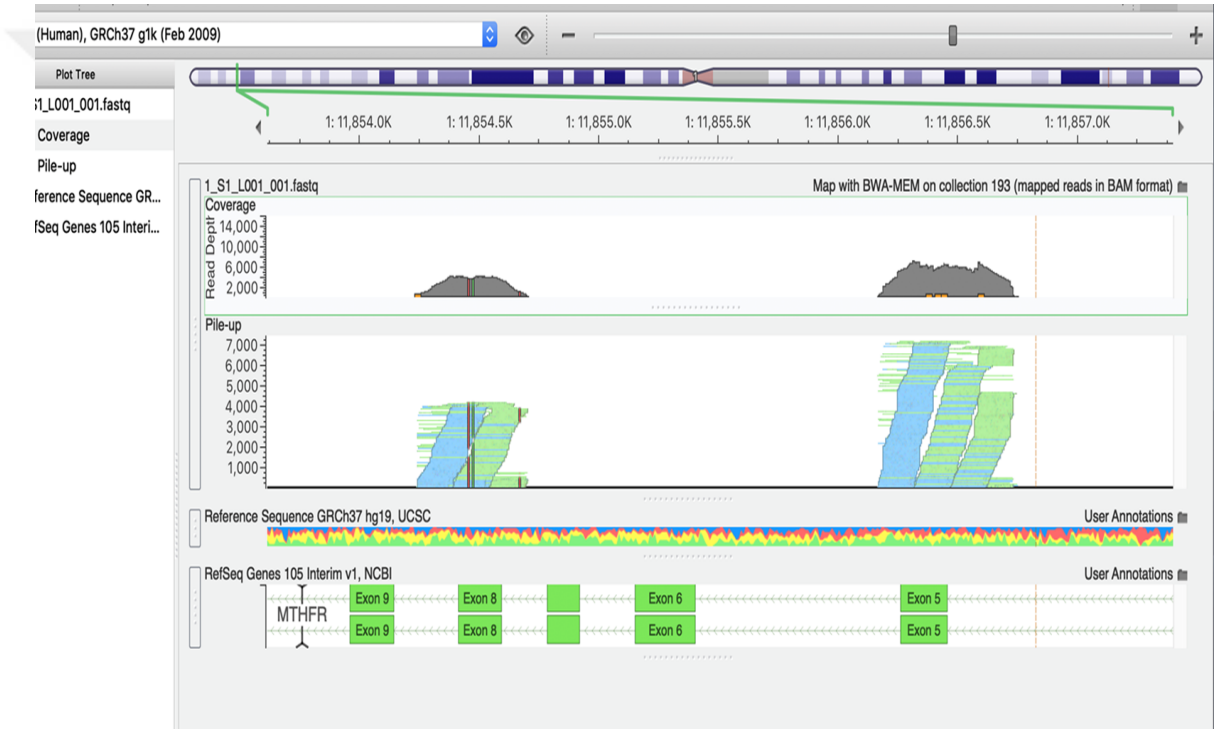
The table was prepared by Rashid Mohiy-Ud-Din

4.2 NGS DATA ANALYZED BY BIOINFORMATIC TOOLS

The data we obtained via performing next generation sequencing was available to us in basic and raw form that was further subjected to bio-informatics processing for further refinement and finally we were successful to obtain the data in interpretable form.

Some of the exome sequences are presented in the following form as:

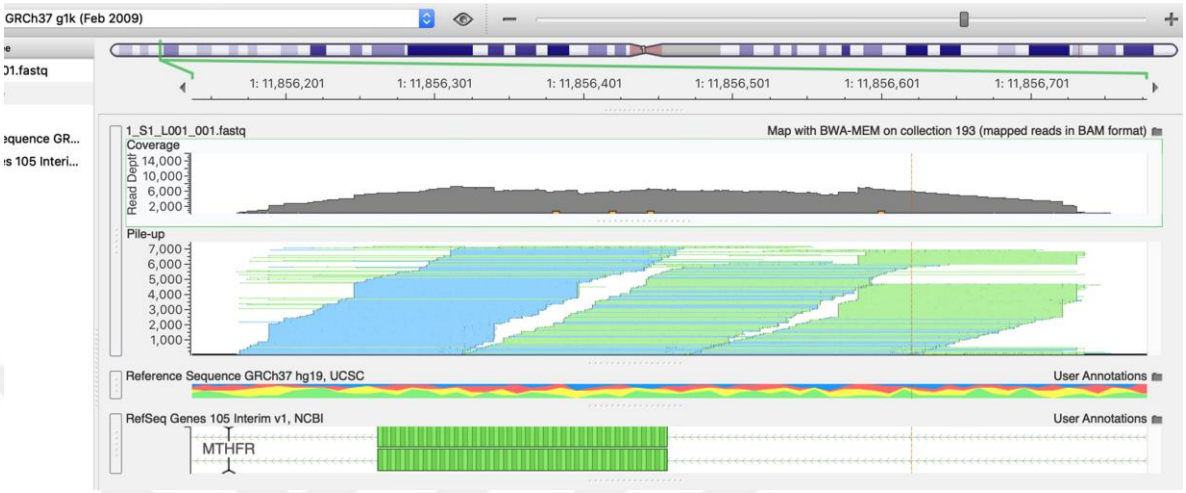
Fig 4.1: Genome sequence MTHFR gene



Reference: The figure was prepared by Rashid Mohiy-Ud-Din

The above figure describes the post Next Generation Sequencing related to a particular exon of MTHFR gene. The first bands or peak segment is basically in raw form that is shown as refined in the second graph. The green and blue colours also indicate the forward as well as the revers reads of the sequence. The genomic readings are piled up according to a specific reference spectrum and aligned to eachother. The third segmentt represents the exonic sequence which upon further magnification shows the base and amino acid sequences.

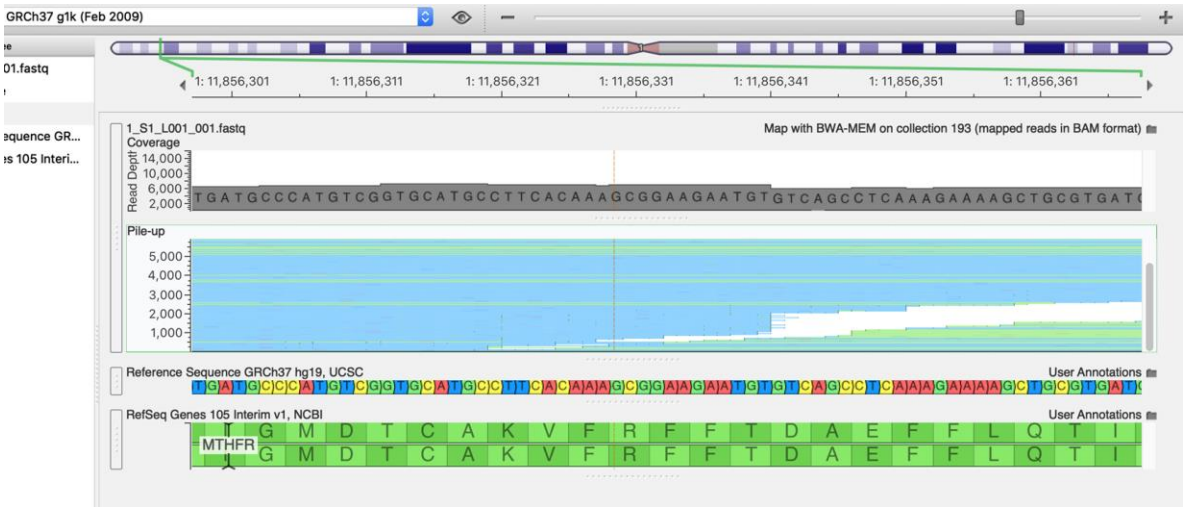
Fig 4.2: Genome sequence MTHFR gene pre-magnified



Reference: The figure was prepared by Rashid Mohiy-Ud-Din

Likewise, Figure 4.2.2. is also referring to the same pattern wise sequence of amino acids in dofferent forms relating to the bands of reference spectruma and aligned accordingly. Here, one can observe the exonic annotations in minute form that upon magnifying gives complete idea about the details of this exonic sequence (Exon 8).

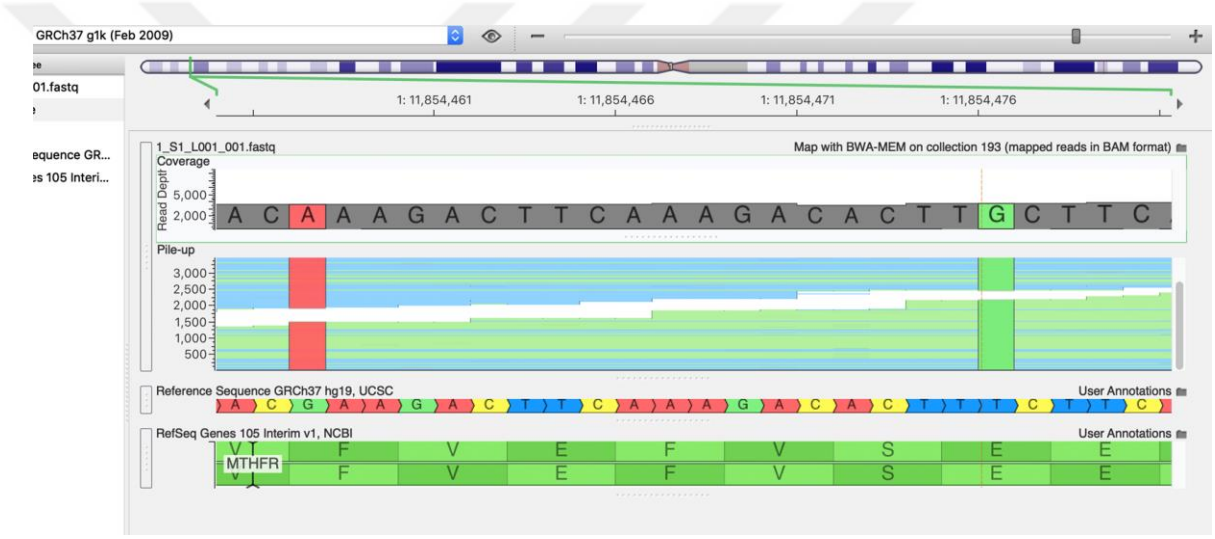
Fig 4.3: Genome sequence MTHFR gene magnified resolution



Reference: The figure was prepared by Rashid Mohiy-Ud-Din

From Figure 4.2.3 one may observe the details of the amino acids in the respective sequences of all of the three segments. Here the bands indicates the differences between the the exonic sequence and that of the reference spectrum if there exist any alteration or single nucleotide polymorphism or a copy number alteration. All of the three peaks are now defined, showing the exact patterns of the amino acid bases.

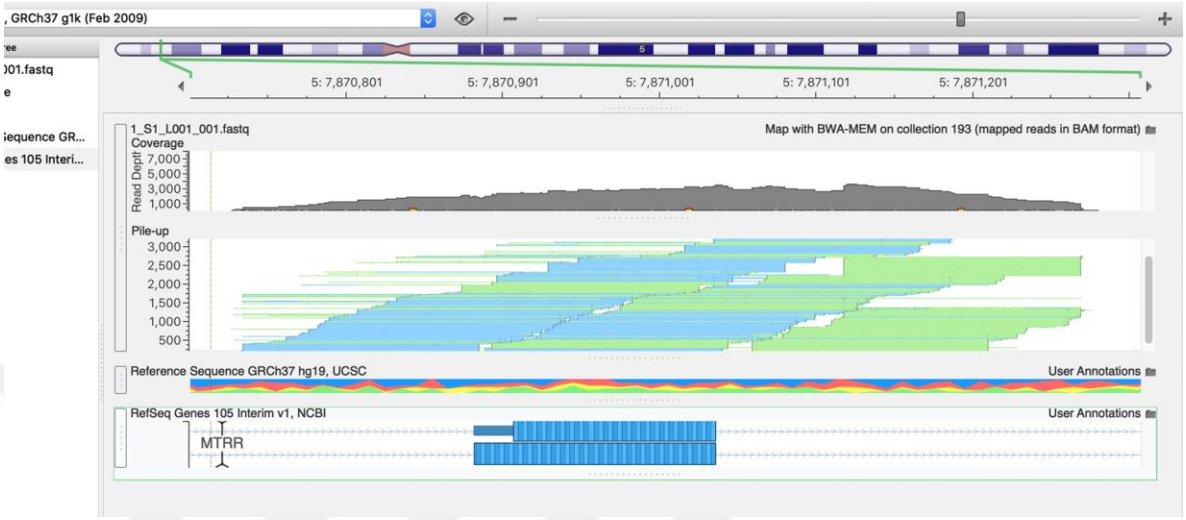
Fig 4.4: Genome sequence MTHFR gene



Reference: The figure was prepared by Rashid Mohiy-Ud-Din

Upon further amplification of the exonic sequences, the genomic alterations or the single nucleotide polymorphisms are clearly indicated among the strands in relevance to the reference spectrum defining the benign alterations as green in colour while the more malignant mutations are described as red colour. It also implies the details about the nature of the amino acid that has been affected and altered as a result of the genetic mutation.

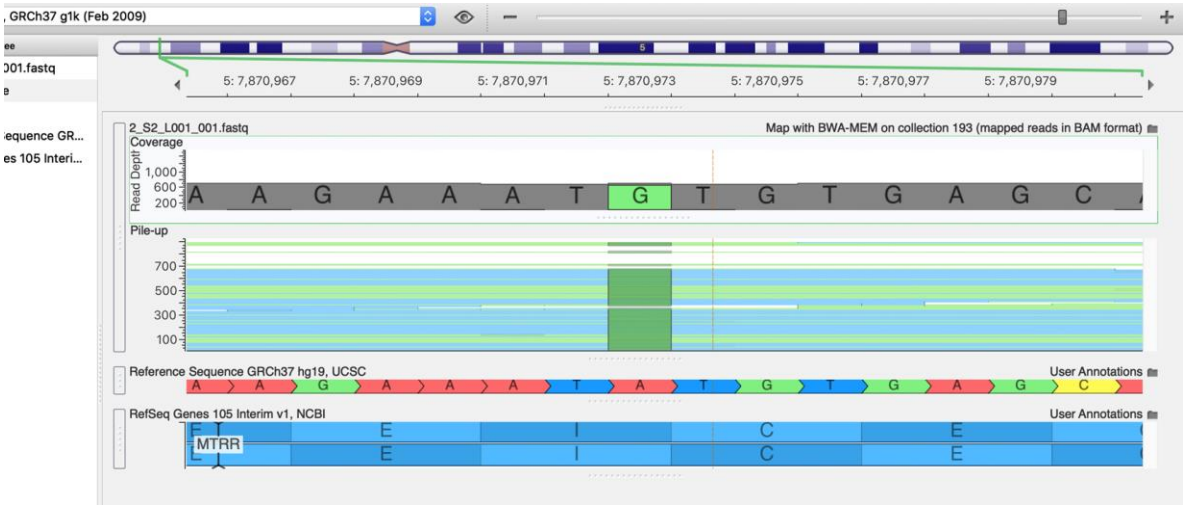
Fig 4.5: Genome Sequence MTRR Gene



Reference: The figure was prepared by Rashid Mohiy-Ud-Din

Fig 4.2.5. shows the post Next Generation Sequencing related to a particular exon of MTRR gene. Again the genome at this exonic level is described as three seperate segments. The green and blue colour differentiation relates to the presence of both forward and reverse readings of the sequenced genome. The reference spectrum is positioned above the exonic segment prior to which the genomic readings are piled up properly according to the reference spectrum for this exon and aligned together.

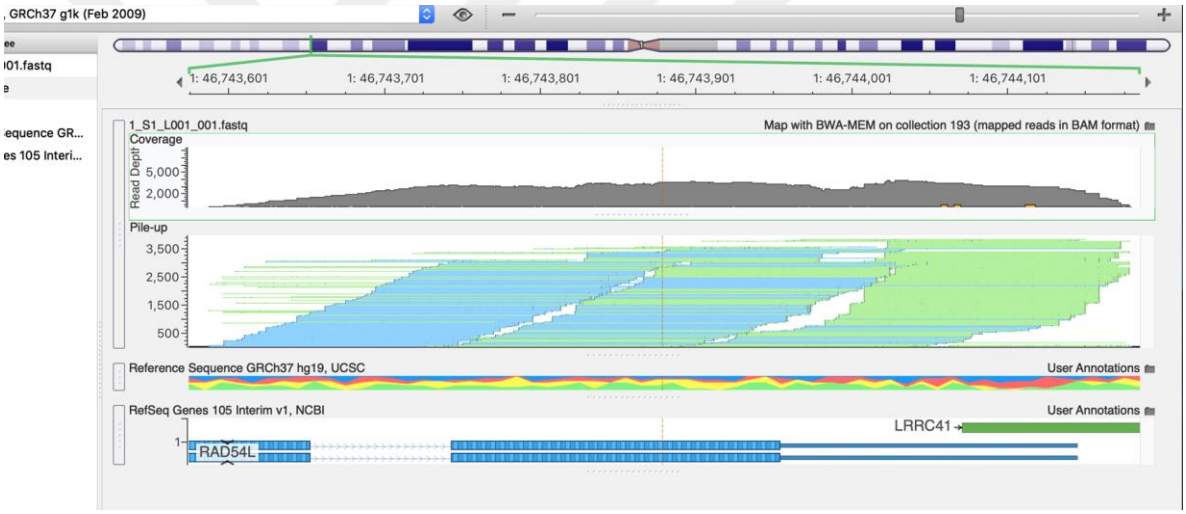
Fig 4.6: Genome Sequence MTRR Gene



Reference: The figure was prepared by Rashid Mohiy-Ud-Din

The figure 4.2.6 is the magnified form and it tends to describe the bases as well as the amino acids of this exonic sequence. In comparison to the data of the reference spectrum for this exon, it also clearly indicates the nature of the amino acid change. Based on the nature of switch of amino acid, the benign ones are indicated as green while the more malignant ones appear as red in colour bands.

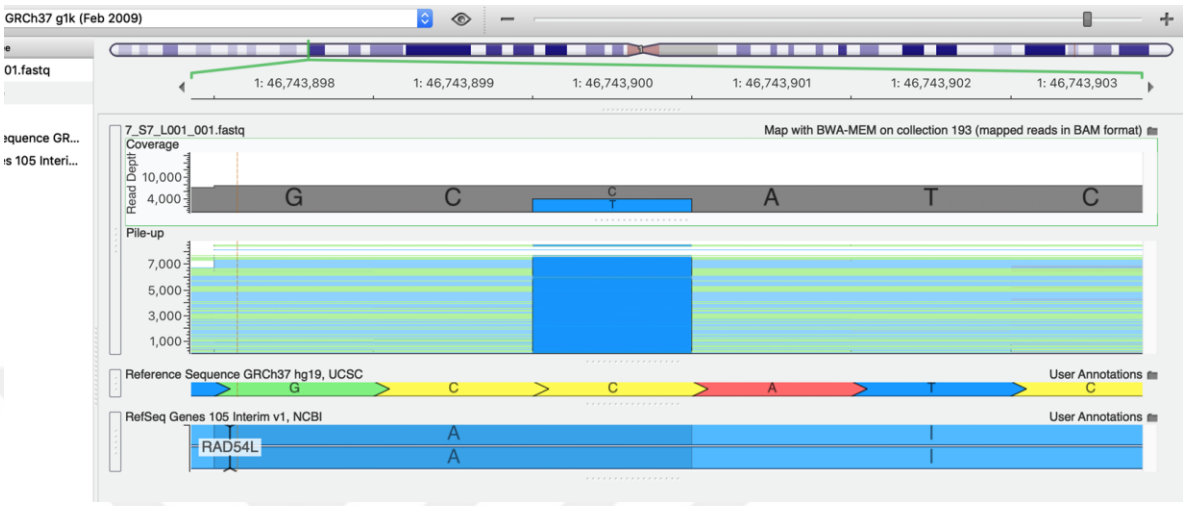
Fig 4.7: Genome Sequence RAD54L Gene



Reference: The figure was prepared by Rashid Mohiy-Ud-Din

Fig 4.2.7. shows the post Next Generation Sequencing related to particular exon of RAD54L gene. Again the genome at this exonic level is described as three seperate segments. The green and blue colour differentiation relates to the presence of both forward and reverse readings of the sequenced genome. The reference spectrum is positioned above the exonic segment prior to which the genomic readings are piled up properly according to the reference spectrum for this exon and aligned together.

Fig 4.8: Genome Sequence RAD54L Gene



Reference: The figure was prepared by Rashid Mohiy-Ud-Din

The figure 4.2.6 is the magnified form and it tends to describe the bases as well as the amino acids of this exonic sequence. In comparison to the data of the reference spectrum for this exon, it also clearly indicates the nature of the amino acid change. Based on the nature of switch of amino acid, the benign ones are indicated as green while the more malignant ones appear as red in colour bands.

The data obtained from the next genenration sequencing method and processed to be described in the form of the above figure is well efficient, however, still for further validation and refinement, the above data required bio-informatics processing that further provides confirmatory assesments of the aforementioned alterations, amino acid and base pairs switching and provides an overall idea whether such mutations lead to benign or malignnat forms.

Therefore, keeping in view the above channel, the refinement was processed by bio-informatics providing us therevised version to be utilized and easily interpret in our results and discussions in the same context.

4.3 STATISTICAL ANALYSIS OF MUTANT SNPs

As a next step, we gathered all the data into tabulation form for further assessment for each mutant SNP with its respective histopathological parmater. We used “GraphPad Prism8” inorder to assess our findings on statistical basis. The statistical tests performed for this study were the following:

(i). Chi Square Test

(ii). Fischer Exact Test.

After applying the above mentioned statistical tests on the data we compiled , the statistical values that we calculated for this purpose includes:

(i). P-Value

(ii). Odds ratio

(iii). Sensitivity Values.

The p-value less than 0.05 was considered as significant and the statistical results for each of the SNP against each the parameter of histopathological data that we collected (including Meningioma types, Meningioma subtypes, Meningioma’s WHO grading, Proliferative index Ki67 scores, Necrosis level and the Recurrence) for each of the patient was analyzed.

Further, the results obtained were compiled in the form of tables and each tables was processed to a graphical form by using the same software tool of GraphPad Prism8. Those results for each of the mutant are now described here in the continuous manner according to the genetic SNP variant and the histopathological feature type.

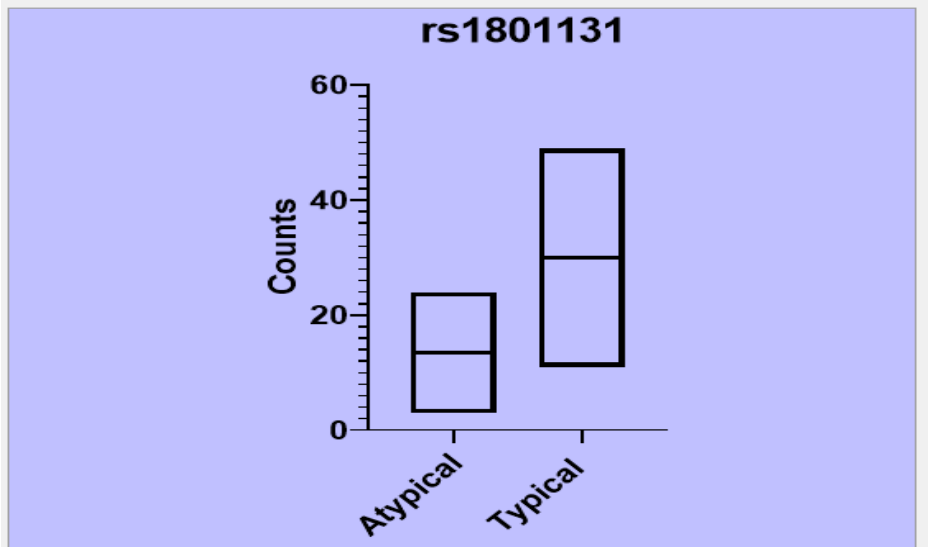
Table 4.2: Statisitcal Findings For rs- 1801131 With Meningioma Types

The statistical findings for MTHFR rs- 1801131 via Fisher Exact test with Meningioma types are as follows:

	Atypical Meningioma	Typical Meningioma
SNP Type T to G (Mis-Sense) rs1801131	3	11
Wild Type	24	49
P value	0.5347	
Odds ratio	0.5568	
Sensitivity	0.1111	
Statistical Test	Fisher's exact test	

Reference: The table has been prepared by Rashid Mohiy-Ud-Din

Figure 4.9: Graphical representation of Table 4.2



Reference: The graph has been prepared by Rashid Mohiy-Ud-Din

The MTHFR variant type (rs1801131) that we assessed for a corelation with meningioma types are presented in table form along with the subsequent graph, however upon the Fisher exact test, the p value was exceeding and we couldn't observe a significant association between the meningioma types and particular genetic variant.

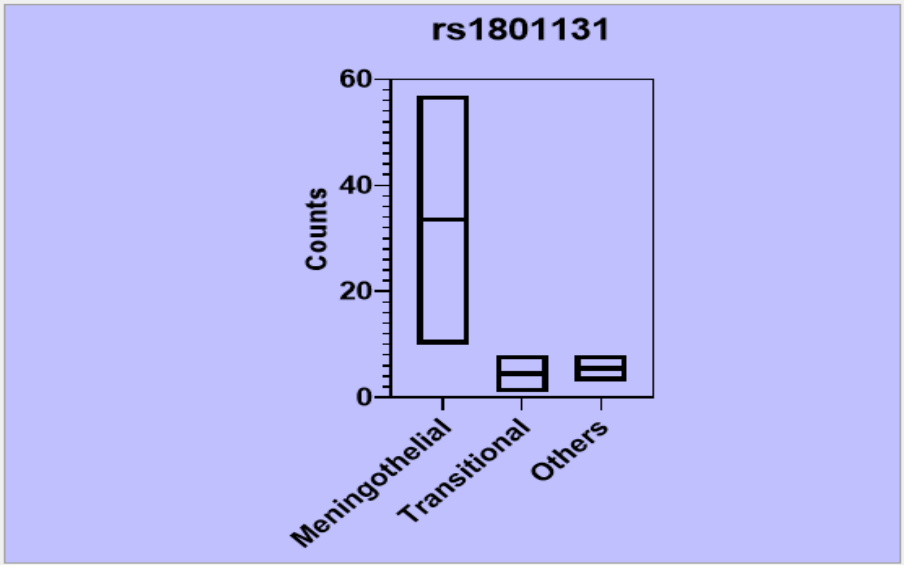
Table 4.3: Statisitcal Findings For rs- 1801131 With Meningioma Subtypes

The statistical findings for MTHFR rs- 1801131 via Chi square test with Meningioma types are as follows:

	Meningothelial	Transitional	Other Sub-types (Chordoid, Angimatous etc)
Mutant SNP Type T to G (Mis-Sense) rs1801131	10	1	3
Wild Type	57	8	8
P value	0.5349		
Statistical Test	Chi-square		

Reference: The table has been prepared by Rashid Mohiy-Ud-Din

Figure 4.10: Graphical representation of Table 4.3



Reference: The graph has been prepared by Rashid Mohiy-Ud-Din

The MTHFR variant type (rs1801131) that we assessed for a corelation with meningioma subtypes are presented in table form along with the subsequent graph, however upon the Chi square test, the p value was exceeding and we couldn't observe a significant association between the meningioma subtypes and particular genetic variant.

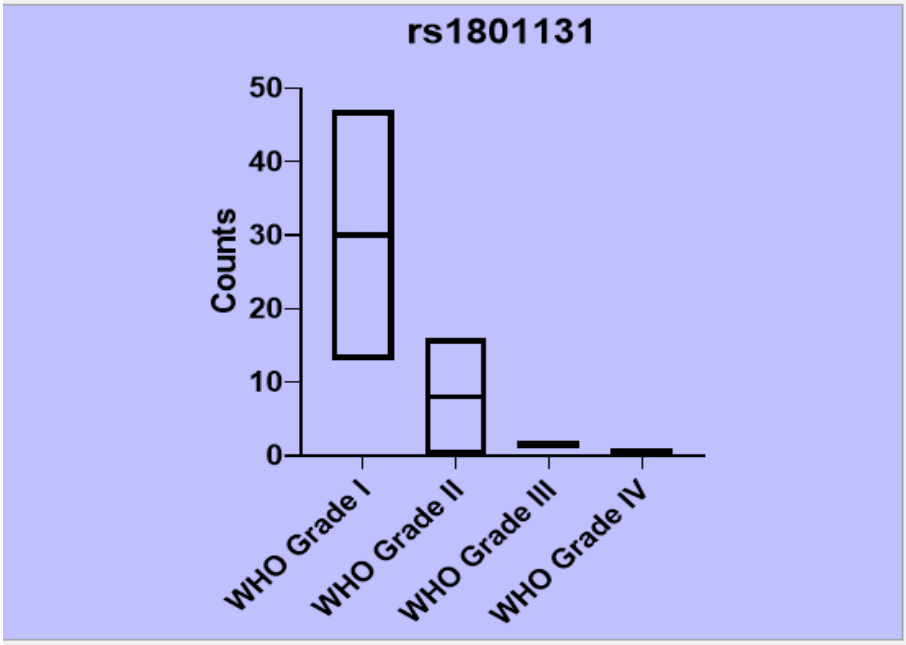
Table 4.4: Statisitcal Findings For rs- 1801131 With WHO Grading

The statistical findings for MTHFR rs- 1801131 via Chi square test with Meningioma types are as follows:

	WHO Grade I	WHO Grade II	WHO Grade III	WHO Grade IV
Mutant SNP Type T to G (Mis-Sense) rs1801131	13	0	1	0
Wild Type	47	16	2	1
P value	0.1832			
Statistical test	Chi-square			

Reference: The table has been prepared by Rashid Mohiy-Ud-Din

Figure 4.11: Graphical representation of Table 4.4



Reference: The graph has been prepared by Rashid Mohiy-Ud-Din

The MTHFR variant type (rs1801131) that we assessed for a corelation with WHO grades are presented in table form along with the subsequent graph, however upon the Chi square test, the p value was exceeding and we couldn't observe a significant association between the meningioma WHO grades and particular genetic variant.

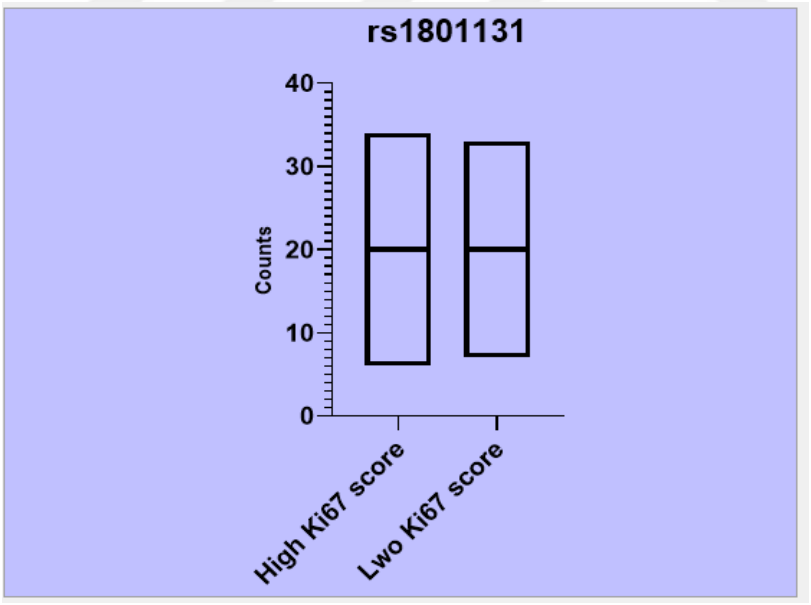
Table 4.5: Statisitcal Findings For rs- 1801131 With Ki67 Scores

The statistical findings for MTHFR rs- 1801131 via Fisher Exact test with Meningioma types are as follows:

	Proliferative index High Ki67 score	Proliferative index Low Ki67 score
Mutant SNP Type T to G (Mis-Sense) rs1801131	6	7
Wild Type	34	33
P value	0.9999	
Odds ratio	0.8319	
Sensitivity	0.15	
Statistical Test	Fisher's exact test	

Reference: The table has been prepared by Rashid Mohiy-Ud-Din

Figure 4.12: Graphical representation of Table 4.5



Reference: The graph has been prepared by Rashid Mohiy-Ud-Din

The MTHFR variant type (rs1801131) that we assessed for a corelation with Ki67 scores are presented in table form along with the subsequent graph, however upon the Fisher exact test, the p value was exceeding and we couldn't observe a significant association between the meningioma Ki67 scores and particular genetic variant.

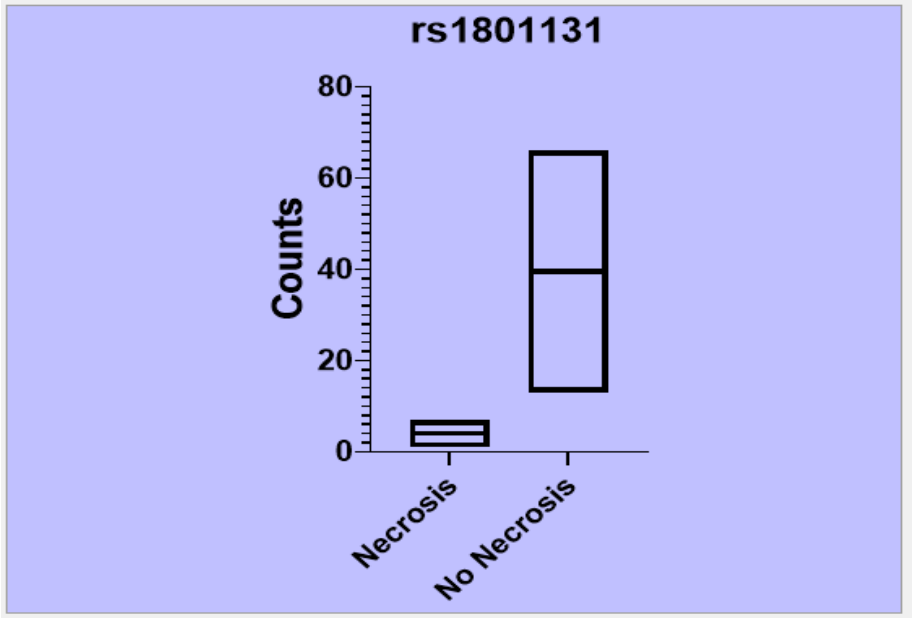
Table 4.6: Statisitcal Findings For rs- 1801131 With Necrosis

The statistical findings for MTHFR rs- 1801131 via Fisher Exact test with Meningioma types are as follows:

	Necrosis	No Necrosis
Mutant SNP Type T to G (Mis-Sense) rs1801131	1	13
Wild Type	7	66
P value	0.9999	
Odds ratio	0.7253	
Sensitivity	0.125	
Statistical Test	Fisher's exact test	

Reference: The table has been prepared by Rashid Mohiy-Ud-Din

Figure 4.13: Graphical representation of Table 4.6



Reference: The graph has been prepared by Rashid Mohiy-Ud-Din

The MTHFR variant type (rs1801131) that we assessed for a corelation with necrosis are presented in table form along with the subsequent graph, however upon the Fisher exact test, the p value was exceeding and we couldn't observe a significant association between the meningioma necrosis and particular genetic variant.

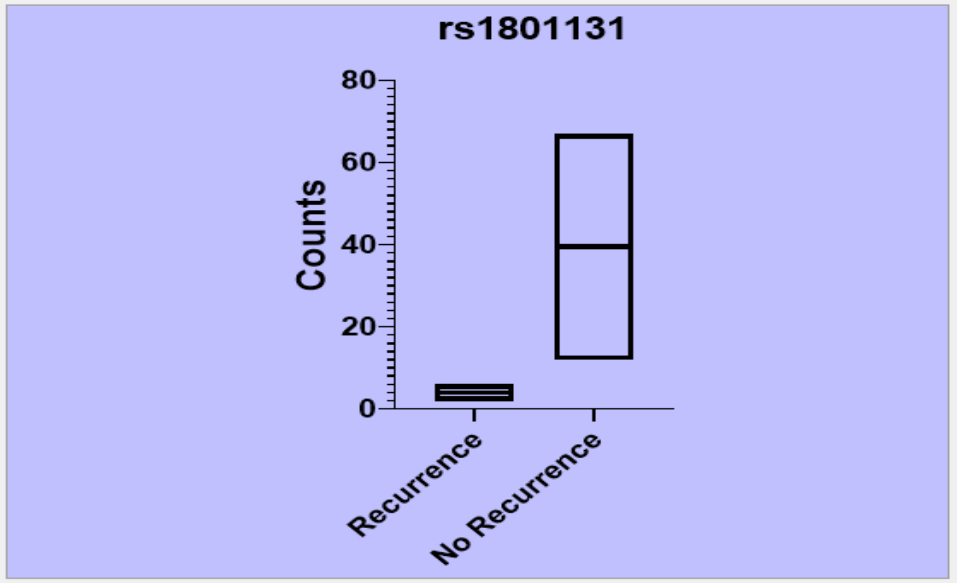
Table 4.7: Statisitcal Findings For rs- 1801131 With Recurrence

The statistical findings for MTHFR rs- 1801131 via Fisher Exact test with Meningioma types are as follows:

	Recurrence	No Recurrence
Mutant SNP Type T to G (Mis-Sense) rs1801131	2	12
Wild Type	6	67
P value	0.6096	
Odds ratio	1.861	
Sensitivity	0.25	
Statistical Test	Fisher's exact test	

Reference: The table has been prepared by Rashid Mohiy-Ud-Din

Figure 4.14: Graphical representation of Table 4.7



Reference: The graph has been prepared by Rashid Mohiy-Ud-Din

The MTHFR variant type (rs1801131) that we assessed for a corelation with recurrence are presented in table form along with the subsequent graph, however upon the Fisher exact test, the p value was exceeding and we couldn't observe a significant association between the meningioma recurrence and this particular genetic variant.

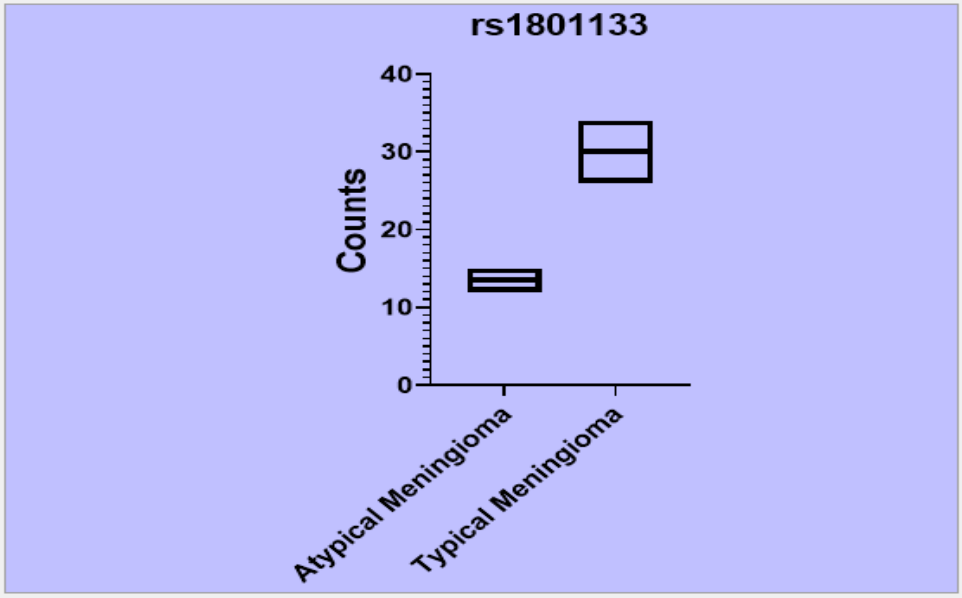
Table 4.8: Statisitcal Findings For rs- 1801133 With Meningioma Types

The statistical findings for MTHFR rs- 1801133 via Fisher Exact test with Meningioma types are as follows:

	Atypical Meningioma	Typical Meningioma
Mutant SNP Type G to A (Mis-Sense) rs1801133	12	34
Wild Type	15	26
P value	0.3557	
Odds ratio	0.6118	
Sensitivity	0.4444	
Statistical Test	Fisher's exact test	

Reference: The table has been prepared by Rashid Mohiy-Ud-Din

Figure 4.15: Graphical representation of Table 4.8



Reference: The graph has been prepared by Rashid Mohiy-Ud-Din

The MTHFR variant type (rs1801133) that we assessed for a corelation with meningioma types are presented in table form along with the subsequent graph, however upon the Fisher exact test, the p value was exceeding and we couldn't observe a significant association between the meningioma types and particular genetic variant.

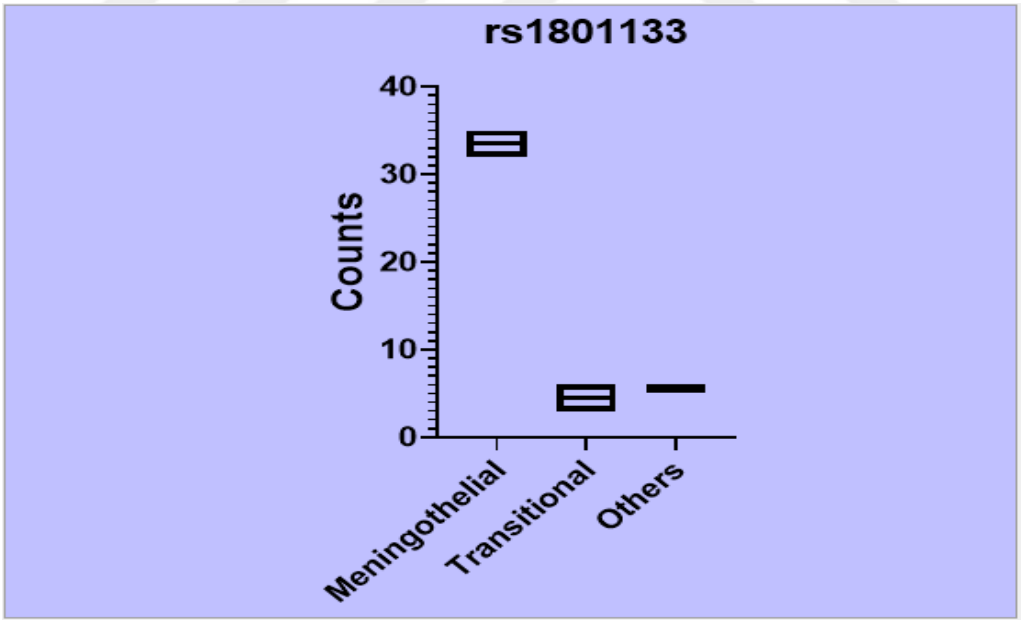
Table 4.9: Statisitcal Findings For rs- 1801133 With Meningioma Subtypes

The statistical findings for MTHFR rs- 1801133 via Chi square test with Meningioma types are as follows:

	Meningothelial	Transitional	Other Sub-types (Chordoid, Angimatous etc)
Mutant SNP Type G to A (Mis-Sense rs1801133	35	6	5
Wild Type	32	3	6
P value	0.6247		
Statistical Test	Chi-square		

Reference: The table has been prepared by Rashid Mohiy-Ud-Din

Figure 4.16: Graphical representation of Table 4.9



Reference: The graph has been prepared by Rashid Mohiy-Ud-Din

The MTHFR variant type (rs1801133) that we assessed for a corelation with meningioma subtypes are presented in table form along with the subsequent graph, however upon the Chi square test, the p value was exceeding and we couldn't observe a significant association between the meningioma subtypes and this particular genetic variant.

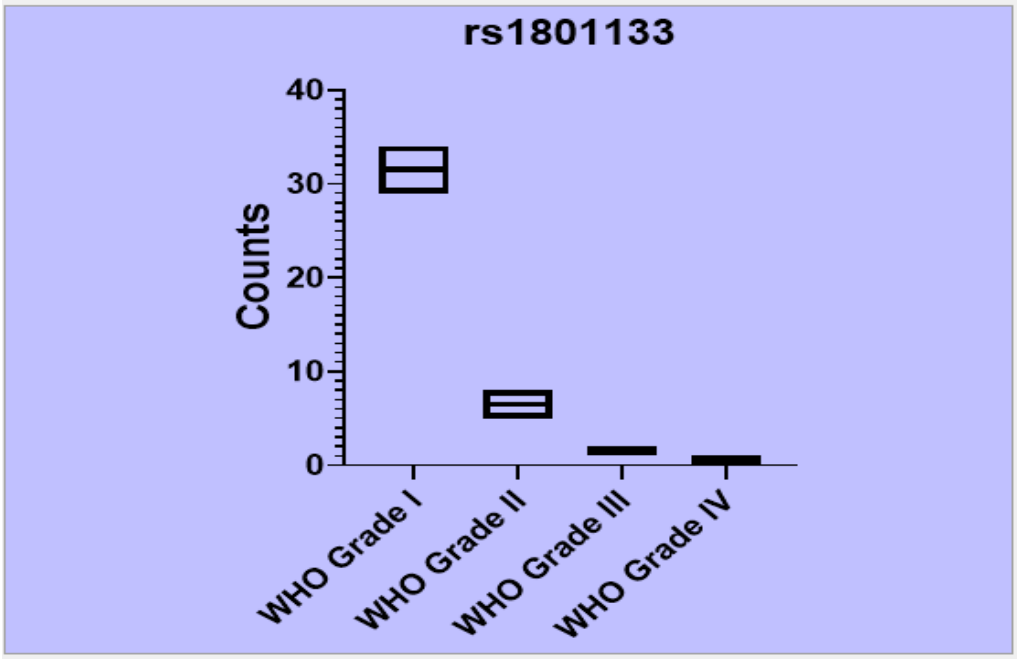
Table 4.10: Statisitcal Findings For rs- 1801133 With WHO Grading

The statistical findings for MTHFR rs- 1801133 via Chi square test with Meningioma types are as follows:

	WHO Grade I	WHO Grade II	WHO Grade III	WHO Grade IV
Mutant SNP Type G to A (Mis-Sense) rs1801133	34	5	1	0
Wild Type	29	8	2	1
P value	0.4895			
Statistical test	Chi-square			

Reference: The table has been prepared by Rashid Mohiy-Ud-Din

Figure 4.17: Graphical representation of Table 4.10



Reference: The graph has been prepared by Rashid Mohiy-Ud-Din

The MTHFR variant type (rs1801133) that we assessed for a corelation with WHO grades are presented in table form along with the subsequent graph, however upon the Chi square test, the p value was exceeding and we couldn't observe a significant association between the meningioma WHO grades and this particular genetic variant.

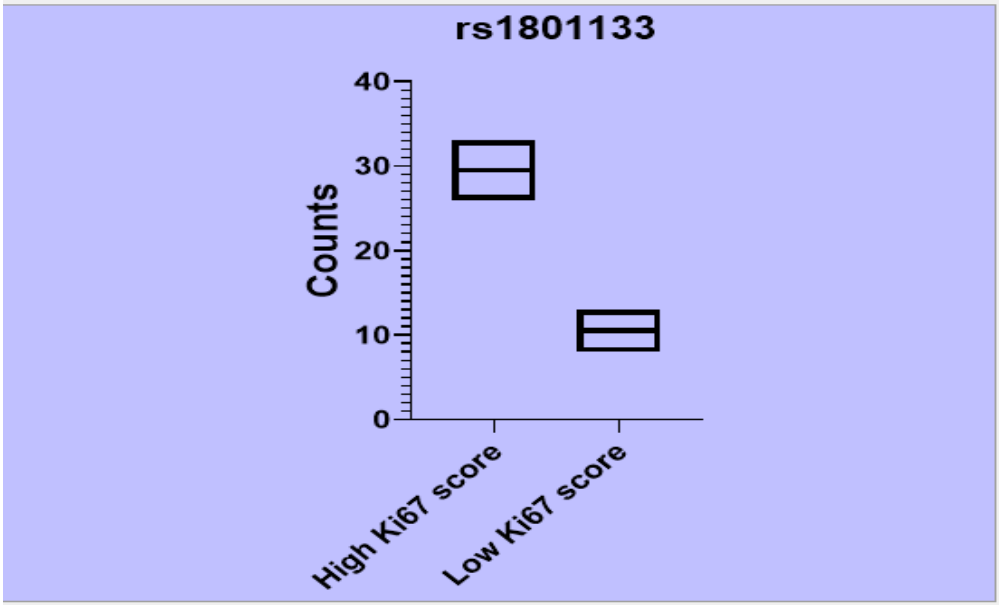
4.11: Statisitcal Findings For rs- 1801133 With Ki76 Scores

The statistical findings for MTHFR rs- 1801133 via Fisher Exact test with Meningioma types are as follows:

	Proliferative index High Ki67 score	Proliferative index Low Ki67 score
Mutant SNP Type G to A (Mis-Sense) rs1801133	26	13
Wild Type	33	8
P value	0.2064	
Odds ratio	0.4848	
Sensitivity	0.4407	
Statisitical Test	Fisher's exact test	

Reference: The table has been prepared by Rashid Mohiy-Ud-Din

Figure 4.18: Graphical representation of Table 4.11



Reference: The graph has been prepared by Rashid Mohiy-Ud-Din

The MTHFR variant type (rs1801133) that we assessed for a corelation with Ki67 scores are presented in table form along with the subsequent graph, however upon the Fisher exact test, the p value was exceeding and we couldn't observe a significant association between the meningioma Ki67 scores and particular genetic variant.

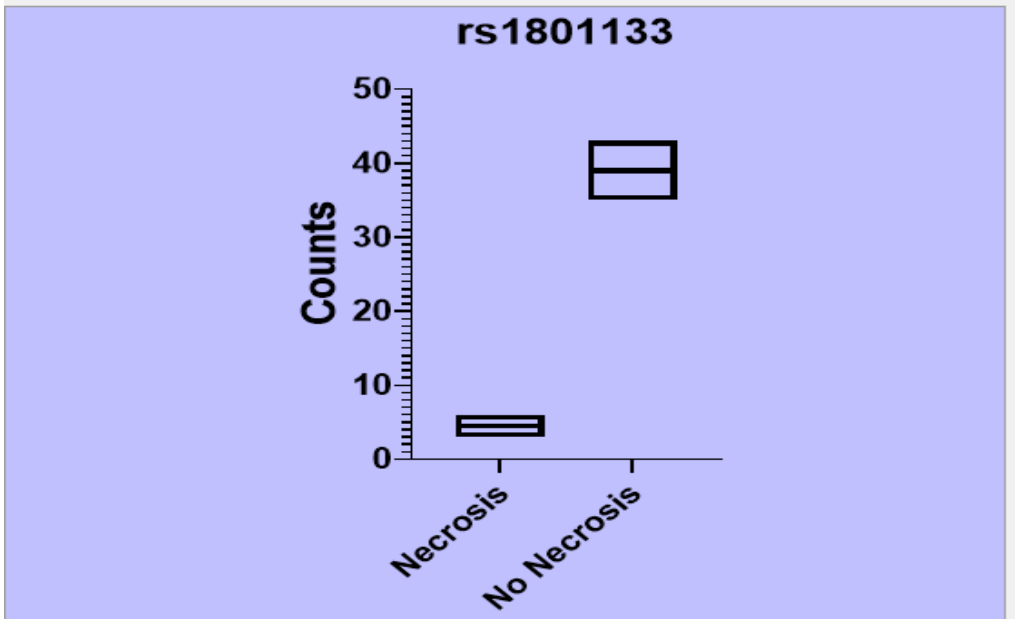
4.12: Statisitcal Findings For rs- 1801133 With Necrosis

The statistical findings for MTHFR rs- 1801133 via Fisher Exact test with Meningioma types are as follows:

	Necrosis	No Necrosis
Mutant SNP Type G to A (Mis-Sense) rs1801133	3	43
Wild Type	6	35
P value	0.2963	
Odds ratio	0.407	
Sensitivity	0.3333	
Statistical Test	Fisher's exact test	

Reference: The table has been prepared by Rashid Mohiy-Ud-Din

Figure 4.19: Graphical representation of Table 4.12



Reference: The graph has been prepared by Rashid Mohiy-Ud-Din

The MTHFR variant type (rs1801133) that we assessed for a corelation with necrosis are presented in table form along with the subsequent graph, however upon the Fisher exact test, the p value was exceeding and we couldn't observe a significant association between the meningioma necrosis and this particular genetic variant.

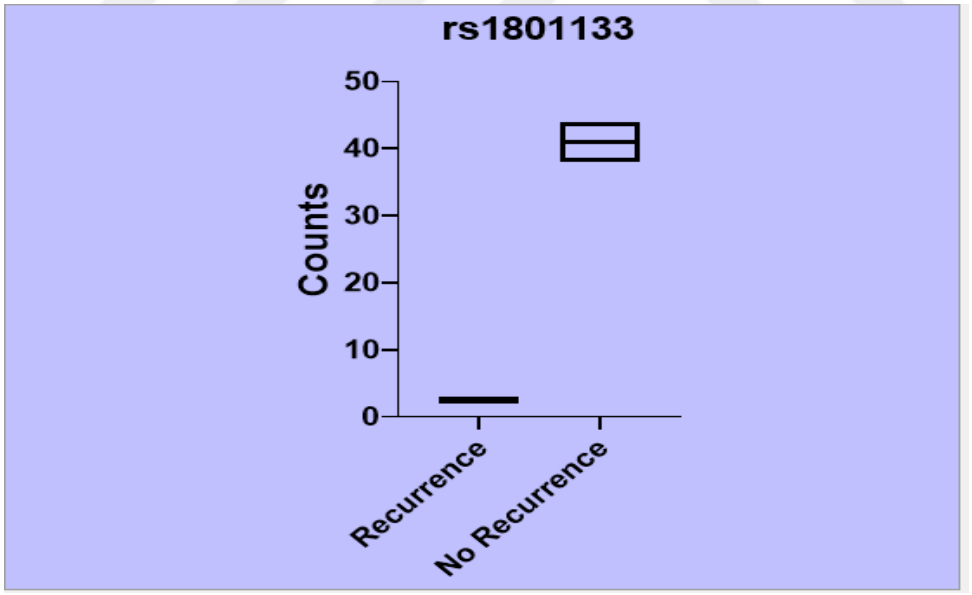
Table 4.13: Statisitcal Findings For rs- 1801133 With Recurrence

The statistical findings for MTHFR rs- 1801133 via Fisher Exact test with Meningioma types are as follows:

	Recurrence	No Recurrence
Mutant SNP Type G to A (Mis-Sense) rs1801133	2	44
Wild Type	3	38
P value	0.6631	
Odds ratio	0.5758	
Sensitivity	0.4	
Statistical Test	Fisher's exact test	

Reference: The table has been prepared by Rashid Mohiy-Ud-Din

Figure 4.20: Graphical representation of Table 4.13



Reference: The graph has been prepared by Rashid Mohiy-Ud-Din

The MTHFR variant type (rs1801133) that we assessed for a corelation with recurrence are presented in table form along with the subsequent graph, however upon the Fisher exact test, the p value was exceeding and we couldn't observe a significant association between the meningioma recurrence and particular genetic variant.

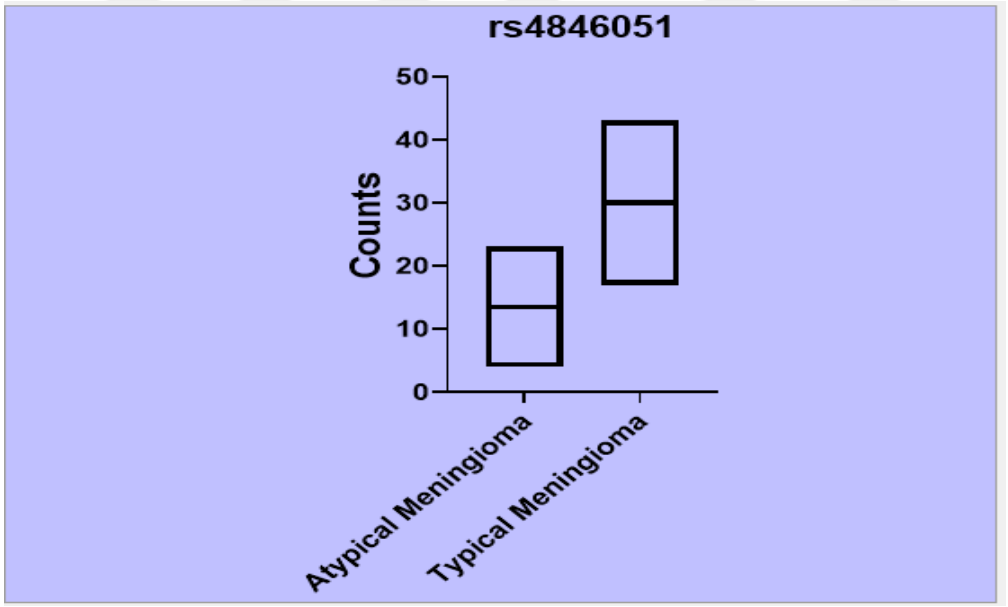
Table 4.14: Statisitcal Findings For rs- 4846051 With Meningioma Types

The statistical findings for MTHFR rs- 4846051 via Fisher Exact test with Meningioma types are as follows:

	Atypical Meningioma	Typical Meningioma
Mutant SNP Type G to A (Syn) rs4846051	4	17
Wild Type	23	43
P value	0.2783	
Odds ratio	0.4399	
Sensitivity	0.1481	
Statisitical Test	Fisher's exact test	

Reference: The table has been prepared by Rashid Mohiy-Ud-Din

Figure 4.21: Graphical representation of Table 4.14



Reference: The graph has been prepared by Rashid Mohiy-Ud-Din

The MTHFR variant type (rs4846051) that we assessed for a corelation with meningioma types are presented in table form along with the subsequent graph, however upon the Fisher exact test, the p value was exceeding and we couldn't observe a significant association between the meningioma types and this particular genetic variant.

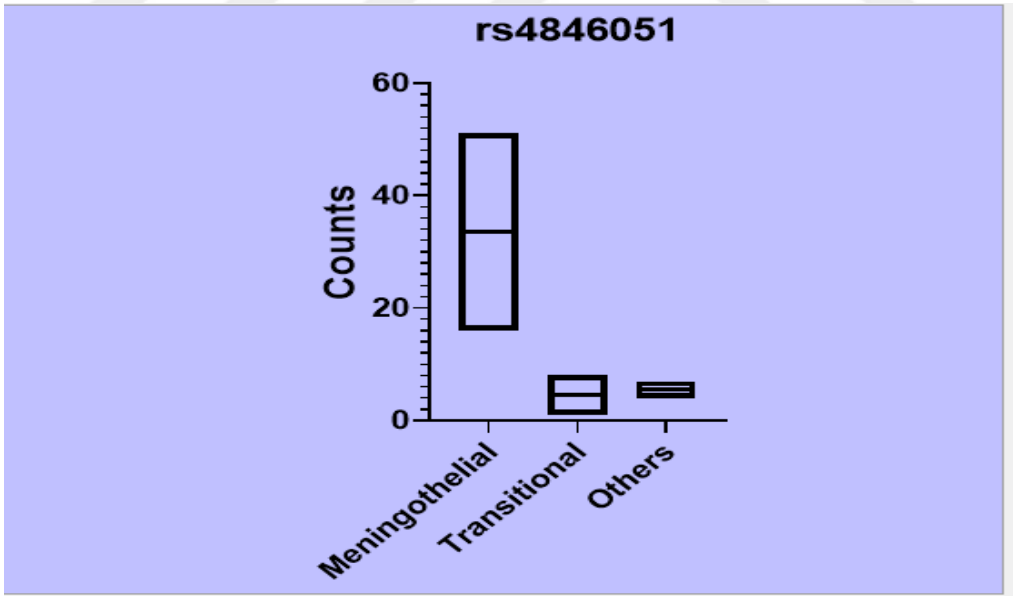
Table 4.15: Statisitcal Findings For rs- 4846051 With Meningioma Subtypes

The statistical findings for MTHFR rs- 4846051 via Chi square test with Meningioma types are as follows:

	Meningothelial	Transitional	Other Sub-types (Chordoid, Angimatous etc)
Mutant SNP Type G to A (Syn) rs4846051	16	1	4
Wild Type	51	8	7
P value	0.4201		
Statistical Test	Chi-square		

Reference: The table has been prepared by Rashid Mohiy-Ud-Din

Figure 4.22: Graphical representation of Table 4.15



Reference: The graph has been prepared by Rashid Mohiy-Ud-Din

The MTHFR variant type (rs4846051) that we assessed for a corelation with meningioma subtypes are presented in table form along with the subsequent graph, however upon the Chi square test, the p value was exceeding and we couldn't observe a significant association between the meningioma subtypes and this particular genetic variant.

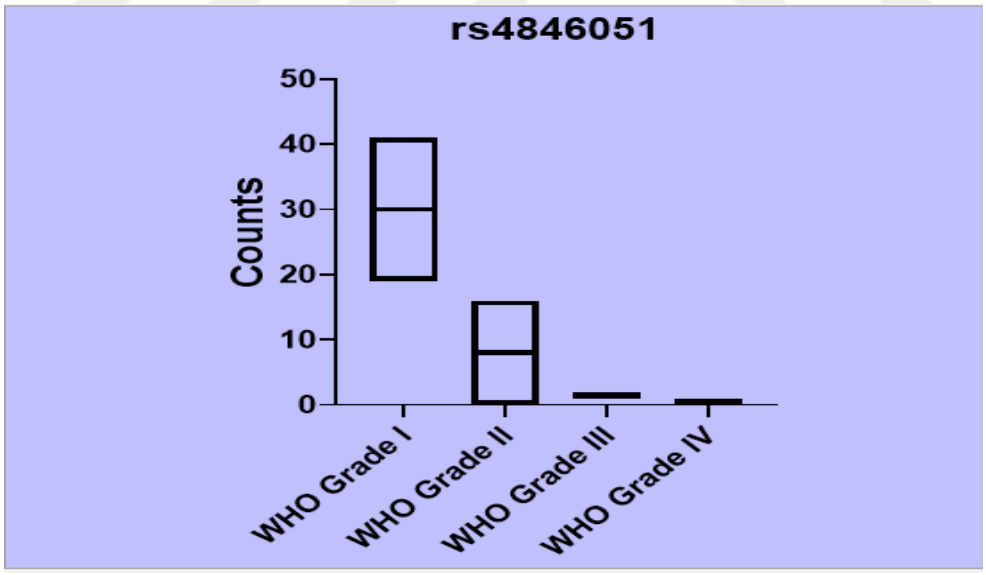
Table 4.16: Statisitcal Findings For rs- 4846051 With WHO Grading

The statistical findings for MTHFR rs- 4846051 via Chi square test with Meningioma types are as follows:

	WHO Grade I	WHO Grade II	WHO Grade III	WHO Grade IV
Mutant SNP Type G to A (Syn) rs4846051	19	0	2	0
Wild Type	41	16	1	1
P value	0.0234 (Significant)			
Statistical test	Chi-square			

Reference: The table has been prepared by Rashid Mohiy-Ud-Din

Figure 4.23: Graphical representation of Table 4.16



Reference: The graph has been prepared by Rashid Mohiy-Ud-Din

*The MTHFR variant type (rs4846051) that we assessed for a corelation with WHO grades are presented in table form along with the subsequent graph, however upon the Chi square test, the p value was 0.02 and found a significant association between WHO grades of meninioma types and this particular genetic variant.

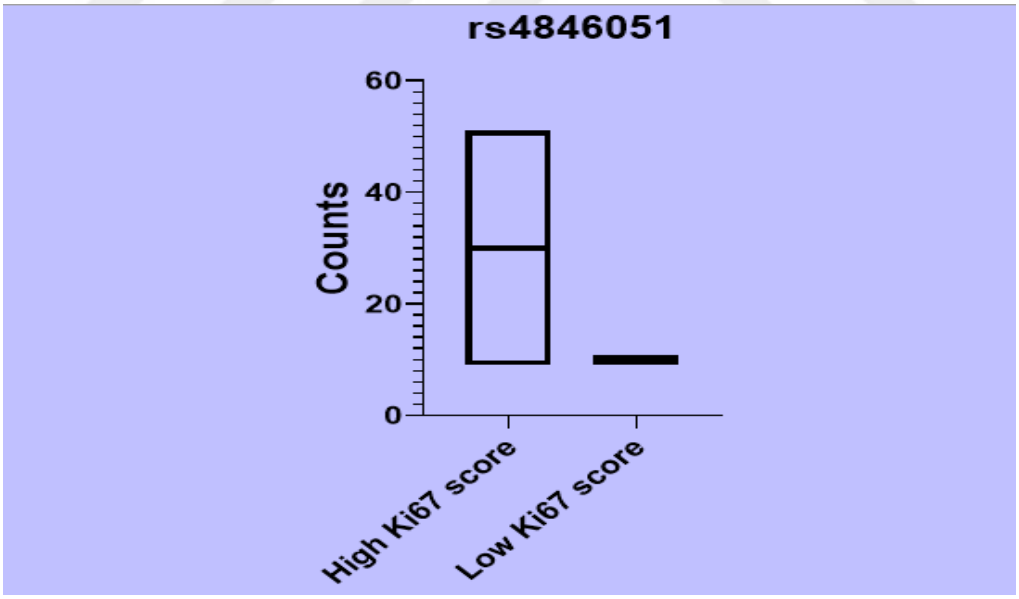
Table 4.17: Statisitcal Findings For rs- 4846051 With Ki67 Scores

The statistical findings for MTHFR rs- 4846051 via Fisher Exact test with Meningioma types are as follows:

	Proliferative index High Ki67 score	Proliferative index Low Ki67 score
Mutant SNP Type G to A (Syn) rs4846051	9	10
Wild Type	51	10
P value	0.0045 (Significant)	
Odds ratio	0.1765	
Sensitivity	0.15	
Statisitical Test	Fisher's exact test	

Reference: The table has been prepared by Rashid Mohiy-Ud-Din

Figure 4.24: Graphical representation of Table 4.17



Reference: The graph has been prepared by Rashid Mohiy-Ud-Din

*The MTHFR variant type (rs4846051) that we assessed for a corelation with Ki67 scores are presented in table form along with the subsequent graph, however upon the Fisher exact test, the p value was 0.04 and found a significant association between Ki67 scores and this particular genetic variant.

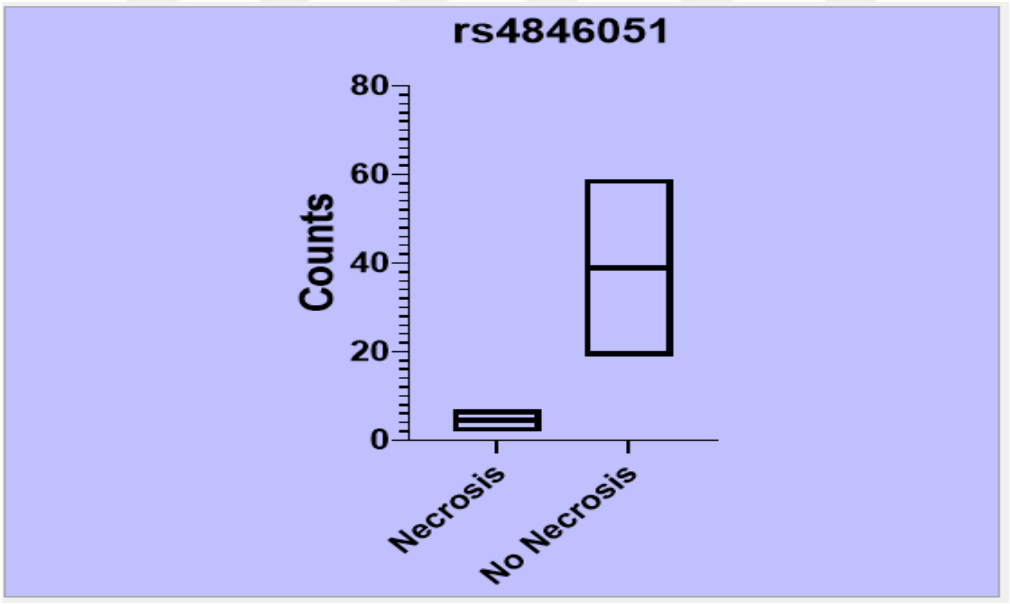
Table 4.18: Statisitcal Findings For rs- 4846051 With Necrosis

The statistical findings for MTHFR rs- 4846051 via Fisher Exact test with Meningioma types are as follows:

	Necrosis	No Necrosis
Mutant SNP Type G to A (Syn) rs4846051	2	19
Wild Type	7	59
P value	0.9999	
Odds ratio	0.8872	
Sensitivity	0.2222	
Statistical Test	Fisher's exact test	

Reference: The table has been prepared by Rashid Mohiy-Ud-Din

Figure 4.25: Graphical representation of Table 4.18



Reference: The graph has been prepared by Rashid Mohiy-Ud-Din

The MTHFR variant type (rs4846051) that we assessed for a corelation with necrosis are presented in table form along with the subsequent graph, however upon the Fisher exact test, the p value was exceeding and we couldn't observe a significant association between the meningioma necrosis and this particular genetic variant.

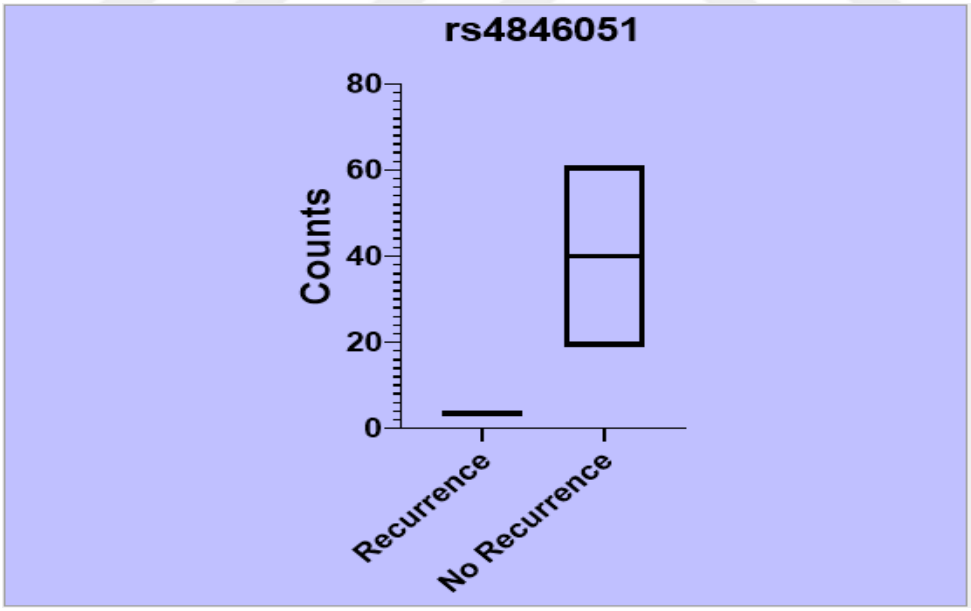
Table 4.19: Statisitcal Findings For rs- 4846051 With Recurrence

The statistical findings for MTHFR rs- 4846051 via Fisher Exact test with Meningioma types are as follows:

	Recurrence	No Recurrence
Mutant SNP Type G to A (Syn) rs4846051	3	19
Wild Type	4	61
P value	0.3625	
Odds ratio	2.408	
Sensitivity	0.4286	
Statistical Test	Fisher's exact test	

Reference: The table has been prepared by Rashid Mohiy-Ud-Din

Figure 4.26: Graphical representation of Table 4.19



Reference: The graph has been prepared by Rashid Mohiy-Ud-Din

The MTHFR variant type (rs4846051) that we assessed for a corelation with recurrence are presented in table form along with the subsequent graph, however upon the Fisher exact test, the p value was exceeding and we couldn't observe a significant association between the meningioma recurrence and this particular genetic variant.

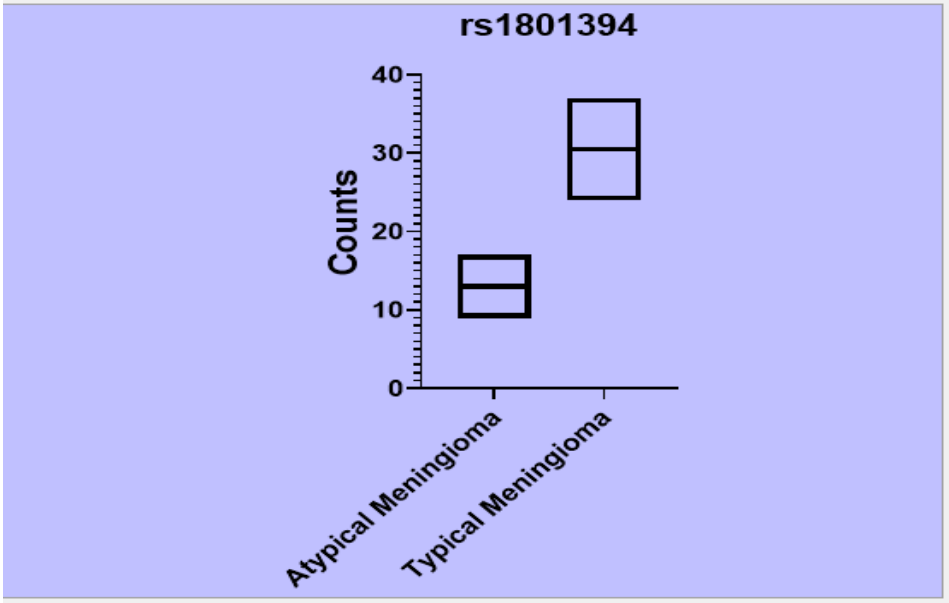
Table 4.20: Statisitcal Findings For rs- 1801394 With Meningioma Types

The statistical findings for MTHFR rs- 1801394 via Fisher Exact test with Meningioma types are as follows:

	Atypical Meningioma	Typical Meningioma
Mutant SNP Type A to G (Mis-Sense) rs1801394	17	37
Wild Type	9	24
P value	0.8104	
Odds ratio	1.225	
Sensitivity	0.6538	
Statisitical Test	Fisher's exact test	

Reference: The table has been prepared by Rashid Mohiy-Ud-Din

Figure 4.27: Graphical representation of Table 4.20



Reference: The graph has been prepared by Rashid Mohiy-Ud-Din

The MTRR variant type (rs1801394) that we assessed for a corelation with meningioma types are presented in table form along with the subsequent graph, however upon the Fisher exact test, the p value was exceeding and we couldn't observe a significant association between the meningioma types and this particular genetic variant.

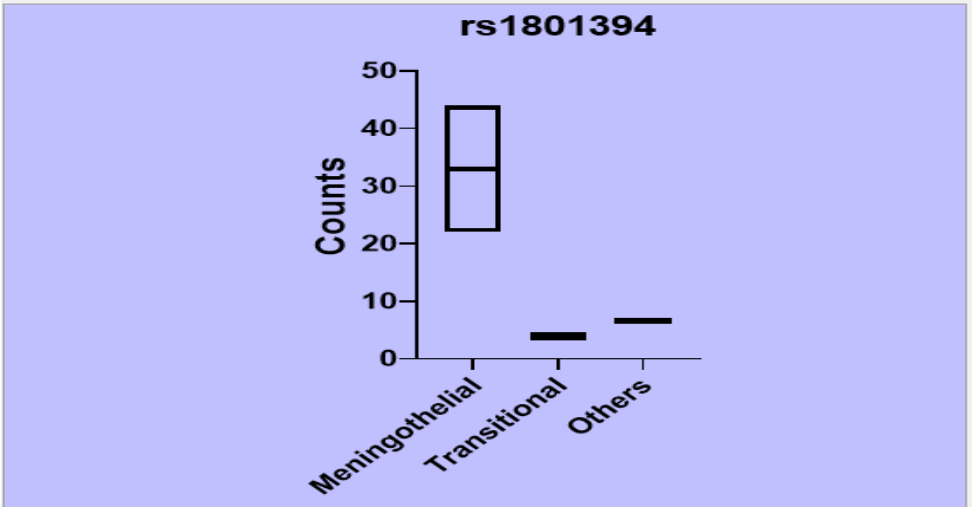
Table 4.21: Statisitcal Findings For rs- 1801394 With Meningioma Subtypes

The statistical findings for MTHFR rs- 1801394 via Chi square test with Meningioma types are as follows:

	Meningothelial	Transitional	Other Sub-types (Chordoid, Angimatous etc)
Mutant SNP Type A to G (Mis-Sense) rs1801394	44	4	6
Wild Type	22	4	7
P value	0.2885		
Statistical Test	Chi-square		

Reference: The table has been prepared by Rashid Mohiy-Ud-Din

Figure 4.28: Graphical representation of Table 4.21



Reference: The graph has been prepared by Rashid Mohiy-Ud-Din

The MTRR variant type (rs1801394) that we assessed for a corelation with meningioma subtypes are presented in table form along with the subsequent graph, however upon the Chi square test, the p value was exceeding and we couldn't observe a significant association between the meningioma subtypes and particular genetic variant.

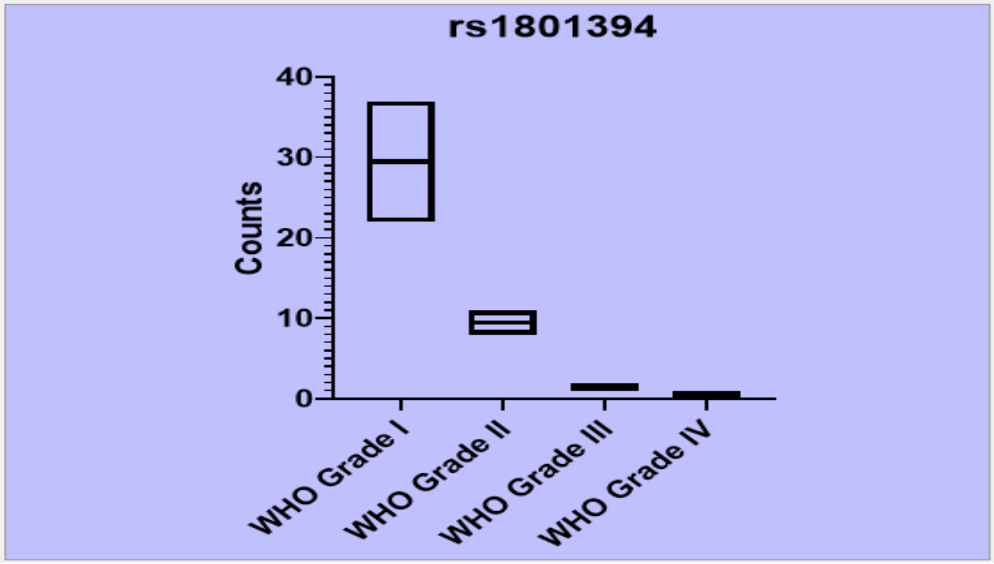
Table 4.22: Statisitcal Findings For rs- 1801394 With WHO Grading

The statistical findings for MTHFR rs- 1801394 via Chi square test with Meningioma types are as follows:

	WHO Grade I	WHO Grade II	WHO Grade III	WHO Grade IV
Mutant SNP Type A to G (Mis-Sense) rs1801394	37	11	1	0
Wild Type	22	8	2	1
P value	0.4579			
Statistical test	Chi-square			

Reference: The table has been prepared by Rashid Mohiy-Ud-Din

Figure 4.29: Graphical representation of Table 4.22



Reference: The graph has been prepared by Rashid Mohiy-Ud-Din

The MTRR variant type (rs1801394) that we assessed for a corelation with WHO grades are presented in table form along with the subsequent graph, however upon the Chi square test, the p value was exceeding and we couldn't observe a significant association between the WHO grades and particular genetic variant.

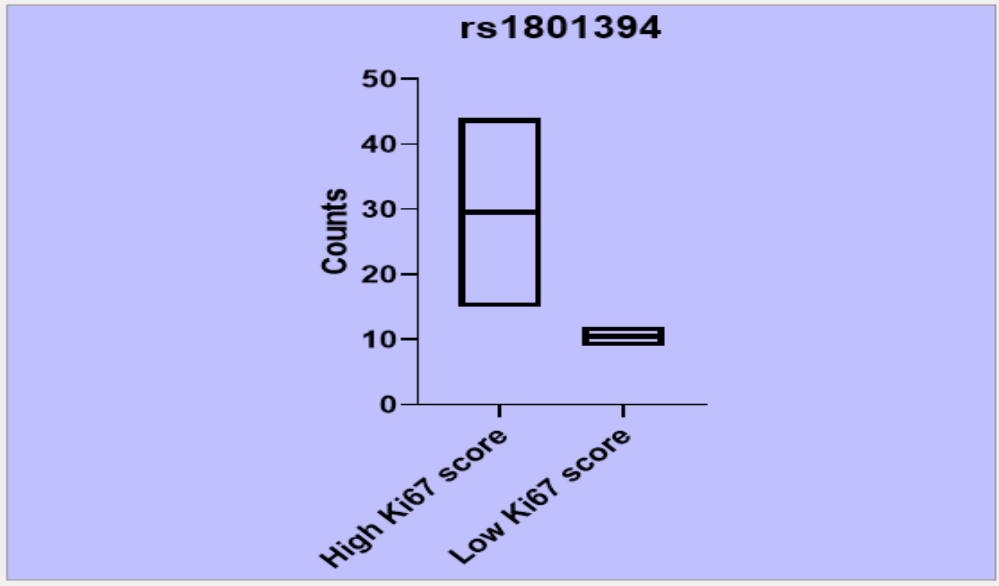
Table 4.23: Statisitcal Findings For rs- 1801394 With Ki67 Scores

The statistical findings for MTHFR rs- 1801394 via Fisher Exact test with Meningioma types are as follows:

	Proliferative index High Ki67 score	Proliferative index Low Ki67 score
Mutant SNP Type A to G (Mis-Sense) rs1801394	44	9
Wild Type	15	12
P value	0.0144 (Significant)	
Odds ratio	3.911	
Sensitivity	0.7458	
Statistical Test	Fisher's exact test	

Reference: The table has been prepared by Rashid Mohiy-Ud-Din

Figure 4.30: Graphical representation of Table 4.23



Reference: The graph has been prepared by Rashid Mohiy-Ud-Din

*The MTRR variant type (rs1801394) that we assessed for a corelation with Ki67 scores are presented in table form along with the subsequent graph. Upon the Fisher exact test, the p value was 0.01 and we observed a significant association between the Ki67 scores and this particular genetic variant.

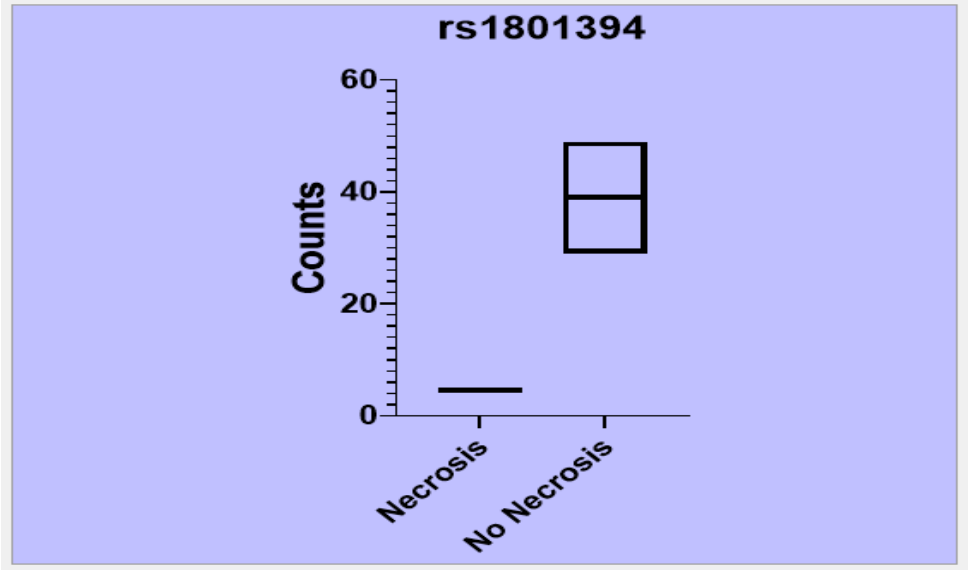
Table 4.24: Statisitcal Findings For rs- 1801394 With Necrosis

The statistical findings for MTHFR rs- 1801394 via Fisher Exact test with Meningioma types are as follows:

	Necrosis	No Necrosis
Mutant SNP Type A to G (Mis-Sense) rs1801394	5	49
Wild Type	4	29
P value	0.7253	
Odds ratio	0.7398	
Sensitivity	0.5556	
Statistical Test	Fisher's exact test	

Reference: The table has been prepared by Rashid Mohiy-Ud-Din

Figure 4.31: Graphical representation of Table 4.24



Reference: The graph has been prepared by Rashid Mohiy-Ud-Din

The MTRR variant type (rs1801394) that we assessed for a corelation with necrosis are presented in table form along with the subsequent graph, however upon the Fisher exact test, the p value was exceeding and we couldn't observe a significant association between the meningioma necrosis and this particular genetic variant.

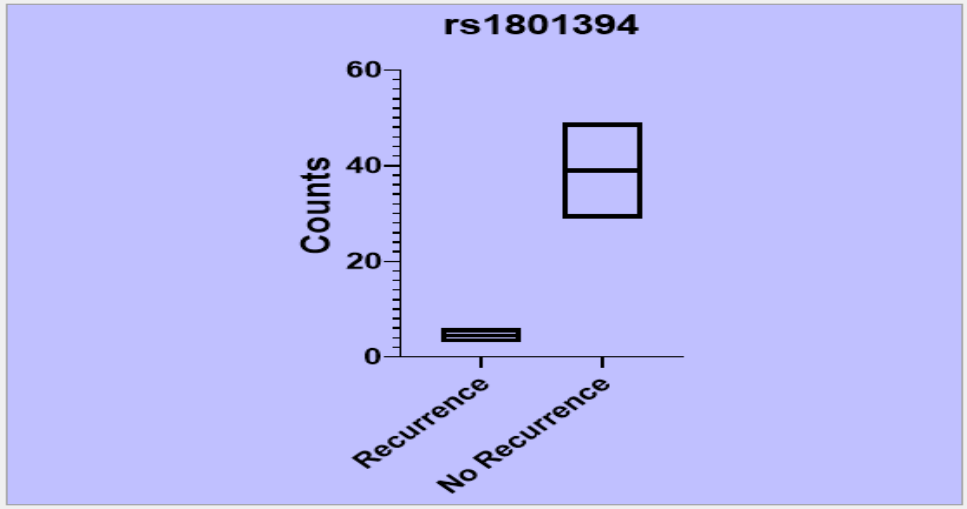
Table 4.25: Statisitcal Findings For rs- 1801394 With Recurrence

The statistical findings for MTHFR rs- 1801394 via Fisher Exact test with Meningioma types are as follows:

	Recurrence	No Recurrence
Mutant SNP Type A to G (Mis-Sense) rs1801394	6	49
Wild Type	3	29
P value	0.9999	
Odds ratio	1.184	
Sensitivity	0.6667	
Statistical Test	Fisher's exact test	

Reference: The table has been prepared by Rashid Mohiy-Ud-Din

Figure 4.32: Graphical representation of Table 4.25



Reference: The graph has been prepared by Rashid Mohiy-Ud-Din

The MTRR variant type (rs1801394) that we assessed for a corelation with recurrence are presented in table form along with the subsequent graph, however upon the Fisher exact test, the p value was exceeding and we couldn't observe a significant association between the meningioma recurrence and this particular genetic variant.

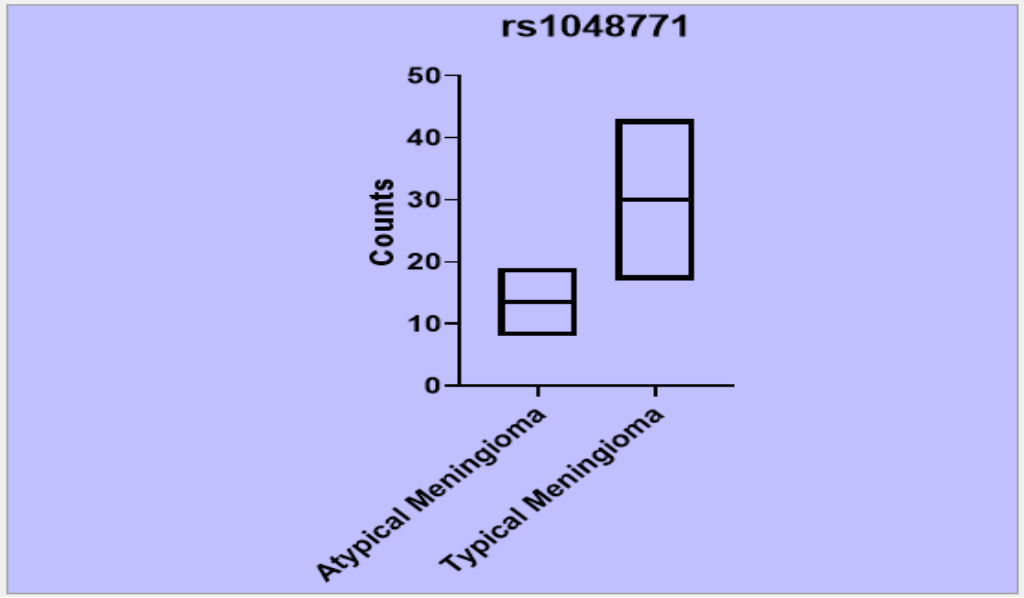
Table 4.26: Statisitcal Findings For rs- 1048771 With Meningioma Types

The statistical findings for MTHFR rs- 1801131 via Fisher Exact test with Meningioma types are as follows:

	Atypical Meningioma	Typical Meningioma
Mutant SNP Type C to T (Syn) rs1048771	8	17
Wild Type	19	43
P value	0.9999	
Odds ratio	1.065	
Sensitivity	0.2963	
Statisitical Test	Fisher's exact test	

Reference: The table has been prepared by Rashid Mohiy-Ud-Din

Figure 4.33: Graphical representation of Table 4.26



Reference: The graph has been prepared by Rashid Mohiy-Ud-Din

The RAD54L variant type (rs1048771) that we assessed for a corelation with meningioma types are presented in table form along with the subsequent graph, however upon the Fisher exact test, the p value was exceeding and we couldn't observe a significant association between the meningioma types and this particular genetic variant.

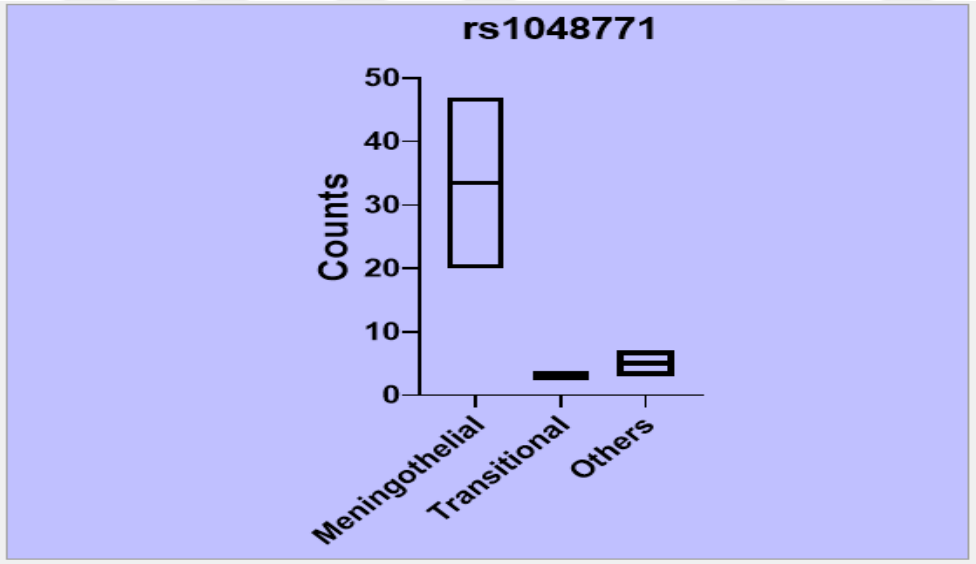
Table 4.27: Statisitcal Findings For rs- 1048771 With Meningioma Subtypes

The statistical findings for MTHFR rs- 1801131 via Chi square test with Meningioma types are as follows:

	Meningothelial	Transitional	Other Sub-types (Chordoid, Angimatous etc)
Mutant SNP Type C to T (Syn) rs1048771	20	3	3
Wild Type	47	3	7
P value	0.592		
Statistical Test	Chi-square		

Reference: The table has been prepared by Rashid Mohiy-Ud-Din

Figure 4.34: Graphical representation of Table 4.27



Reference: The graph has been prepared by Rashid Mohiy-Ud-Din

The RAD54L variant type (rs1048771) that we assessed for a corelation with meningioma subtypes are presented in table form along with the subsequent graph, however upon the Chi square test, the p value was exceeding and we couldn't observe a significant association between the meningioma subtypes and particular genetic variant.

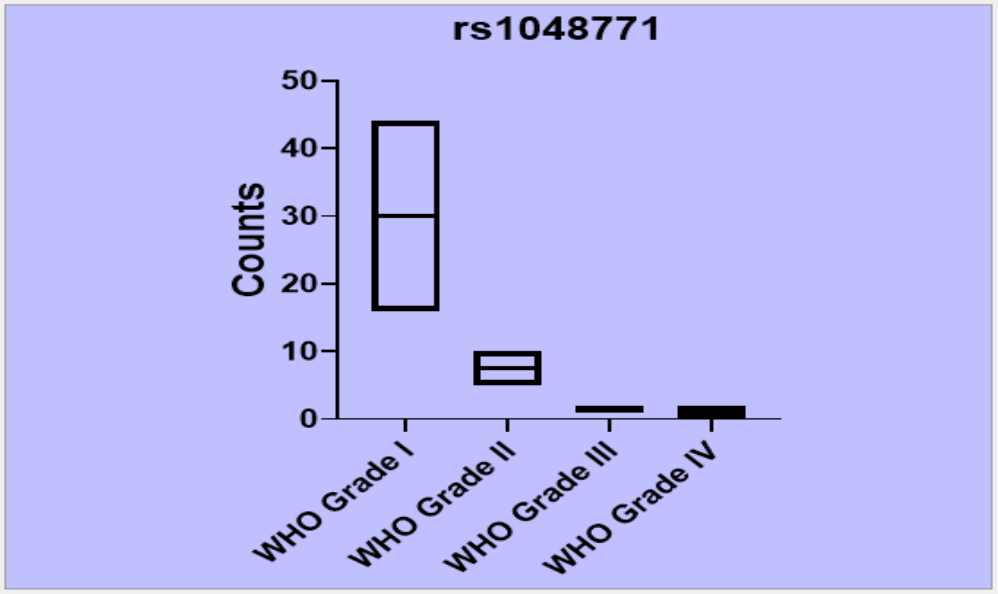
Table 4.28: Statisitcal Findings For rs- 1048771 With WHO Grading

The statistical findings for MTHFR rs- 1801131 via Chi square test with Meningioma types are as follows:

	WHO Grade I	WHO Grade II	WHO Grade III	WHO Grade IV
Mutant SNP Type C to T (Syn) rs1048771	16	5	1	0
Wild Type	44	10	2	2
P value	0.7803			
Statistical test	Chi-square			

Reference: The table has been prepared by Rashid Mohiy-Ud-Din

Figure 4.35: Graphical representation of Table 4.28



Reference: The graph has been prepared by Rashid Mohiy-Ud-Din

The RAD54L variant type (rs1048771) that we assessed for a corelation with WHO grades are presented in table form along with the subsequent graph, however upon the Chi square test, the p value was exceeding and we couldn't observe a significant association between the meningioma types and this particular genetic variant.

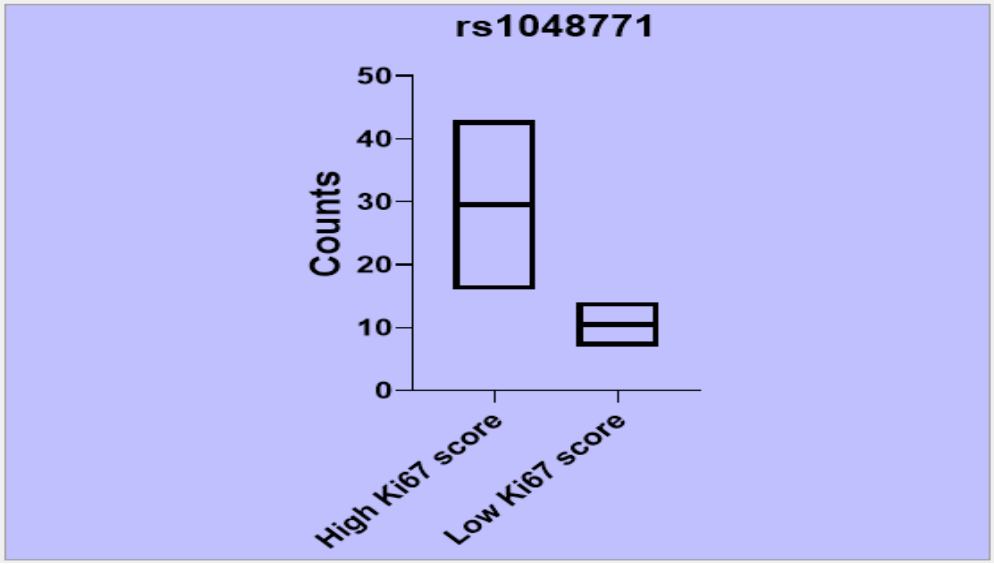
Table 4.29: Statisitcal Findings For rs- 1048771 With Ki67 Scores

The statistical findings for MTHFR rs- 1801131 via Fisher Exact test with Meningioma types are as follows:

	Proliferative index High Ki67 score	Proliferative index Low Ki67 score
Mutant SNP Type C to T (Syn) rs1048771	16	7
Wild Type	43	14
P value	0.5867	
Odds ratio	0.7442	
Sensitivity	0.2712	
Statisitical Test	Fisher's exact test	

Reference: The table has been prepared by Rashid Mohiy-Ud-Din

Figure 4.36: Graphical representation of Table 4.29



Reference: The graph has been prepared by Rashid Mohiy-Ud-Din

The RAD54L variant type (rs1048771) that we assessed for a corelation with Ki67 scores are presented in table form along with the subsequent graph, however upon the Fisher exact test, the p value was exceeding and we couldn't observe a significant association between the meningioma Ki67 scores and this particular genetic variant.

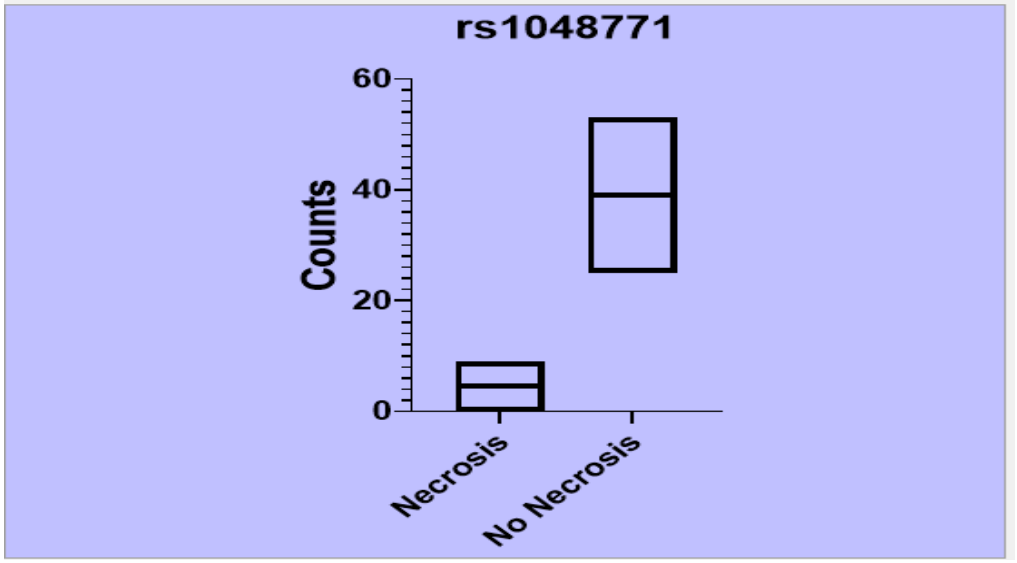
Table 4.30: Statisitcal Findings For rs- 1048771 With Necrosis

The statistical findings for MTHFR rs- 1801131 via Fisher Exact test with Meningioma types are as follows:

	Necrosis	No Necrosis
Mutant SNP Type C to T (Syn) rs1048771	0	25
Wild Type	9	53
P value	0.0545 (Almost Significant)	
Odds ratio	0,00	
Sensitivity	0,00	
Statistical Test	Fisher's exact test	

Reference: The table has been prepared by Rashid Mohiy-Ud-Din

Figure 4.37: Graphical representation of Table 4.30



Reference: The graph has been prepared by Rashid Mohiy-Ud-Din

*The RAD54L variant type (rs1048771) that we assessed for a corelation with necrosis are presented in table form along with the subsequent graph, however upon the Fisher exact test, the p value was 0.05 and we noted a significant association between the meningioma necrosis and this particular genetic variant.

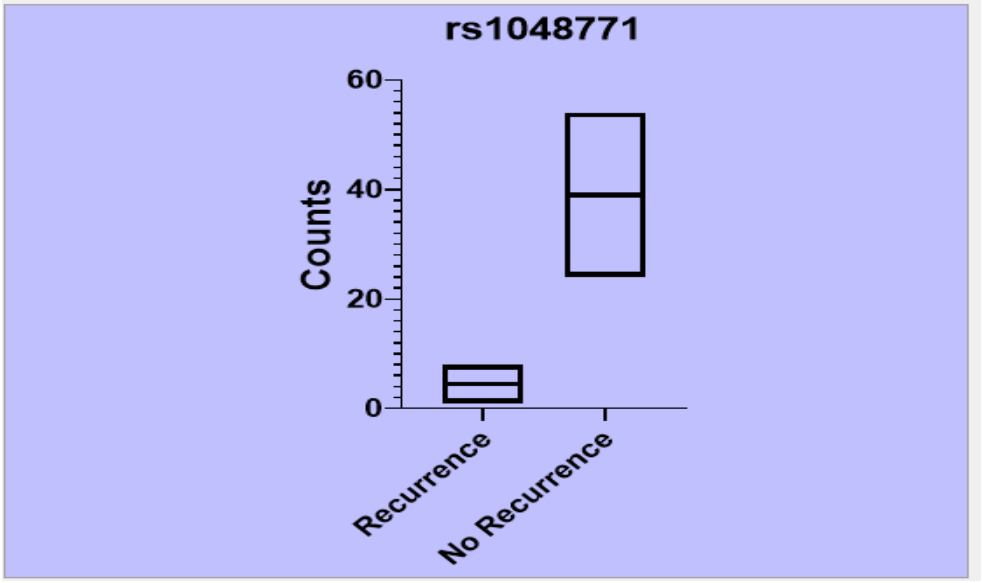
Table 4.31: Statisitcal Findings For rs- 1048771 With Recurrence

The statistical findings for MTHFR rs- 1048771 via Fisher Exact test with Meningioma types are as follows:

	Recurrence	No Recurrence
Mutant SNP Type C to T (Syn) rs1048771	1	24
Wild Type	8	54
P value	0.4367	
Odds ratio	0.2813	
Sensitivity	0.1111	
Statistical Test	Fisher's exact test	

Reference: The table has been prepared by Rashid Mohiy-Ud-Din

Figure 4.38: Graphical representation of Table 4.31



Reference: The graph has been prepared by Rashid Mohiy-Ud-Din

The RAD54L variant type (rs1048771) that we assessed for a corelation with recurrence are presented in table form along with the subsequent graph, however upon the Fisher exact test, the p value was exceeding and we couldn't observe a significant association between the meningioma recurrence and this particular genetic variant.

4.4. GENETIC AND HISTOPATHOLOGICAL PARAMETERS CORELATION

Meningiomas a re classified as second most common among the primary brain tumors. The recent version of classification provided by World Health Organization (WHO) is considered as a hallmark for classification of neuropathological tumors in relevance to the previous version of 2007. Yet it mainly applies the consideration of cytological, anatomical locations and histopathological factors in regard of classifying the meningioma tumors accordingly. The recent advancements in meningioma research has led to reveal some of the very important connections between the genetic profiles and the histopathological features.

The risk of meningioma and its relation with the genes MTHFR, MTRR and RAD54L has been investigated and depicted in many studies. A number of studies also suggests the association of MTHFR polymorphism to the risk of meningioma. Kumawat et al (2018) performed two seperate studies in different time periods to evaluate the risk of MTHFR and MTRR genes in the north and south Indian ethinicties of their population respectively. Although, Kumawat et al in their study couldn't find any significance for allelic polymorphic SNP of MTHFR C677T and MTHFR A1298C. The only statistically significant ploymorphic alteration was noted the CC genotype. While for MTRR A66G SNP, AG genotype was more likely significant (p 0.07).

In the same context, a study was performed by Zhang et al (2013) in China, for northern chinese han population suggesting a close association of MTHFR (C677T) polymorphism with meningioma. From his findings, the TT allele was found to be significantly associated with Meningioma as compared to CT. His studies also indicate significant association of MTRR (A66G) variant with meningioma risk.

Functional Polymorphism in MTHFR (C677T and A1298C) as risk for meningioma was also investigated by Bethke et al (2008) at United Kingdom. Their study clearly pointed that MTHFR A1298C (rs1801131) was associated with an increased risk of meningioma in their cohort as compared to C677T (rs1801133). Influence of the MTRR A66G (rs1801394) genotype on risk of meningioma was clearly observed.

Kafadar et al (2006) at Turkey performed a study to evaluate and suggest the susceptible risk for Meningioma via MTHFR Polymorphism. Inspite of established risk for CT variant, Kafadar et al, couldn't find a significant link for TT 677 variant (rs1801131).

Several studies were also performed likewise in a similar pattern considering the genetic spectrum for both NF2 and non-NF2 meningiomas by several researchers assessing their corelation with the anatomical locations of their origin and the typical and non-typical behaviour they tend to shown.

Clark et al, has reported an extensive analysis upon three hundred genomes of non NF2 meningiomas and the major mutation findings consisted of TRAF7, KLF4, AKT1, and SMO. They noted the TRAF7 mutations to be occurring in corelation with KLF4 recurrence or with AKT1 mutation. SMO mutations were the least they found in their cohort. Clark et al, reported in regard of these non NF2 mutations that although being distinctive, they had corelations with the benign and malignant nature of the tumor type and also with the their anatomical location of origin. Their findings indicate that non NF2 meningiomas came out to be benign and have medial skull base as their origin commonly, while the NF2 related ones were having atypical behaviours and the cerebellar hemispheres as their anatomical locations.

Linking the aforementioned findings to our findings, implies that the non NF2 meningiomas that we considered were usually benign however in case of two of our genes i-e MTHFR (rs1801131) and MTRR (rs4840851) we tried to indocate that the non NF2 mutations in our case were not completely benign and showed a varied tendency for risks association with meningioma.

Kros, et al also studied the status of meningioma further and its association specifically with the localization of tumors and their histology. As in these kinds of meningioma the inactivation of NF2 gene (which is located on Chromosome 22q) is involved, the meningioma sub-types in their study mostly reported to be transitional and fibrous and interestingly not among the meningothelial group. Kros et al further reported a potential corelation between the NF2 disruption at 22q and anatomical location of anterior skull base for their tumors (p-value being 0.03). Furthermore their study revealed that meningioma histological subtype was successfully corelated with disruption of NF2 at Chromosome 22q in fibrous and transitional types but very rare effect on meningothelial ones.

As we tried to assess the relationship of meningioma subtypes with their respective genetic mutations in MTHFR, MTRR and RAD54L, however we also couldn't find any possible link or association between these genetic polymorphs and the meningioma subypes. However our frequency indicated the Meningothelial to be the most common, unlike the results from Kros et al.

Brastianos, et al also analyzed the oncogenic mutations in SMO and AKT1 and with their link with the anatomical locations mainly but also with the possible grade of meningioma by performing whole exome sequencing for seventeen meningiomas and then adding further fourty eight tumors to

their cohort for further validation of those genetic alterations. The oncogenic mutations that they noticed upon histochemical studies for AKT1 and SMO indicated significant links with the anatomical locality of skull base for these mutations and also found to be assoicated with the higher grades of meningioma , which is quite similar to our findings upon MTHFR (rs1801131) showing a significant corelation between this polymorphic mutant to the WHO grading.

Reuss et al, further screened the oncogenic mutations in KLF4 and TRAF7 and revealed their link in secretory and glandular meningiomas. His findings upon Sanger sequencing reveals the KLF4 and TRAF7 mutations to follow the same polymorphic sequencing pattern. However the KLF4 mutations were not noticed to be involved in non-secretory meningiomas while TRAF7 mutations were observed in non-secretory meningiomas, however the KF$ mutations were quite exclusive for secretory meningiomas only. According to our results from a retrospective study, we couldn't be able to come acroos such type of meningiomas where this urges a need to include and further validare our stduy upon these basis.

A distinct subset of meningioma's association with the genetic mutations was also studied by Clark et al in POLR2A which is noted to be involved in RNA Polymerase II mediation of transcription processes for protein coding. This genomic mutation of POLR2A wasn't yet been explored if it has any association with pathological aspects in humans. From thorough analysis of genome via next generation sequencing analysis, Clark et al via their study, a cohort consisiting of more than seven hundred meningiomas, tried to provide the answer to the relation between the POLR2A mutants and the meningioma subgroups with definite clinical and pathological features. POLR2A mutations were solely detected in WHO grade I meningiomas that are mostly beenign in nature and there was no detection in grade II or grade III meningiomas (that are atypical and malignnat in nature) screened for these variants.

However, in our study upon the genes of MTHFR, MTRR and RAD54L, there wasn't any significance observed for the corelation between the genetic variants that we screened for these genes and the subsequent subtypes of meningiomas.

In light of previous studies performed, in this retrospective study we tried to evaluate and reveal if there exists an association or link between the genetic alterations (objective parameters) and the histopathological characteristics (subjective parameters) of the meningioma tumors. We found four siginifcant corelations in our study from all of the four genes that we included in our study which includes the following:

MTHFR rs4846051: Significant correlation found with grading scheme provided by WHO.

MTHFR rs4846051: Significant correlation found with Proliferative index (Ki67 scores).

MTRR rs1801394: Significantly correlated with Proliferative index (Ki67 scores).

RAD54L rs1048771: Significant correlation found with level of Necrosis.

Our study clearly indicates a sort of relationship between the genentic and histopathological factors. The proper diagnosis requires more objective approach and parameter as compared to the subjective guidelines. The objective markers has always been proved fruitful in the precise prognosis of the patient assessment which leads to designing of a good treatment model by health care professionals. The results we tried to reveal in this study are quite promising and emphaiszed to be considered for a larger cohort of studies for a variety of genes involved in meningiomas.

5. CONCLUSION

Meningiomas are classified as second most common among the primary brain tumors. Up till now, the meningioma grading categorization is minly and mostly relied upon the subjective parameters that includes the anatomical, cytological and histopathological characteristics as criterion. No doubt the above factors and the criteria established on these basis lead to a correct prognosis that inturn leads to designong of a precise treatment plan for the respective meningioma patient. Many patients are misgraded most likely due to limitations of the applied histopathological criteria during the assessment of their proper prognosis affecting the outcome of treatment plan suggested by the clinician.

In this retrospective study we tried to reveal if there exists an association or link between the genetic alterations (objective parameters) and the histopathological characteristics (subjective parameters) of the meningioma tumors. We found four siginifcant corelations in our study from all of the four genes that we included in our study which includes the following:

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MTRR rs1801394: Significantly correlated with Proliferative index (Ki67 scores).

RAD54L rs1048771: Significant correlation found with level of Necrosis.

The genetic SNPs of the above genes have been significantly corelated with the afore mentioned subjective parameters. The 2016 CNS WHO represents a substantial step forward over its 2007 and sets the stage for such progress. It is hoped that these more objective and more precisely defined parameters will allow for making improvements to tailor the patient therapy and provide better classification for meningioma tumors.

Valuable diagnosis and therapy of meningioma patients need a multidisciplinary approach, which ideally should include information on microscopy (subjective histological parameters) and genetic changes (objective paramters). This provides an insight if the classifications of meningioma tumors needs to re-assessed and further revised according to their genetic landscape and clinical histopathology.

Further large scale studies are required to validate our findings and also a further genetic testing is recommended as a direction for furture studies, a genetic library needs to established consisting of larger cohorts and data for their genetic alterations and thier assessment for corelation woth the histopathological parameters.

REFERENCES

Periodicals

Antinheimo, J., Haapasalo, H., Haltia, M., Tatagiba, M., Thomas, S., Brandis, A., Sainio, M., Carpen, O., Samii, M. and Jääskeläinen, J., 1997. Proliferation potential and histological features in neurofibromatosis 2-associated and sporadic meningiomas. Journal of neurosurgery, 87(4), pp.610-614.

Ayerbe, J., Lobato, R.D., De la Cruz, J., Alday, R., Rivas, J.J., Gomez, P.A. and Cabrera, A., 1999. Risk factors predicting recurrence in patients operated on for intracranial meningioma. A multivariate analysis. *Acta neurochirurgica*, *141*(9), pp.921-932.

Aboukais, R., Zairi, F., Le Rhun, E., Lejeune, J.P., Devos, P. and Reyns, N., 2015. Radiation-associated grade 2 meningiomas: A nine patient-series and review of the literature. Clinical neurology and neurosurgery, 136, pp.10-14.

Buerki, R.A., Horbinski, C.M., Kruser, T., Horowitz, P.M., James, C.D. and Lukas, R.V., 2018. An overview of meningiomas. *Future Oncology*, *14*(21), pp.2161-2177.

Brassesco, M.S., Valera, E.T., Neder, L., Castro-Gamero, A.M., De Oliveira, F.M., Santos, A.C., Scrideli, C.A., Oliveira, R.S., Machado, H.R. and Tone, L.G., 2009. Childhood radiation-associated atypical meningioma with novel complex rearrangements involving chromosomes 1 and 12. *Neuropathology*, *29*(5), pp.585-590.

Banerjee, R., Lohse, C.M., Kleinschmidt-DeMasters, B.K. and Scheithauer, B.W., 2002. A role for chromosome 9p21 deletions in the malignant progression of meningiomas and the prognosis of anaplastic meningiomas. *Brain pathology*, *12*(2), pp.183-190.

Boström, J., Cobbers, J.L., Wolter, M., Tabatabai, G., Weber, R.G., Lichter, P., Collins, V.P. and Reifenberger, G., 1998. Mutation of the PTEN (MMAC1) tumor suppressor gene in a subset of glioblastomas but not in meningiomas with loss of chromosome arm 10q. *Cancer research*, *58*(1), pp.29-33.

Büschges, R., Boström, J., Wolter, M., Blaschke, B., Weber, R.G., Lichter, P., Collins, V.P. and Reifenberger, G., 2001. Analysis of human meningiomas for aberrations of the MADH2, MADH4, APM-1 and DCC tumor suppressor genes on the long arm of chromosome 18. *International journal of cancer*, *92*(4), pp.551-554.

Bello, M.J., Pestaña, A., Rey, J.A., De Campos, J.M., Kusak, M.E., Vaquero, J. and Sarasay, J.L., 1994. Allelic loss at 1 p is associated with tumor progression of meningiomas. *Genes, Chromosomes and Cancer*, *9*(4), pp.296-298.

Boström, J., Mühlbauer, A. and Reifenberger, G., 1997. Deletion mapping of the short arm of chromosome 1 identifies a common region of deletion distal to D1S496 in human meningiomas. *Acta neuropathologica*, *94*(5), pp.479-485.

Boström, J., Meyer-Puttlitz, B., Wolter, M., Blaschke, B., Weber, R.G., Lichter, P., Ichimura, K., Collins, V.P. and Reifenberger, G., 2001. Alterations of the tumor suppressor genes CDKN2A (p16INK4a), p14ARF, CDKN2B (p15INK4b), and

CDKN2C (p18INK4c) in atypical and anaplastic meningiomas. *The American journal of pathology*, *159*(2), pp.661-669.

Bello, M.J., de Campos, J.M., Vaquero, J., Kusak, M.E., Sarasa, J.L. and Rey, J.A., 2000. High-resolution analysis of chromosome arm 1p alterations in meningioma. *Cancer genetics and cytogenetics*, *120*(1), pp.30-36.

Büschges, R., Ichimura, K., Weber, R.G., Reifenberger, G. and Collins, V.P., 2002. Allelic gain and amplification on the long arm of chromosome 17 in anaplastic meningiomas. *Brain pathology*, *12*(2), pp.145-153.

Bitzer, M., Opitz, H., Popp, J., Morgalla, M., Gruber, A., Heiss, E. and Voigt, K., 1998. Angiogenesis and brain oedema in intracranial meningiomas: influence of vascular endothelial growth factor. *Acta neurochirurgica*, *140*(4), pp.333-340.

Bitzer, M., Wöckel, L., Luft, A.R., Wakhloo, A.K., Petersen, D., Opitz, H., Sievert, T., Ernemann, U. and Voigt, K., 1997. The importance of pial blood supply to the development of peritumoral brain edema in meningiomas. *Journal of neurosurgery*, *87*(3), pp.368-373.

Black, P., Carroll, R. and Zhang, J., 1996. The molecular biology of hormone and growth factor receptors in meningiomas. In *Modern neurosurgery of meningiomas and pituitary adenomas* (pp. 50-53). Springer, Vienna.

Bethke, L., Webb, E., Murray, A., Schoemaker, M., Feychting, M., Lönn, S., Ahlbom, A., Malmer, B., Henriksson, R., Auvinen, A. and Kiuru, A., 2008. Functional polymorphisms in folate metabolism genes influence the risk of meningioma and glioma. Cancer Epidemiology and Prevention Biomarkers, 17(5), pp.1195-1202.

Backer-Grøndahl, T., Moen, B.H. and Torp, S.H., 2012. The histopathological spectrum of human meningiomas. International journal of clinical and experimental pathology, 5(3), p.231.

Bhat, A.R., Wani, M.A., Kirmani, A.R. and Ramzan, A.U., 2014. Histological-subtypes and anatomical location correlated in meningeal brain tumors (meningiomas). Journal of neurosciences in rural practice, 5(3), p.244.

Babu, S., Uppin, S.G., Uppin, M.S., Panigrahi, M.K., Saradhi, V., Bhattacharjee, S., Sahu, B.P., Purohit, A.K. and Challa, S., 2011. Meningiomas: correlation of Ki67 with histological grade. Neurology India, 59(2), p.204.

Brastianos, P.K., Horowitz, P.M., Santagata, S., Jones, R.T., McKenna, A., Getz, G., Ligon, K.L., Palescandolo, E., Van Hummelen, P., Ducar, M.D. and Raza, A., 2013. Genomic sequencing of meningiomas identifies oncogenic SMO and AKT1 mutations. Nature genetics, 45(3), pp.285-289.

Bi, W.L., Mei, Y., Agarwalla, P.K., Beroukhim, R. and Dunn, I.F., 2016. Genomic and epigenomic landscape in meningioma. Neurosurgery Clinics, 27(2), pp.167-179.

Banerjee, J., Pääkkö, E., Harila, M., Herva, R., Tuominen, J., Koivula, A., Lanning, M. and Harila-Saari, A., 2009. Radiation-induced meningiomas: a shadow in the success story of childhood leukemia. Neuro-oncology, 11(5), pp.543-549.

Baser, M.E., Kuramoto, L., Woods, R., Joe, H., Friedman, J.M., Wallace, A.J., Ramsden, R.T., Olschwang, S., Bijlsma, E., Kalamarides, M. and Papi, L., 2005. The location of constitutional neurofibromatosis 2 (NF2) splice site mutations is associated with the severity of NF2. Journal of medical genetics, 42(7), pp.540-546.

Collins, V.P., Nordenskjold, M. and Dumanski, J.P., 1990. The molecular genetics of meningiomas. *Brain pathology*, *1*(1), pp.19-24.

Cai, D.X., Banerjee, R., Scheithauer, B.W., Lohse, C.M., Kleinschmidt-Demasters, B.K. and Perry, A., 2001. Chromosome 1p and 14q FISH analysis in clinicopathologic subsets of meningioma: diagnostic and prognostic implications. *Journal of Neuropathology & Experimental Neurology*, *60*(6), pp.628-636.

Cai, D.X., James, C.D., Scheithauer, B.W., Couch, F.J. and Perry, A., 2001. PS6K amplification characterizes a small subset of anaplastic meningiomas. *American journal of clinical pathology*, *115*(2), pp.213-218.

Carlson, K.M., Bruder, C., Nordenskjöld, M. and Dumanski, J.P., 1997. 1p and 3p deletions in meningiomas without detectable aberrations of chromosome 22 identified by comparative genomic hybridization. *Genes, Chromosomes and Cancer*, *20*(4), pp.419-424.

Carroll, R.S., Glowacka, D., Dashner, K. and Black, P.M., 1993. Progesterone receptor expression in meningiomas. *Cancer research*, *53*(6), pp.1312-1316.

Carroll, R.S., Zhang, J., Dashner, K., Sar, M., Wilson, E.M. and Black, P.M., 1995. Androgen receptor expression in meningiomas. *Journal of neurosurgery*, *82*(3), pp.453-460.

Clark, V.E., Erson-Omay, E.Z., Serin, A., Yin, J., Cotney, J., Özduman, K., Avşar, T., Li, J., Murray, P.B., Henegariu, O. and Yilmaz, S., 2013. Genomic analysis of non-NF2 meningiomas reveals mutations in TRAF7, KLF4, AKT1, and SMO. Science, 339(6123), pp.1077-1080.

Clark, V.E., Harmancı, A.S., Bai, H., Youngblood, M.W., Lee, T.I., Baranoski, J.F., Ercan-Sencicek, A.G., Abraham, B.J., Weintraub, A.S., Hnisz, D. and Simon, M., 2016. Recurrent somatic mutations in POLR2A define a distinct subset of meningiomas. Nature genetics, 48(10), pp.1253-1259.

Claus, E.B., Calvocoressi, L., Bondy, M.L., Schildkraut, J.M., Wiemels, J.L. and Wrensch, M., 2011. Family and personal medical history and risk of meningioma. Journal of neurosurgery, 115(6), pp.1072-1077.

Dumanski, J.P., Carlbom, E., Collins, V.P. and Nordenskjöld, M., 1987. Deletion mapping of a locus on human chromosome 22 involved in the oncogenesis of meningioma. *Proceedings of the National Academy of Sciences*, *84*(24), pp.9275-9279.

Deimling, V., 1998. Analysis of the PTEN gene in human meningiomas. *Neuropathology and applied neurobiology*, *24*(1), pp.3-8.

Decimo, I., Fumagalli, G., Berton, V., Krampera, M. and Bifari, F., 2012. Meninges: from protective membrane to stem cell niche. *American journal of stem cells*, *1*(2), p.92.

Evans, J.J., Jeun, S.S., Lee, J.H., Harwalkar, J.A., Shoshan, Y., Cowell, J.K. and Golubic, M., 2001. Molecular alterations in the neurofibromatosis type 2 gene and its protein rarely occurring in meningothelial meningiomas. *Journal of neurosurgery*, *94*(1), pp.111-117.

Eom, K.S., Kim, H.S., Kim, T.Y. and Kim, J.M., 2009. Intraventricular malignant meningioma with CSF-disseminated spinal metastasis: case report and literature review. Journal of Korean Neurosurgical Society, 45(4), p.256. Falchetti, M., Larocca, L. and Pallini, R., 2002. Telomerase in brain tumors. *Child's nervous system*, *18*(3-4), pp.112-117.

Fathallah-Shaykh, H.M., He, B., Zhao, L.J., Engelhard, H.H., Cerullo, L., Lichtor, T., Byrne, R., Munoz, L., Von Roenn, K., Rosseau, G.L. and Glick, R., 2003. Genomic expression discovery predicts pathways and opposing functions behind phenotypes. *Journal of Biological Chemistry*, *278*(26), pp.23830-23833.

Gusella, J.F., Ramesh, V., MacCollin, M. and Jacoby, L.B., 1999. Merlin: the neurofibromatosis 2 tumor suppressor. *Biochimica et biophysica acta*, *1423*(2), pp.M29-36.

Gutmann, D.H., Donahoe, J., Perry, A., Lemke, N., Gorse, K., Kittiniyom, K., Rempel, S.A., Gutierrez, J.A. and Newsham, I.F., 2000. Loss of DAL-1, a protein 4.1-related tumor suppressor, is an important early event in the pathogenesis of meningiomas. *Human molecular genetics*, *9*(10), pp.1495-1500.

Goldman, C.K., Bharara, S., Palmer, C.A., Vitek, J., Tsai, J.C., Weiss, H.L. and Gillespie, G.Y., 1997. Brain edema in meningiomas is associated with increased vascular endothelial growth factor expression. *Neurosurgery*, *40*(6), pp.1269-1277.

Hitotsumatsu, T., Iwaki, T., Kitamoto, T., Mizoguchi, M., Suzuki, S.O., Hamada, Y., Fukui, M. and Tateishi, J., 1997. Expression of neurofibromatosis 2 protein in human brain tumors: an immunohistochemical study. *Acta neuropathologica*, *93*(3), pp.225-232.

Huynh, D.P., Mautner, V., Baser, M.E., Stavrou, D. and Pulst, S.M., 1997. Immunohistochemical detection of schwannomin and neurofibromin in vestibular schwannomas, ependymomas and meningiomas. *Journal of Neuropathology & Experimental Neurology*, *56*(4), pp.382-390.

Heinrich, B., Hartmann, C., Stemmer-Rachamimov, A.O., Louis, D.N. and MacCollin, M., 2003. Multiple meningiomas: Investigating the molecular basis of sporadic and familial forms. *International journal of cancer*, *103*(4), pp.483-488.

Hsu, D.W., Efird, J.T. and Hedley-Whyte, E.T., 1997. Progesterone and estrogen receptors in meningiomas: prognostic considerations. *Journal of neurosurgery*, *86*(1), pp.113-120.

Ikeda, K., Saeki, Y., Gonzalez-Agosti, C., Ramesh, V. and Chiocca, E.A., 1999. Inhibition of NF2-negative and NF2-positive primary human meningioma cell proliferation by overexpression of merlin due to vector-mediated gene transfer. *Journal of neurosurgery*, *91*(1), pp.85-92.

Joseph, J.T., Lisle, D.K., Jacoby, L.B., Paulus, W., Barone, R., Cohen, M.L., Roggendorf, W.H., Bruner, J.M., Gusella, J.F. and Louis, D.N., 1995. NF2 gene analysis distinguishes hemangiopericytoma from meningioma. *The American journal of pathology*, *147*(5), p.1450.

Jenny, Y.M., Ng, H.K., Lau, K.M., Lo, K.W., Poon, W.S. and Huang, D.P., 1997. Loss of heterozygosity of chromosome 14q in low-and high-grade meningiomas. *Human pathology*, *28*(7), pp.779-785.

Joachim, T., Ram, Z., Rappaport, Z.H., Simon, M., Schramm, J., Wiestler, O.D. and von Deimling, A., 2001. Comparative analysis of the NF2, TP53, PTEN, KRAS, NRAS and HRAS genes in sporadic and radiation-induced human meningiomas. *International journal of cancer*, *94*(2), pp.218-221.

Jenny, Y.M., Ng, H.K., Lo, K.W., Chong, E.Y., Lam, P.Y., Ng, E.K., Poon, W.S. and Huang, D.P., 1998. Analysis of cell cycle regulators: p16INK4A, pRb, and CDK4 in low-and high-grade meningiomas. *Human pathology*, *29*(11), pp.1200-1207.

Jääskeläinen, J., 1986. Seemingly complete removal of histologically benign intracranial meningioma: late recurrence rate and factors predicting recurrence in 657 patients. A multivariate analysis. *Surgical neurology*, *26*(5), pp.461-469.

Krampla, W., Newrkla, S., Pfisterer, W., Jungwirth, S., Fischer, P., Leitha, T., & Tragl, K. H. (2004). Frequency and risk factors for meningioma in clinically healthy 75-year-old patients: Results of the Transdanube Ageing Study (VITA). *Cancer: Interdisciplinary International Journal of the American Cancer Society*, *100*(6), 1208-1212

Kleihues, P., Louis, D.N., Scheithauer, B.W., Rorke, L.B., Reifenberger, G., Burger, P.C. and Cavenee, W.K., 2002. The WHO classification of tumors of the nervous system. *Journal of Neuropathology & Experimental Neurology*, *61*(3), pp.215-225.

Kimura, Y., Koga, H., Araki, N., Mugita, N., Fujita, N., Takeshima, H., Nishi, T., Yamashima, T., Saido, T.C., Yamasaki, T. and Moritake, K., 1998. The involvement of calpain-independent proteolysis of the tumor suppressor NF2 (merlin) in schwannomas and meningiomas. *Nature medicine*, *4*(8), p.915.

Kedra, D., Peyrard, M., Fransson, I., Collins, J.E., Dunham, I., Roe, B.A. and Dumanski, J.P., 1996. Characterization of a second human clathrin heavy chain polypeptide gene (CLH-22) from chromosome 22q11. *Human molecular genetics*, *5*(5), pp.625-631.

Karamitopoulou, E., Perentes, E., Tolnay, M. and Probst, A., 1998. Prognostic significance of MIB-1, p53, and bcl-2 immunoreactivity in meningiomas. *Human pathology*, *29*(2), pp.140-145.

Kumawat, R., Gowda, S.H., Debnath, E., Rashid, S., Niwas, R., Suri, A., Sarkar, C., Sinha, S. and Chosdol, K., 2018. 64P Association of MTHFR gene polymorphisms with glioma and meningioma patients in Indian population. Annals of Oncology, 29(suppl_9), pp.mdy 429-003.

Kumawat, R., Gowda, S.H., Debnath, E., Rashid, S., Niwas, R., Gupta, Y., Upadaya, A.D., Suri, A., Chandra, P.S., Gupta, D.K. and Lakshmy, R., 2018. Association of Single Nucleotide Polymorphisms (SNPs) in Genes Encoding for Folate Metabolising Enzymes with Glioma and Meningioma in Indian Population. Asian Pacific journal of cancer prevention: APJCP, 19(12), p.3415.

Kros, J., de Greve, K., van Tilborg, A., Hop, W., Pieterman, H., Avezaat, C., Lekanne dit Deprez, R. and Zwarthoff, E., 2001. NF2 status of meningiomas is associated with tumour localization and histology. The Journal of pathology, 194(3), pp.367-372.

Louis, D.N., 2000. Meningiomas. *WHO Classification of Tumours of the Central Nervous System*.

Lomas, J., Bello, M.J., Alonso, M.E., Gonzalez-Gomez, P., Arjona, D., Kusak, M.E., De Campos, J.M., Sarasa, J.L. and Rey, J.A., 2002. Loss of chromosome 22 and absence of NF2 gene mutation in a case of multiple meningiomas. *Human pathology*, *33*(3), pp.375-378.

Li, X., & Zhao, J. (2009). Intracranial meningiomas of childhood and adolescence: report of 34 cases with follow-up. *Child's Nervous System*, *25*(11), 1411.

Lamszus, K., Vahldiek, F., Mautner, V.F., Schichor, C., Tonn, J., Stavrou, D., Fillbrandt, R., Westphal, M. and Kluwe, L., 2000. Allelic losses in neurofibromatosis 2-associated meningiomas. *Journal of Neuropathology & Experimental Neurology*, *59*(6), pp.504-512.

Louis, D.N., Ramesh, V. and Gusella, J.F., 1995. Neuropathology and molecular genetics of neurofibromatosis 2 and related tumors. *Brain Pathology*, *5*(2), pp.163-172.

Larson, J.J., Tew, J.M., Simon, M. and Menon, A.G., 1995. Evidence for clonal spread in the development of multiple meningiomas. *Journal of neurosurgery*, *83*(4), pp.705-709.

Lekanne, R.D., Riegman, P.H., Groen, N.A., Warringa, U.L., Molijn, A.C., Bootsma, D., Menon, A.G. and Kley, N.A., 1995. Cloning and characterization of MN1, a gene from chromosome 22q11, which is disrupted by a balanced translocation in a meningioma. *Oncogene*, *10*(8), pp.1521-1528.

Leone, P.E., Bello, M.J., de Campos, J.M., Vaquero, J., Sarasa, J.L., Pestaña, A. and Rey, J.A., 1999. NF2 gene mutations and allelic status of 1p, 14q and 22q in sporadic meningiomas. *Oncogene*, *18*(13), p.2231.

Lamszus, K., Kluwe, L., Matschke, J., Meissner, H., Laas, R. and Westphal, M., 1999. Allelic losses at 1p, 9q, 10q, 14q, and 22q in the progression of aggressive meningiomas and undifferentiated meningeal sarcomas. *Cancer genetics and cytogenetics*, *110*(2), pp.103-110.

Leuraud, P., Marie, Y., Robin, E., Huguet, S., He, J., Mokhtari, K., Cornu, P., Hoang-Xuan, K. and Sanson, M., 2000. Frequent Loss of 1p32 Region but no Mutation if the p18 Tumor Suppressor Gene in Meningiomas. *Journal of neuro-oncology*, *50*(3), pp.207-213.

Leone, P.E., Mendiola, M., Alonso, J., Paz-y-Miño, C. and Pestaña, A., 2003. Implications of a RAD54L polymorphism (2290C/T) in human meningiomas as a risk factor and/or a genetic marker. *BMC cancer*, *3*(1), p.6.

Lomas, J., Bello, M.J., Arjona, D., Gonzalez-Gomez, P., Alonso, M.E., de Campos, J.M., Vaquero, J., Ruiz-Barnes, P., Sarasa, J.L., Casartelli, C. and Rey, J.A., 2001. Analysis of p73 gene in meningiomas with deletion at 1p. *Cancer genetics and cytogenetics*, *129*(1), pp.88-91.

Lekanne Deprez, R.H., Riegman, P.H., van Drunen, E., Warringa, U.L., Groen, N.A., Stefanko, S.Z., Koper, J.W., Avezaat, C.J., Mulder, P.G., Zwarthoff, E.C. and Hagemeijer, A., 1995. Cytogenetic, molecular genetic and pathological analyses in 126 meningiomas. *Journal of Neuropathology & Experimental Neurology*, *54*(2), pp.224-235.

Langford, L.A., Piatyszek, M.A., Xu, R., Schold Jr, S.C., Wright, W.E. and Shay, J.W., 1997. Telomerase activity in ordinary meningiomas predicts poor outcome. *Human pathology*, *28*(4), pp.416-420.

Lamszus, K., Lengler, U., Schmidt, N.O., Stavrou, D., Ergün, S. and Westphal, M., 2000. Vascular endothelial growth factor, hepatocyte growth factor/scatter factor, basic fibroblast growth factor, and placenta growth factor in human meningiomas and their relation to angiogenesis and malignancy. *Neurosurgery*, *46*(4), pp.938-948.

Li, R., Wang, R., Li, Y., Li, X., Feng, Y., Li, Y. and Jiang, C., 2013. Association study on MTHFR polymorphisms and meningioma in northern China. Gene, 516(2), pp.291-293.

Leone, P.E., Mendiola, M., Alonso, J., Paz-y-Miño, C. and Pestaña, A., 2003. Implications of a RAD54L polymorphism (2290C/T) in human meningiomas as a risk factor and/or a genetic marker. BMC cancer, 3(1), p.6.

Lin, J.W., Lu, C.H., Lin, W.C., Wu, Y.T., Huang, Y.J., Shih, F.Y., Ho, J.T. and Chuang, M.J., 2012. A clinicopathological study of the significance of the proportion of choroid morphology in chordoid meningioma. Journal of Clinical Neuroscience, 19(6), pp.836-843.

Lacruz, C.R., de Santamaría, J.S. and Bardales, R.H., 2014. Central nervous system intraoperative cytopathology. New York, NY: Springer.

Lamszus, K., 2004. Meningioma pathology, genetics, and biology. Journal of Neuropathology & Experimental Neurology, 63(4), pp.275-286.

McLendon, R.E., 1998. Genetic syndromes asso-ciated with tumors and/or hamartomas. *Russel and Rubinstein's Pathology of Tumors of the Nervous System*, *2*, pp.371-417.

Menon, G., Nair, S., Sudhir, J., Rao, B. R. M., Mathew, A., & Bahuleyan, B. (2009). Childhood and adolescent meningiomas: a report of 38 cases and review of literature. *Acta neurochirurgica*, *151*(3), 239.

Mihaila, D., Jankowski, M., Gutiérrez, J.A., Rosenblum, M.L., Newsham, I.F., Bögler, O. and Rempel, S.A., 2003. Meningiomas: loss of heterozygosity on chromosome 10 and marker-specific correlations with grade, recurrence, and survival. *Clinical cancer research*, *9*(12), pp.4443-4451.

Maxwell, M., Shih, S.D., Galanopoulos, T., Hedley-Whyte, E.T. and Cosgrove, G.R., 1998. Familial meningioma: analysis of expression of neurofibromatosis 2 protein merlin: report of two cases. *Journal of neurosurgery*, *88*(3), pp.562-569.

Mendiola, M., Bello, M.J., Alonso, J., Leone, P.E., Vaquero, J., Sarasa, J.L., Kusak, M.E., De Campos, J.M., Pestaña, A. and Rey, J.A., 1999. Search for mutations of the hRAD54 gene in sporadic meningiomas with deletion at 1p32. *Molecular Carcinogenesis: Published in cooperation with the University of Texas MD Anderson Cancer Center*, *24*(4), pp.300-304.

Müller, P., Henn, W., Niedermayer, I., Ketter, R., Feiden, W., Steudel, W.I., Zang, K.D. and Steilen-Gimbel, H., 1999. Deletion of chromosome 1p and loss of expression of alkaline phosphatase indicate progression of meningiomas. *Clinical cancer research*, *5*(11), pp.3569-3577.

Menon, A.G., Rutter, J.L., von Sattel, J.P., Synder, H., Murdoch, C., Blumenfeld, A., Martuza, R.L., von Deimling, A., Gusella, J.F. and Houseal, T.W., 1997. Frequent loss of chromosome 14 in atypical and malignant meningioma: identification of a putativetumor progression'locus. *Oncogene*, *14*(5), p.611.

Maíllo, A., Orfao, A., Sayagués, J.M., Díaz, P., Gómez-Moreta, J.A., Caballero, M., Santamarta, D., Santos-Briz, A., Morales, F. and Tabernero, M.D., 2003. New classification scheme for the prognostic stratification of meningioma on the basis of chromosome 14 abnormalities, patient age, and tumor histopathology. *Journal of clinical oncology*, *21*(17), pp.3285-3295.

Mihaila, D., Gutiérrez, J.A., Rosenblum, M.L., Newsham, I.F., Bögler, O. and Rempel, S.A., 2003. Meningiomas: analysis of loss of heterozygosity on chromosome 10 in tumor progression and the delineation of four regions of chromosomal deletion in common with other cancers. *Clinical cancer research*, *9*(12), pp.4435-4442.

Matsuno, A., Nagashima, T., Matsuura, R., Tanaka, H., Hirakawa, M., Murakami, M., Tamura, A. and Kirino, T., 1996. Correlation between MIB-1 staining index and the immunoreactivity of p53 protein in recurrent and non-recurrent meningiomas. *American journal of clinical pathology*, *106*(6), pp.776-781.

McCutcheon, I.E., 1996. The biology of meningiomas. *Journal of Neuro-oncology*, *29*(3), pp.207-216.

Niedermayer, I., Feiden, W., Henn, W., Steilen-Gimbel, H., Steudel, W.I. and Zang, K.D., 1997. Loss of alkaline phosphatase activity in meningiomas: a rapid histochemical technique indicating progression-associated deletion of a putative tumor suppressor gene on the distal part of the short arm of chromosome 1. *Journal of Neuropathology & Experimental Neurology*, *56*(8), pp.879-886.

Nozaki, M., Tada, M., Kashiwazaki, H., Hamou, M.F., Diserens, A.C., Shinohe, Y., Sawamura, Y., Iwasaki, Y., de Tribolet, N. and Hegi, M.E., 2001. p73 is not mutated in meningiomas as determined with a functional yeast assay but p73 expression increases with tumor grade. *Brain Pathology*, *11*(3), pp.296-305.

Ohgaki, H., Eibl, R.H., Reichel, M.B., Mariani, L., Petersen, I., Höll, T., Wiestler, O.D., Kleihues, P., Schwab, M. and Gehring, M., 1993. Mutations of the p53 tumor suppressor gene in neoplasms of the human nervous system. *Molecular carcinogenesis*, *8*(2), pp.74-80.

Perry, A., Stafford, S.L., Scheithauer, B.W., Suman, V.J. and Lohse, C.M., 1997. Meningioma grading: an analysis of histologic parameters. *The American journal of surgical pathology*, *21*(12), pp.1455-1465.

Perry, A., Scheithauer, B.W., Stafford, S.L., Lohse, C.M. and Wollan, P.C., 1999. "Malignancy" in meningiomas: a clinicopathologic study of 116 patients, with grading implications. *Cancer: Interdisciplinary International Journal of the American Cancer Society*, *85*(9), pp.2046-2056.

Perry, A., Giannini, C., Raghavan, R., Scheithauer, B.W., Banerjee, R., Margraf, L., Bowers, D.C., Lytle, R.A., Newsham, I.F. and Gutmann, D.H., 2001. Aggressive phenotypic and genotypic features in pediatric and NF2-associated meningiomas: a clinicopathologic study of 53 cases. *Journal of Neuropathology & Experimental Neurology*, *60*(10), pp.994-1003.

Pelz, A.F., Klawunde, P., Skalej, M., Wieacker, P., Kirches, E., Schneider, T. and Mawrin, C., 2007. Novel chromosomal aberrations in a recurrent malignant meningioma. *Cancer genetics and cytogenetics*, *174*(1), pp.48-53.

Peyrard, M., Pan, H.Q., Kedra, D., Fransson, I., Swahn, S., Hartman, K., Clifton, S.W., Roe, B.A. and Dumanski, J.P., 1996. Structure of the Promoter and Genomic Organization of the Human β′-Adaptin Gene (BAM22) from Chromosome 22q12. *Genomics*, *36*(1), pp.112-117.

Pulst, S.M., Rouleau, G.A., Marineau, C., Fain, P. and Sieb, J.P., 1993. Familial meningioma is not allelic to neurofibromatosis 2. *Neurology*, *43*(10), pp.2096-2096.

Peyrard, M., Seroussi, E., Sandberg-Nordqvist, A.C., Xie, Y.G., Han, F.Y., Fransson, I., Collins, J., Dunham, I., Kost-Alimova, M., Imreh, S. and Dumanski, J.P., 1999. The human LARGE gene from 22q12. 3-q13. 1 is a new, distinct member of the glycosyltransferase gene family. *Proceedings of the National Academy of Sciences*, *96*(2), pp.598-603.

Perry, A., Cai, D.X., Scheithauer, B.W., Swanson, P.E., Lohse, C.M., Newsham, I.F., Weaver, A. and Gutmann, D.H., 2000. Merlin, DAL-1, and progesterone receptor expression in clinicopathologic subsets of meningioma: a correlative immunohistochemical study of 175 cases. *Journal of Neuropathology & Experimental Neurology*, *59*(10), pp.872-879.

Perry, A., Stafford, S.L., Scheithauer, B.W., Suman, V.J. and Lohse, C.M., 1998. The prognostic significance of MIB-1, p53, and DNA flow cytometry in completely resected primary meningiomas. *Cancer: Interdisciplinary International Journal of the American Cancer Society*, *82*(11), pp.2262-2269.

Perry A, Louis DN, Scheithauer BW, Budka H, Deimling VA. Meningiomas. In: Louis DN, Ohgaki H, Wiestler OD, Cavenee WK, editors. World Health Organization classification of the central nervous system. 2007. p. 164-72.

Perry A. Meningiomas. In: McLendon RE, Rosenblum MK, Bigner DD, editors. Russell & Rubinstein's Pathology of Tumors of the Nervous System. Seventh. Oxford University Press Inc. 2006; pp: 427-474.

Provias, J., Claffey, K., delAguila, L., Lau, N., Feldkamp, M., Guha, A. and Guha, A., 1997. Meningiomas: role of vascular endothelial growth factor/vascular permeability factor in angiogenesis and peritumoral edema. *Neurosurgery*, *40*(5), pp.1016-1026.

Paz-y-Miño, C., López-Cortés, A., Muñoz, M.J., Castro, B., Cabrera, A. and Sánchez, M.E., 2010. Relationship of an hRAD54 gene polymorphism (2290 C/T) in an Ecuadorian population with chronic myelogenous leukemia. Genetics and molecular biology, 33(4), pp.646-649.

Ruttledge, M.H., Sarrazin, J., Rangaratnam, S., Phelan, C.M., Twist, E., Merel, P., Delattre, O., Thomas, G., Nordenskjöld, M., Collins, V.P. and Dumanski, J.P., 1994. Evidence for the complete inactivation of the NF2 gene in the majority of sporadic meningiomas. *Nature genetics*, *6*(2), p.180.

Ruttledge, M.H., Xie, Y.G., Han, F.Y., Peyrard, M., Collins, V.P., Nordenskjöld, M. and Dumanski, J.P., 1994. Deletions on chromosome 22 in sporadic meningioma. *Genes, Chromosomes and Cancer*, *10*(2), pp.122-130.

Rempel, S.A., Schwechheimer, K., Davis, R.L., Cavenee, W.K. and Rosenblum, M.L., 1993. Loss of heterozygosity for loci on chromosome 10 is associated with morphologically malignant meningioma progression. *Cancer research*, *53*(10), pp.2386-2392.

Rajcan-Separovic, E., Maguire, J., Loukianova, T., Nisha, M. and Kalousek, D., 2003. Loss of 1p and 7p in radiation-induced meningiomas identified by comparative genomic hybridization. *Cancer genetics and cytogenetics*, *144*(1), pp.6-11.

Ragel, B. and Jensen, R.L., 2003. New approaches for the treatment of refractory meningiomas. *Cancer Control*, *10*(2), pp.148-158.

Reuss, D.E., Piro, R.M., Jones, D.T., Simon, M., Ketter, R., Kool, M., Becker, A., Sahm, F., Pusch, S., Meyer, J. and Hagenlocher, C., 2013. Secretory meningiomas are defined by combined KLF4 K409Q and TRAF7 mutations. Acta neuropathologica, 125(3), pp.351-358.

Rienstein, S., Loven, D., Israeli, O., Ram, Z., Rappaport, Z.H., Barkai, G., Goldman, B., Aviram-Goldring, A. and Friedman, E., 2001. Comparative genomic hybridization analysis of radiation-associated and sporadic meningiomas. Cancer genetics and cytogenetics, 131(2), pp.135-140.

Sadetzki, S., Modan, B., Chetrit, A., & Freedman, L. (2000). An latrogenic Epidemic of Benign Meningioma. *American journal of epidemiology*, *151*(3), 266-272. (Sadetzki et al, 2000).

Simpson, D., 1957. The recurrence of intracranial meningiomas after surgical treatment. *Journal of neurology, neurosurgery, and psychiatry*, *20*(1), p.22.

Seizinger, B.R., De La Monte, S., Atkins, L., Gusella, J.F. and Martuza, R.L., 1987. Molecular genetic approach to human meningioma: loss of genes on chromosome 22. *Proceedings of the National Academy of Sciences*, *84*(15), pp.5419-5423.

Stangl, A.P., Wellenreuther, R., Lenartz, D., Kraus, J.A., Menon, A.G., Schramm, J., Wiestler, O.D. and von Deimling, A., 1997. Clonality of multiple meningiomas. *Journal of neurosurgery*, *86*(5), pp.853-858.

Santarius, T., Kirsch, M., Nikas, D.C., Imitola, J. and Black, P.M., 2000. Molecular analysis of alterations of the p18INK4c gene in human meningiomas. *Neuropathology and applied neurobiology*, *26*(1), pp.67-75.

Sulman, E.P., Dumanski, J.P., White, P.S., Zhao, H., Maris, J.M., Mathiesen, T., Bruder, C., Cnaan, A. and Brodeur, G.M., 1998. Identification of a consistent region of allelic loss on 1p32 in meningiomas: correlation with increased morbidity. *Cancer research*, *58*(15), pp.3226-3230.

Simon, M., Park, T.W., Köster, G., Mahlberg, R., Hackenbroch, M., Boström, J., Löning, T. and Schramm, J., 2001. Alterations of INK4a p16—p14ARF/INK4b p15 Expression and Telomerase Activation in Meningioma Progression. *Journal of neuro-oncology*, *55*(3), pp.149-158.

Sayagués, J.M., Tabernero, M.D., Maillo, A., Díaz, P., Rasillo, A., Bortoluci, A., Gomez-Moreta, J., Santos-Briz, A., Morales, F. and Orfao, A., 2002. Incidence of numerical chromosome aberrations in meningioma tumors as revealed by fluorescence in situ hybridization using 10 chromosome-specific probes. *Cytometry: The Journal of the International Society for Analytical Cytology*, *50*(3), pp.153-159.

Sato, K., Schäuble, B., Kleihues, P. and Ohgaki, H., 1996. Infrequent alterations of the p15, p16, CDK4 and CYCLIN D1 genes in non-astrocytic human brain tumors. *International journal of cancer*, *66*(3), pp.305-308.

Shoshan, Y., Chernova, O., Jeun, S.S., Somerville, R.P., Israel, Z., Barnett, G.H. and Cowell, J.K., 2000. Radiation-induced meningioma: a distinct molecular genetic pattern?. *Journal of Neuropathology & Experimental Neurology*, *59*(7), pp.614-620.

Simon, M., Park, T.W., Leuenroth, S., Hans, V.H., Löning, T. and Schramm, J., 2000. Telomerase activity and expression of the telomerase catalytic subunit, hTERT, in meningioma progression. *Journal of neurosurgery*, *92*(5), pp.832-840.

Samoto, K., Ikezaki, K., Ono, M., Shono, T., Kohno, K., Kuwano, M. and Fukui, M., 1995. Expression of vascular endothelial growth factor and its possible relation with neovascularization in human brain tumors. *Cancer research*, *55*(5), pp.1189-1193.

Schmitz, U., Mueller, W., Weber, M., Sevenet, N., Delattre, O. and von Deimling, A., 2001. INI1 mutations in meningiomas at a potential hotspot in exon 9. *British Journal of Cancer*, *84*(2), p.199.

Simon, M., von Deimling, A., Larson, J.J., Wellenreuther, R., Kaskel, P., Waha, A., Warnick, R.E., Tew, J.M. and Menon, A.G., 1995. Allelic losses on chromosomes 14, 10, and 1 in atypical and malignant meningiomas: a genetic model of meningioma progression. *Cancer research*, *55*(20), pp.4696-4701.

Sanson, M. and Cornu, P., 2000. Biology of meningiomas. *Acta neurochirurgica*, *142*(5), pp.493-505.

Sirachainan, N., Wongruangsri, S., Kajanachumpol, S., Pakakasama, S., Visudtibhan, A., Nuchprayoon, I., Lusawat, A., Phudhicharoenrat, S., Shuangshoti, S. and Hongeng, S., 2008. Folate pathway genetic polymorphisms and susceptibility of central nervous system tumors in Thai children. Cancer detection and prevention, 32(1), pp.72-78.

Sun, S.Q., Hawasli, A.H., Huang, J., Chicoine, M.R. and Kim, A.H., 2015. An evidence-based treatment algorithm for the management of WHO Grade II and III meningiomas. Neurosurgical focus, 38(3), p.E3.

Turgut, M., 2018. Tumour stem cells in meningioma: A review. Journal of clinical neuroscience: official journal of the Neurosurgical Society of Australasia, 53, pp.280-281.

Ueki, K., Wen-Bin, C., Narita, Y., Asai, A. and Kirino, T., 1999. Tight association of loss of merlin expression with loss of heterozygosity at chromosome 22q in sporadic meningiomas. *Cancer research*, *59*(23), pp.5995-5998.

Vernooij, M. W., Ikram, M. A., Tanghe, H. L., Vincent, A. J., Hofman, A., Krestin, G. P., ... & van der Lugt, A. (2007). Incidental findings on brain MRI in the general population. *New England Journal of Medicine*, *357*(18), 1821-1828.

Von Deimling, A., Kraus, J.A., Stangl, A.P., Wellenreuther, R., Lenartz, D., Schramm, J., Louis, D.N., Ramesh, V., Gusella, J.F. and Wiestler, O.D., 1995. Evidence for subarachnoid spread in the development of multiple meningiomas. *Brain Pathology*, *5*(1), pp.11-14.

Von Deimling, A., Larson, J., Wellenreuther, R., Stangl, A.P., van Velthoven, V., Warnick, R., Tew Jr, J., Balko, G. and Menon, A.G., 1999. Clonal origin of recurrent meningiomas. *Brain pathology*, *9*(4), pp.645-650.

Von Deimling, A., Fimmers, R., Schmidt, M.C., Bender, B., Fassbender, F., Nagel, J., Jahnke, R., Kaskel, P., Duerr, E.M., Koopmann, J. and Maintz, D., 2000. Comprehensive allelotype and genetic analysis of 466 human nervous system tumors. *Journal of Neuropathology & Experimental Neurology*, *59*(6), pp.544-558.

Wiemels, J., Wrensch, M. and Claus, E.B., 2010. Epidemiology and etiology of meningioma. *Journal of neuro-oncology*, *99*(3), pp.307-314.

Weber, R.G., Boström, J., Wolter, M., Baudis, M., Collins, V.P., Reifenberger, G. and Lichter, P., 1997. Analysis of genomic alterations in benign, atypical, and anaplastic meningiomas: toward a genetic model of meningioma progression. *Proceedings of the National Academy of Sciences*, *94*(26), pp.14719-14724.

Wellenreuther, R., Kraus, J.A., Lenartz, D., Menon, A.G., Schramm, J., Louis, D.N., Ramesh, V., Gusella, J.F., Wiestler, O.D. and von Deimling, A., 1995. Analysis of the neurofibromatosis 2 gene reveals molecular variants of meningioma. *The American journal of pathology*, *146*(4), p.827.

Watson, M.A., Gutmann, D.H., Peterson, K., Chicoine, M.R., Kleinschmidt-DeMasters, B.K., Brown, H.G. and Perry, A., 2002. Molecular characterization of human meningiomas by gene expression profiling using high-density oligonucleotide microarrays. *The American journal of pathology*, *161*(2), pp.665-672.

Wang, J.L., Zhang, Z.J., Hartman, M., Smits, A., Westermark, B., Muhr, C. and Nistér, M., 1995. Detection of TP53 gene mutation in human meningiomas: A study using immunohistochemistry, polymerase chain reaction/single-strand conformation polymorphism and dna sequencing techniques on paraffin-embedded samples. *International journal of cancer*, *64*(4), pp.223-228.

Yamasaki, F., Yoshioka, H., Hama, S., Sugiyama, K., Arita, K. and Kurisu, K., 2000. Recurrence of meningiomas: influence of vascular endothelial growth factor expression. *Cancer: Interdisciplinary International Journal of the American Cancer Society*, *89*(5), pp.1102-1110.

Yuzawa, S., Nishihara, H. and Tanaka, S., 2016. Genetic landscape of meningioma. *Brain tumor pathology*, *33*(4), pp.237-247.

Zankl, H. and Zang, K.D., 1972. Cytological and cytogenetical studies on brain tumors. *Humangenetik*, *14*(2), pp.167-169.

Zucman-Rossi, J., Legoix, P. and Thomas, G., 1996. Identification of new members of the Gas2 and Ras families in the 22q12 chromosome region. *Genomics*, *38*(3), pp.247-254.

Zang, K.D., 2001. Meningioma: a cytogenetic model of a complex benign human tumor, including data on 394 karyotyped cases. *Cytogenetic and Genome Research*, *93*(3-4), pp.207-220.

Zhang, J., Zhou, Y.W., Shi, H.P., Wang, Y.Z., Li, G.L., Yu, H.T. and Xie, X.Y., 2013. 5, 10-Methylenetetrahydrofolate reductase (MTHFR), methionine synthase (MTRR), and methionine synthase reductase (MTR) gene polymorphisms and adult meningioma risk. Journal of neuro-oncology, 115(2), pp.233-239.

Other Publications

Berg E, 2017, MTHFR explained in simple terms), [online], Dr. Eric Berg's DC Advanced Evaluation, https://www.youtube.com/watch?v=L76PaoGaPx0 [accessed 12 th September, 2019].

Cara G and Teresa E, 2015, MTHFR, Folate, and The Autism Gene [online], Health, Home, and Happiness, (www.healthhomeandhappiness.com/folate-vs-folic-acid-mthfr-and-why-i-regret-taking-my-prenatal-vitamin.html), [Accessed 12th September, 2019].

Kresak J, 2014, Anaplastic meningioma, [online], PathologyOutlines. http://www.pathologyoutlines.com/topic/cnstumoranaplasticmeningioma.html [accessed 24th November, 2019]

Hinsdale, IL, 2010, CBTRUS statistical report: primary brain and central nervous system tumors diagnosed in the United States in 2004-2006, [online], Central Brain Tumor Registry of the United States. *Central Brain Tumor Registry of the United States*.

Katrin Lamszus, 2007, Christian Hagel, Manfred Westphal. "Meningioma", [online] Elsevier BV www.clinmedjournals.org [Accessed 19th September, 2019]