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Article: **Analysis of Functional Polymorphisms in Meningioma-associated Genes**

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Analysis of Functional Polymorphisms in Meningioma-Associated Genes

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Abstract

Meningioma is the most common benign intracranial tumour that develops in the meningeal protective covering of the central nervous system (CNS). Globally, every nine individuals out of 100,000 are diagnosed with this cancer. Basic risk factors of meningioma comprise ionizing radiation, hormonal imbalance, and genetic aberrations. In this study, various bioinformatics tools, specialized for consensus-based identification, sequence-homology, and supervised learning, were employed to analyze and screen the deleterious mutational landscape of commonly associated genes of meningioma/genes commonly associated with meningioma. This study employed an in-silico approach aimed to utilize thirteen different tools to benchmark pathogenic single nucleotide polymorphisms (SNPs) in SMARCB1, AKT1, SMO, SUFU, NF2 and MTHFR genes related to meningioma. We identified six highly pathogenic SNPs related to meningioma: SMARCB1 (rs387906812, rs387906811, rs267607072), AKT1 (rs121434592), SMO (rs121918347), and SUFU (rs202247756). Additionally, several deleterious missense variants of NF2 and MTHFR genes were also identified. Hence, this study is a gateway for research on SNPs since they can be utilized to conduct a type-based diagnosis of meningioma for its early prognosis. They can also be utilized as genomic targets for a targeted therapy by developing inhibitors against mutated proteins. For this purpose, further wet-lab experiments and genome-wide association studies are required to genotype these SNPs in a large number of samples, collected from different populations belonging to various ethnicities, for the development of SNP(s) gene panels.

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Keywords: biomarker, consensus-based, meningioma, sequence-homology, single nucleotide polymorphisms (SNPs), supervised learning

Introduction

Meningioma is a primary intracranial tumour that originates from the arachnoid cap cells located in the meningeal layers of the central nervous system (CNS) [1, 2]. At times, it can also develop on some parts of the spinal cord. It is the most common primary brain tumour, accounting for ~30% of all primary CNS tumours in adults; it rarely develops in children [3, 4]. Meningioma is categorized as a non-glioma tumour that can be either benign (non-cancerous as 90%) or malignant; it has a high prevalence rate in females as compared to males [5], with a sex ratio of 2-4:1 [6, 7]. Additionally, females are 2.8 times more at risk of developing meningioma than men [8]. Globally, the annual age-standardized incidence ratio (ASIR) for meningioma is approximately 8.83/100,000 [9, 10]. In Pakistan, the incidence rate of CNS tumours is around 2.03% and the ratio between males to females is 2.3:1.3; whereas, in paediatrics, the incidence rate is 15% [5]. The 5-year survival rate for malignant and non-malignant meningiomas is 97-87.3% and 85-50.2%, respectively [11].

Meningioma is prevalent in individuals between 20-40 years of age; however, it is even more prevalent in individuals between 60-65 years of age [12, 13] This shows that its prevalence is positively related to age [9]. World Health Organization (WHO) defines 15 subtypes of meningiomas and divides them into 3 grades of malignancy based on various histopathological characteristics. The most common type of meningioma accounts for grade 1 (80%), atypical grade 2 (18%), and anaplastic grade 3 (2%), [9, 14, 15]. When left unattended, it grows slowly with poor prognosis. Complications of meningioma include neurological symptoms such as severe headache, tinnitus, epilepsy, sensory deficits such as blurred vision and speech problems, increased intracranial pressure, weakness, seizures, and numbness [12, 16]. Conventional approaches for diagnosing, locating, and measuring meningeal tumours include MRI (magnetic resonance imaging) and CT (computerized tomography) scans. [16, 17]. Surgical removal and radiotherapy are normally used to treat this type of tumour; however, its recurrence occurs in 20% of the cases. The recurrence can be treated with repeated therapies and other systemic approaches such

as molecular personalized targeted therapy [18, 19]. Hence, due to the inconsistencies in the prognostic relevance of the WHO grade, the demand for more accurate and reliable biomarkers for the diagnosis of low-grade meningioma has increased [15, 20]. NGS (next-generation sequencing), WGS (whole genome sequencing), WES (whole exome sequencing), DNA methylation assays, and transcriptome analysis are examples of new molecular techniques with better characterization. They can retrieve relatively more reliable prognostic biomarkers as well as potent druggable targets. [21-23]. The exact causes behind meningioma are not yet known; however, radiation exposure, genetic disorders (Neurofibromatosis type2), injuries in the skull and surrounding membranes, and hormonal imbalance (progesterone) are some of the risk factors [24-26]. Single nucleotide polymorphisms (SNPs) are important molecular markers that occur abundantly throughout the genome; they are sufficient for disrupting a protein's structural and functional viability [27]. Based on the above-mentioned mutational landscape, meningiomas can be either *NF2*-mutated or non-*NF2*-mutated [28].

Prior studies reported that individuals having loss of q arm of chromosome 22 suffer from meningioma. *NF2* gene, located on 22q12.2, acts as a tumour suppressor and is related to Neurofibromatosis type 2 (NF2) which is a tumour predisposition syndrome. Due to the inactivation of the NF2 gene, about 50% of all *NF2* patients develop more than one type of meningioma [29, 30]. *NF2* gene produces a merlin protein that links actin filaments, transmembrane proteins, and signalling molecules to regulate various cell signalling pathways. The inactivation of this gene due to nonsense, missense, and frameshift mutations affect the structure and function of the protein [28, 31]. In the case of non-NF2 mutated meningioma, mutations in other genes, such as *SMO, TRAF7, KLF4, POLR2A, AKT1, KLF4,* and *PIK3CA,* are involved, this is true especially in the case of grade 1 meningioma [32, 33]. Missense mutations in *SMARCB1, AKT1, SMO, SUFU, KLF4, TRAF7, MTHFR, MTRR, BRIP1, AKAP9, MAP9, TET2, SLX4, PRDM2, DDX43, MYH9, SRSF2,* and *UNC13C* are also associated with meningioma [34, 35].

Hence, this *in-silico* study aimed to identify an association between meningioma and the most commonly reported genes, namely *NF2, AKT1,*

SMO, SUFU, SMARCB1, and *MTHFR,* via SNP analysis based on various *in-silico* methods. For this purpose, various bioinformatics tools were used to report the effect of missense and deleterious SNPs on the structure and function of proteins.

Methodology

Protein Sequence Retrieval and 3D Model Retrieval

The protein sequence of *NF2, MTHFR, SMARCB1, AKT1, SMO,* and *SUFU* gene (s) were retrieved from NCBI (https://www.ncbi.nlm.nih.gov/) in FASTA format [36] for future inputs in various SNP databases; whereas, protein 3D models were retrieved from the PDB database (https://www.rcsb.org/) [37] for future inputs in various SNP databases.

Missense SNPs Retrieval

The SNPs reported in *NF2* and *MTHFR* were retrieved from Ensembl Human (GRCh38.p13) database (https://www.ensembl.org/Homo_sapiens/Info/Annotation?redirect=no) [38]. It is a genome browser dealing with vertebrate genomes, it supports research in comparative genomics, evolution, sequence variation and transcriptional regulation. Ensembl annotates genes, computes multiple alignments, predicts regulatory function, and collects disease data. The SNPs of *SUFO*, *SMARCB1*, *SMO*, and *AKT1* were obtained from a literature survey of the reported articles.

Identification of Deleterious Missense SNPs

Deleterious missense mutations were identified using three methods.

Sequence Homology Based Method

In this method, three online tools, namely SIFT [39], Mutation Assessor [40], and PROVEAN [41], were used to screen deleterious SNPs by giving protein sequences as input. These homology-based approaches use sequence similarities between known and unknown proteins to transfer a function to an unknown protein.

Supervised Learning Method

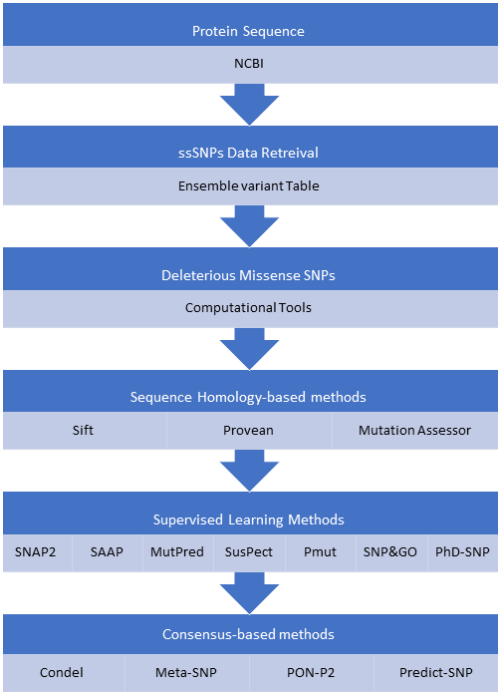
This method is based on three types of algorithms: random forests [42], neural networks [43], and support vector machines [44]. It was used to identify the amino acid changes that were deleterious. When given never-

before-seen data, supervised learning allows a model to recognize the underlying patterns and relationships between the input data and the output labels, allowing it to produce accurate labelling results. For this purpose, seven online tools were used: SNAP2 [45], SAAP [46], SusPect [47], SNP & GO [48], Pmut [49], MutPred2 [50], and PhD-SNP [51].

Consensus-based Method

Three online web tools, namely Condel [52], Predict-SNP [53], and Meta-SNP [54,] were used to further analyze the most deleterious missense SNPs. These tools work in an integrated manner and transfer the results from one tool to the next. Consensus-based prediction combines different strengths and weaknesses of several predictors. After the combination, a prediction is made based on a meta-level. This meta-result, representing the consensus of all methods, is potentially more reliable than a single prediction. Hence, Hence, the results were a combined consensus score obtained from all the applied tools. The methodology has been summarized in Figure 1.

Figure 1. Chart Representation of the Methodology Adopted

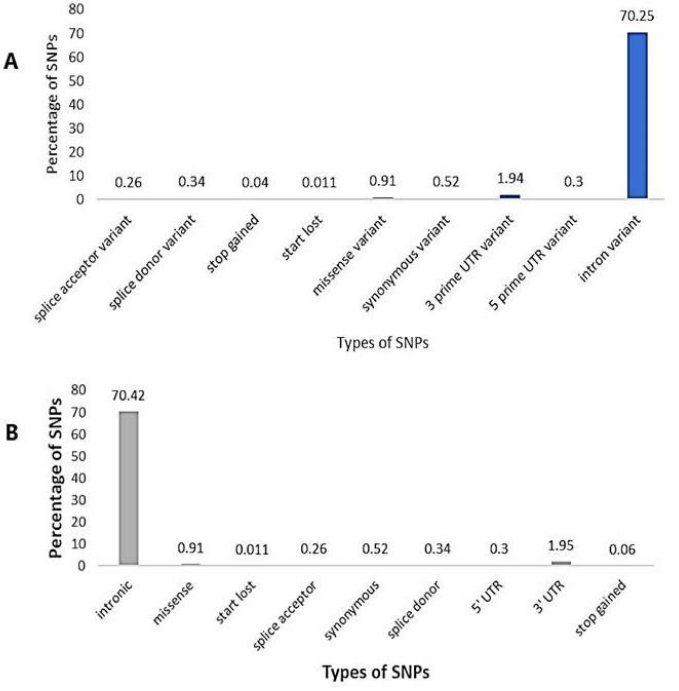


Results

Missense SNPs Retrieval

The variation table (s) comprising missense SNPs in *NF2* (Ensemble ID ENSG00000186575) and *MTHFR* (Ensemble ID ENSG00000177000) genes were retrieved from the Ensembl database. The percentage division of different types of all the reported SNPs in *NF2* and *MTHFR* are graphically represented in Figure 2. While, missense SNPs in *AKTI*, *SMO*, *SUFU*, and *SMARCB1* genes, associated with meningioma, were retrieved from a literature survey. Only 694 missense variants in the *NF2* gene and 347 missense variants in the *MTHFR* gene were considered for further screening of deleterious SNPs through other tools.

Figure 2. Graphical representation of the Percentages of different types of SNPs retrieved from ENSEMBL database; A: percentage of different types of SNPs in *MTHFR* gene; B: percentage of different types of SNPs in *NF2* gene; where the percentage of SNPs are represented on Y-axis and types of SNPs are represented on X-axis



Sequence Homology-based Results

PROVEAN, SIFT, and Mutation Assessor web servers were used to predict the effect of mutation on phenotypic properties of proteins. These computational tools classify mutation into two categories; deleterious or normal. In the case of PROVEAN, a score >2.5 is considered normal, while a score ≤2.5 is considered deleterious [55]. On the other hand, after searching for homologous sequences for the given protein sequence, SIFT tool differentiates/categorizes polymorphism as 'deleterious' if the negative score value ranges between 0 – 0.5 and as neutral or 'tolerated' if the positive value is >0.05 – 1.0 [56]. The Mutation Assessor tool was utilized to find conserved amino acids in the protein's families and subfamilies. For this purpose, it employed entropy measurements on a combination basis and provided a FIS (functional impact score) of/for dealing with variations in amino acids [40]. A bar chart representation is shown in Figure 3, it depicts the number of SNPs predicted to be deleterious for each selected gene(s) via the tools used in this method.

The obtained results, for the SNPs shown to be deleterious in all these tools, are enlisted/illustrated in supplementary Table 1-3. It was determined that since SNPs in *NF2, MHFR, AKTI, SMO, SUFU,* and *SMARCB1* genes are deleterious, they may be linked to meningioma.

Table 1. S1. Sequence Homology-based Predictions of SNPs in *NF2* Gene, using SIFT, PROVEAN, and Mutation Assessor Webservers

Sr. No.	Variant ID	Mutations	SIFT score	SIFT prediction	PROVEAN score	PROVEAN prediction	M.Assessor score	M.Assessor prediction
1	rs1064795612	I26T	0.001	Damaging	-3.07	Deleterious	2.36	Disease
2	rs563168478	D30G	0.001	Damaging	-6.49	Deleterious	2.75	Disease
3	rs764901064	R52W	0	Damaging	-6.58	Deleterious	3.27	Disease
4	rs762440698	L54R	0	Damaging	-4.84	Deleterious	3.415	Disease
5	rs764089682	G55R	0	Damaging	-7.52	Deleterious	3.16	Disease
6	rs1383283673	W60R	0	Damaging	-13.16	Deleterious	3.025	Disease
7	rs780872661	W60C	0	Damaging	-12.22	Deleterious	3.225	Disease
8	rs1286915234	F61S	0	Damaging	-5.81	Deleterious	3.11	Disease
9	rs121434261	F62S	0	Damaging	-7.52	Deleterious	4.285	Disease
10	rs772274240	Y66N	0	Damaging	-7.19	Deleterious	3.575	Disease
11	rs1060503668	A98D	0	Damaging	-5.21	Deleterious	3.33	Disease
12	rs1555987677	F100L	0.023	Damaging	-5.4	Deleterious	3.62	Disease
13	rs1240469044	Y101C	0.001	Damaging	-7.39	Deleterious	4.085	Disease
14	rs781593146	E112K	0.006	Damaging	-3.63	Deleterious	3.175	Disease
15	rs1328289194	I113F	0.001	Damaging	-3.79	Deleterious	2.685	Disease

Sr. No.	Variant ID	Mutations	SIFT score	SIFT prediction	PROVEAN score	PROVEAN prediction	M.Assessor score	M.Assessor prediction
16	rs1569281810	L117V	0.002	Damaging	-2.77	Deleterious	4.565	Disease
17	rs1006294051	Q121E	0.035	Damaging	-2.85	Deleterious	4.515	Disease
18	rs878853927	I131F	0.001	Damaging	-3.49	Deleterious	3.565	Disease
19	rs759344163	C133S	0.003	Damaging	-9.44	Deleterious	4.485	Disease
20	rs1029716358	P134L	0.001	Damaging	-8.28	Deleterious	3.355	Disease
21	rs1388544922	A142V	0.001	Damaging	-3.86	Deleterious	3.305	Disease
22	rs1374299963	Y153H	0.01	Damaging	-3.66	Deleterious	2.31	Disease
23	rs150667239	R160W	0.014	Damaging	-2.57	Deleterious	1.28	Disease
24	rs1303879665	G161R	0.001	Damaging	-7.45	Deleterious	3.465	Disease
25	rs1232015629	L163F	0.004	Damaging	-3.66	Deleterious	2.905	Disease
26	rs779353677	E166V	0.001	Damaging	-5.53	Deleterious	3.415	Disease
27	rs866605024	V173A	0	Damaging	-3.92	Deleterious	3.485	Disease
28	rs1555993319	Y192C	0.003	Damaging	-8.38	Deleterious	3.57	Disease
29	rs749176138	R196Q	0.005	Damaging	-2.55	Deleterious	2.425	Disease
30	rs1487106309	R200G	0.001	Damaging	-6.37	Deleterious	3.135	Disease
31	rs141629512	M205V	0.003	Damaging	-3.49	Deleterious	1.13	Disease
32	rs776818377	G218S	0	Damaging	-5.89	Deleterious	4.555	Disease
33	9rs776818377	G218C	0	Damaging	-8.82	Deleterious	4.555	Disease
34	rs1555994816	V219M	0.035	Damaging	-2.85	Deleterious	3.03	Disease
35	rs746025177	Y221C	0	Damaging	-8.24	Deleterious	4.18	Disease
36	rs1298953582	I224M	0	Damaging	-2.85	Deleterious	3.27	Disease
37	rs1386029079	R225W	0.001	Damaging	-3.95	Deleterious	1.28	Disease
38	rs886057336	N226H	0.001	Damaging	-4.74	Deleterious	3.025	Disease
39	rs1028670573	G229C	0.001	Damaging	-4.99	Deleterious	2.485	Disease
40	rs761195572	A238V	0.001	Damaging	-3.67	Deleterious	3.005	Disease
41	rs1432132718	L250Q	0	Damaging	-5.28	Deleterious	2.785	Disease
42	rs1474769404	F256L	0.001	Damaging	-5.79	Deleterious	3.085	Disease
43	rs753300935	P257R	0.003	Damaging	-6.09	Deleterious	2.73	Disease
44	rs753300935	P257L	0.005	Damaging	-7.34	Deleterious	2.385	Disease
45	rs1555997533	F271C	0	Damaging	-7.13	Deleterious	2.89	Disease
46	rs549225513	L276P	0.001	Damaging	-3.82	Deleterious	1.935	Disease
47	rs754093587	R291C	0	Damaging	-6.79	Deleterious	2.675	Disease
48	rs867836772	C300R	0	Damaging	-11.5	Deleterious	2.87	Disease
49	rs1255367068	G302R	0.013	Damaging	-7.62	Deleterious	2.25	Disease
50	rs1555998800	H304R	0	Damaging	-7.72	Deleterious	2.91	Disease
51	rs200272173	H304Q	0	Damaging	-7.72	Deleterious	2.56	Disease
52	rs1462083103	M308T	0.001	Damaging	-5.49	Deleterious	2.74	Disease
53	rs746175548	K322N	0	Damaging	-4.82	Deleterious	3.48	Disease
54	rs1569302357	R326G	0.001	Damaging	-5.25	Deleterious	2.86	Disease
55	rs200372028	E328V	0	Damaging	-6.52	Deleterious	3.1	Disease
56	rs1305519234	E335G	0	Damaging	-6.14	Deleterious	3.41	Disease
57	rs140266312	R336W	0	Damaging	-6.76	Deleterious	3.31	Disease
58	rs778412102	E350K	0.002	Damaging	-3.3	Deleterious	2.94	Disease
59	rs1465752977	R359G	0.001	Damaging	-5.39	Deleterious	2.65	Disease
60	rs74315492	L360P	0	Damaging	-5.68	Deleterious	3.045	Disease
61	rs1280154812	L374R	0.001	Damaging	-4.97	Deleterious	3.07	Disease

Sr. No.	Variant ID	Mutations	SIFT score	SIFT prediction	PROVEAN score	PROVEAN prediction	M.Assessor score	M.Assessor prediction
62	rs867367858	R376W	0	Damaging	-6.1	Deleterious	2.94	Disease
63	rs1457638896	L458R	0.024	Damaging	-5.27	Deleterious	3.04	Disease
64	rs1556002568	L517P	0.001	Damaging	-4.31	Deleterious	2.205	Disease
65	rs74315493	L535P	0.001	Damaging	-5.27	Deleterious	2.835	Disease
66	rs74315494	Q538P	0.001	Damaging	-5.25	Deleterious	2.97	Disease
67	rs917012886	D559Y	0.008	Damaging	-6.78	Deleterious	2.39	Disease
68	rs878853926	H562L	0.001	Damaging	-6.26	Deleterious	2.8	Disease

Only those SNPs have been enlisted which are commonly predicted deleterious in all webservers

Figure 3: Bar Chart Representation of the Sequence Homology-based Prediction of Snps in Different Genes Associated with Meningioma; A: Represents Snps Count for NF2 Gene (I.E., Out of 346 Missense Snps, 186 In SIFT, 171 In PROVEAN, and 196 in Meta Assessor Predicted were Damaging); B: Represents Snps Count for MTHFR Gene (I.E., Out of 500 Missense Snps, 252 in SIFT, 232 in PROVEAN, 307 in Meta Assessor were Predicted Damaging); C: Represents Snps Count for *SMARCB1*, *AKT1*, *SMO*, *SUFU* Genes (I.E., Out of Total 12 Missense Snps, 10 in SIFT, 9 in PROVEAN, and 9 in Meta Assessor were Predicted Damaging)

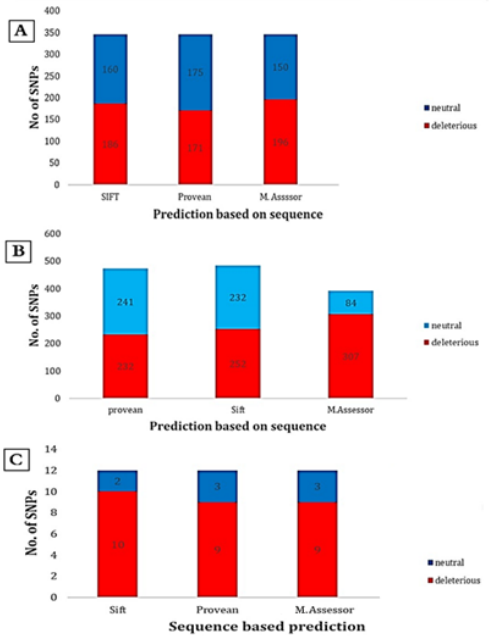


Table 2. S2: Sequence Homology-based Prediction of SNPs in *MTHFR* Gene, using SIFT, PROVEAN, and Mutation Assessor Webservers

Sr. No.	Variant ID	Mutations	SIFT Score	SIFT prediction	PROVEAN score	PROVEAN prediction	M.Assessor score	M.Assessor prediction
1	rs774531580	E63K	0	Damaging	-3.58	Deleterious	5.015	Diseases
2	rs796064512	P66L	0.002	Damaging	-8.95	Deleterious	4.99	Diseases
3	rs763539350	R68G	0.002	Damaging	-5.07	Deleterious	3.86	Diseases
4	rs770151367	T69A	0.004	Damaging	-4.17	Deleterious	3.74	Diseases
5	rs746177570	T69I	0.003	Damaging	-5.05	Deleterious	4.37	Diseases
6	rs786204009	R82W	0	Damaging	-7.38	Deleterious	4.52	Diseases
7	rs1426036757	M83T	0.001	Damaging	-4.96	Deleterious	4.125	Diseases
8	rs147257424	A113P	0.004	Damaging	-4.01	Deleterious	4.535	Diseases
9	rs769381688	H127Y	0	Damaging	-5.88	Deleterious	4.095	Diseases
10	rs786204012	C130R	0	Damaging	-11.37	Deleterious	4.97	Diseases
11	rs761230732	L142P	0	Damaging	-6.79	Deleterious	4.825	Diseases
12	rs761230732	L142R	0	Damaging	-5.85	Deleterious	4.825	Diseases
13	rs1018189670	G149D	0	Damaging	-6.87	Deleterious	3.725	Diseases
14	rs776195746	R157W	0	Damaging	-7.85	Deleterious	5.045	Diseases
15	rs121434295	R157Q	0	Damaging	-3.93	Deleterious	4.7	Diseases
16	rs772932189	G158E	0.001	Damaging	-7.35	Deleterious	5.05	Diseases
17	rs138524217	D159N	0	Damaging	-4.68	Deleterious	5.03	Diseases
18	rs1182635980	A175T	0.002	Damaging	-3.48	Deleterious	3.645	Diseases
19	rs1172158928	D177H	0.001	Damaging	-6.72	Deleterious	4.225	Diseases
20	rs1285236808	V194M	0.002	Damaging	-2.59	Deleterious	4.295	Diseases
21	rs760161369	A195V	0.006	Damaging	-3.45	Deleterious	4.965	Diseases
22	rs786204014	G196D	0.001	Damaging	-6.86	Deleterious	3.74	Diseases
23	rs776901659	Y197D	0.002	Damaging	-9.37	Deleterious	4.985	Diseases
24	rs1291640722	P198A	0.006	Damaging	-7.57	Deleterious	4.01	Diseases
25	rs1302392264	D210H	0.001	Damaging	-6.27	Deleterious	4.63	Diseases
26	rs144920629	A220V	0.002	Damaging	-3.76	Deleterious	3.83	Diseases
27	rs144920629	G221R	0	Damaging	-7.91	Deleterious	4.705	Diseases
28	rs748571395	T227M	0	Damaging	-5.81	Deleterious	4.985	Diseases
29	rs1362436529	L229P	0.006	Damaging	-3.5	Deleterious	4.19	Diseases
30	rs760971789	C243G	0.003	Damaging	-10.61	Deleterious	4.57	Diseases
31	rs774681018	P251S	0.002	Damaging	-7.62	Deleterious	4.675	Diseases
32	rs786204017	P254S	0	Damaging	-7.92	Deleterious	4.975	Diseases
33	rs775829502	G255R	0	Damaging	-7.92	Deleterious	4.705	Diseases
34	rs786204018	G255V	0	Damaging	-8.91	Deleterious	5.05	Diseases
35	rs373398993	I256N	0	Damaging	-6.61	Deleterious	4.88	Diseases
36	rs1553186124	P258R	0	Damaging	-8.91	Deleterious	5.06	Diseases
37	rs925312071	C306Y	0.001	Damaging	-8.7	Deleterious	4.585	Diseases
38	rs200890679	G313V	0.001	Damaging	-6.67	Deleterious	4.605	Diseases
39	rs761392430	L318H	0	Damaging	-5.48	Deleterious	4.51	Diseases
40	rs774104819	H319Y	0	Damaging	-5.71	Deleterious	5.045	Diseases
41	rs121434297	L323P	0	Damaging	-6.08	Deleterious	4.655	Diseases
42	rs778682219	R363C	0	Damaging	-7.91	Deleterious	3.81	Diseases
43	rs121434296	R377C	0	Damaging	-7.91	Deleterious	4	Diseases

Sr. No.	Variant ID	Mutations	SIFT Score	SIFT prediction	PROVEAN score	PROVEAN prediction	M.Assessor score	M.Assessor prediction
44	rs750323424	R377H	0	Damaging	-4.94	Deleterious	4	Diseases
45	rs1406297300	W381R	0	Damaging	-13.83	Deleterious	4.08	Diseases
46	rs370007225	G387S	0	Damaging	-5.93	Deleterious	3.72	Diseases
47	rs1430872491	G387D	0	Damaging	-6.92	Deleterious	4.07	Diseases
48	rs200138092	R388C	0	Damaging	-7.9	Deleterious	4.07	Diseases
49	rs769953411	R388H	0	Damaging	-4.94	Deleterious	4.07	Diseases
50	rs759745583	G390V	0.001	Damaging	-8.26	Deleterious	4.04	Diseases
51	rs754015864	F435S	0	Damaging	-7.68	Deleterious	4.005	Diseases
52	rs1219122595	P454H	0	Damaging	-8.31	Deleterious	4.08	Diseases
53	rs764455987	G478V	0	Damaging	-8.58	Deleterious	4.01	Diseases
54	rs760349899	G490R	0.001	Damaging	-5.86	Deleterious	3.885	Diseases
55	rs748104181	S493T	0	Damaging	-2.86	Deleterious	4.075	Diseases
56	rs1357376759	W500G	0	Damaging	-12.39	Deleterious	4.065	Diseases
57	rs786204026	Y506D	0.002	Damaging	-8.35	Deleterious	3.825	Diseases
58	rs764650203	Y506C	0.002	Damaging	-7.52	Deleterious	3.825	Diseases
59	rs1425929014	K510E	0.001	Damaging	-3.81	Deleterious	4.07	Diseases
60	rs753049408	K510T	0.001	Damaging	-5.72	Deleterious	4.07	Disease
61	rs1464875135	Y512C	0	Damaging	-8.88	Deleterious	3.84	Disease
62	rs995289143	Y538N	0	Damaging	-7.89	Deleterious	3.795	Disease
63	rs995289143	Y538H	0	Damaging	-4.56	Deleterious	3.795	Disease
64	rs1405669115	N550Y	0.001	Damaging	-7.43	Deleterious	3.935	Disease
65	rs1161759917	G562D	0	Damaging	-6.86	Deleterious	4.065	Disease
66	rs2274974	G566E	0.005	Damaging	-6.05	Deleterious	3.94	Disease
67	rs144508139	P572L	0	Damaging	-9.81	Deleterious	4.025	Disease
68	rs786204031	V575A	0.002	Damaging	-3.92	Deleterious	3.295	Disease
69	rs770471347	F580L	0	Damaging	-5.88	Deleterious	3.755	Disease
70	rs149278646	D585N	0	Damaging	-4.9	Deleterious	3.895	Disease
71	rs983672500	E586K	0	Damaging	-3.92	Deleterious	3.685	Disease
72	rs761226286	A587D	0	Damaging	-5.88	Deleterious	3.955	Disease
73	rs761226286	A587G	0	Damaging	-3.92	Deleterious	3.605	Disease
74	rs1030439905	F588S	0	Damaging	-7.47	Deleterious	3.935	Disease
75	rs786204034	L598P	0.001	Damaging	-5.85	Deleterious	3.63	Disease
76	rs758206023	S603C	0.002	Damaging	-4.61	Deleterious	3.475	Disease
77	rs773336859	N624D	0	Damaging	-4.84	Deleterious	3.82	Disease
78	rs747993832	F626L	0.005	Damaging	-5.08	Deleterious	3.835	Disease

Only those SNPs have been enlisted which are commonly predicted deleterious in all webservers

Table 3. S3. Sequence homology-based predictions of SNPs in SMARCB1, AKT1, SUFU, SMO genes, using SIFT, PROVEAN, and Mutation Assessor webservers

Gene	SMARCB1	SMARCB1	SMARCB1	AKT1	SMO	SUFU
Variant ID	rs387906812	rs387906811	rs267607072	rs121434592	rs121918347	rs202247756

Mutation	R377H	P48L	E31V	E17K	W535L	R123C
SIFT score	-3.65	0	0.001	0.004	0.003	0
SIFT prediction	Disease	Disease	Disease	Disease	Disease	Disease
PROVEAN score	0	-7.31	-4.42	-3.68	11	-7.43
PROVEAN prediction	Disease	Disease	Disease	Disease	Disease	Disease
M.Asssesor score	2.045	2.56	2.545	2.47	3.41	3.72
M.Assessor prediction	Disease	Disease	Disease	Disease	Disease	Disease

Only those SNPs have been enlisted which are commonly predicted deleterious in all webservers

Supervised Learning Method-based Results

Based on the algorithms, SNAP2, SAAP, SusPect, SNPs&GO, PMUT, MutPred2, and PhD-SNP online tools were used to screen deleterious SNPs with their predicted effect. SNAP2 tool sorts protein based on neural networks. With this tool, SNPs are predicted as 'neutral' or 'disease' if the score is > 0.5 [45, 57]. The SusPect is another tool used in this method to benchmark phylogenetic variants and to analyze sequence conservation, structure, and protein-protein interaction (PPI). This tool assigns numeric values (0-100), and a value ≥ 50 is assigned to deleterious variants [47]. Similarly, the SNPs&GO webserver is an SVM (support vector machine) based analyzer that was used to predict the functions of a protein based on structure as well as the function. The results indicated the reliability index and probability value for each analysis, they also provided a PhD-SNP score. Hence, it was determined that this webserver predicts the best results when analyzing deleterious SNPs [48]. Then, PMuT, an online tool, was also applied to further analyze deleterious SNPs, it gives values ranging between 0-1. For the analysis, a cut-off value of 0.5 was applied to filter deleterious SNPs (i.e., mutation ≥ 0.5 was considered deleterious). Additionally, the pathogenicity index gives high confidence about its prediction of a variant to be disease-associated [49]. MutPred was utilized since it provides a prioritization process for pathogenic amino acids along with/using probabilistic modelling to analyze the effect of amino acid substitution on the structure of the protein [50]. Finally, PhD-SNP was applied based on three prediction methods, namely sequence-based, hybrid method, and sequence-profile-based, to predict whether a point mutation is disease-related or not. It gives a score value ranging between 0 – 1 and mutations with

> 0.5 value are considered a 'disease' [51]. A bar chart given in Figure 4 depicts the number of SNPs predicted to be deleterious for each selected gene (s) in various tools used in this method. Conclusively, SNPs in *NF2*, *MHFR*, *AKTI*, *SMO*, *SUFU*, and *SMARCB1* genes are deleterious and may be linked to meningioma (Table S4-6).

Table 4. S4: Supervised Learning Method based Predictions of Deleterious SNPs in NF2 Gene, using PMUT, SusPect, SNAP2, SNPs&GO, SAAP, PhD-SNP, and MutPred2 Webservers

Sr. No.	Variant ID	Mutations	PMUT score	PMUT prediction	SNAP2 Score	SNAP2 prediction	PhD-SNP prediction	Mutpred2 prediction
1	rs1383283673	W60R	0.76	Disease	71	Disease	Disease	Disease
2	rs780872661	W60C	0.89	Disease	19	Disease	Disease	Disease
3	rs1286915234	F61S	0.84	Disease	58	Disease	Disease	Disease
4	rs121434261	F62S	0.88	Disease	97	Disease	Disease	Disease
5	rs772274240	Y66N	0.88	Disease	71	Disease	Disease	Disease
6	rs559558673	D70Y	0.62	Disease	41	Disease	Disease	Disease
7	rs1060503668	A98D	0.87	Disease	58	Disease	Disease	Disease
8	rs781593146	E112K	0.75	Disease	53	Disease	Disease	Disease
9	rs1328289194	I113F	0.57	Disease	45	Disease	Disease	Disease
10	rs1388544922	A142V	0.72	Disease	62	Disease	Disease	Disease
11	rs1303879665	G161R	0.67	Disease	52	Disease	Disease	Disease
12	rs779353677	E166V	0.87	Disease	27	Disease	Disease	Disease
13	rs1555993319	Y192C	0.81	Disease	43	Disease	Disease	Disease
14	rs749176138	R196Q	0.76	Disease	15	Disease	Disease	Disease
15	rs1487106309	R200G	0.76	Disease	64	Disease	Disease	Disease
16	rs776818377	G218S	0.86	Disease	64	Disease	Disease	Disease
17	rs776818377	G218C	0.87	Disease	61	Disease	Disease	Disease
18	rs746025177	Y221C	0.85	Disease	65	Disease	Disease	Disease
19	rs1298953582	I224M	0.86	Disease	9	Disease	Disease	Disease
20	rs1028670573	G229C	0.87	Disease	21	Disease	Disease	Disease
21	rs761195572	A238V	0.87	Disease	26	Disease	Disease	Disease
22	rs1432132718	L250Q	0.73	Disease	16	Disease	Disease	Disease
23	rs754093587	R291C	0.87	Disease	46	Disease	Disease	Disease
24	rs867836772	C300R	0.88	Disease	64	Disease	Disease	Disease
25	rs74315492	L360P	0.6	Disease	76	Disease	Disease	Disease
26	rs1280154812	L374R	0.69	Disease	40	Disease	Disease	Disease
27	rs74315493	L535P	0.67	Disease	86	Disease	Disease	Disease

Only those SNPs have been enlisted which are commonly predicted deleterious in all webservers

Table 5. S5: Supervised Learning Method based Predictions of Deleterious SNPs in *MTHFR* Gene, using PMUT, SusPect, SNAP2, SNPs&GO, SAAP, PhD-SNP, and MutPred2 Webservers

Sr. No.	Variant ID	Mutations	PMUT score	PMUT prediction	SusSpect Score	SusPect prediction	PhD-SNP score	PhD-SNP prediction	SNPs&GO Score	SNPs&GO prediction	MutPred score	MutPred prediction
1	rs774531580	E63K	0.93	Disease	97	Disease	8	Disease	0.547	Disease	0.912	Disease
2	rs796064512	P66L	0.93	Disease	97	Disease	9	Disease	0.525	Disease	0.883	Disease
3	rs763539350	R68G	0.83	Disease	67	Disease	8	Disease	0.764	Disease	0.879	Disease
4	rs770151367	T69A	0.85	Disease	55	Disease	6	Disease	0.629	Disease	0.774	Disease
5	rs746177570	T69I	0.92	Disease	64	Disease	8	Disease	0.833	Disease	0.859	Disease
6	rs786204009	R82W	0.87	Disease	84	Disease	9	Disease	0.615	Disease	0.643	Disease
7	rs1426036757	M83T	0.91	Disease	85	Disease	8	Disease	0.583	Disease	0.928	Disease
8	rs147257424	A113P	0.92	Disease	87	Disease	7	Disease	0.602	Disease	0.841	Disease
9	rs769381688	H127Y	0.92	Disease	94	Disease	6	Disease	0.661	Disease	0.936	Disease
10	rs786204012	C130R	0.93	Disease	96	Disease	9	Disease	0.527	Disease	0.952	Disease
11	rs761230732	L142P	0.92	Disease	97	Disease	8	Disease	0.851	Disease	0.96	Disease
12	rs761230732	L142R	0.93	Disease	96	Disease	9	Disease	0.783	Disease	0.959	Disease
13	rs1018189670	G149D	0.81	Disease	62	Disease	8	Disease	0.643	Disease	0.952	Disease
14	rs776195746	R157W	0.92	Disease	97	Disease	8	Disease	0.928	Disease	0.948	Disease
15	rs121434295	R157Q	0.89	Disease	88	Disease	7	Disease	0.56	Disease	0.912	Disease
16	rs772932189	G158E	0.93	Disease	95	Disease	8	Disease	0.887	Disease	0.949	Disease
17	rs138524217	D159N	0.92	Disease	88	Disease	6	Disease	0.743	Disease	0.913	Disease
18	rs1182635980	A175T	0.81	Disease	77	Disease	7	Disease	0.62	Disease	0.857	Disease
19	rs1172158928	D177H	0.88	Disease	72	Disease	8	Disease	0.509	Disease	0.927	Disease

Sr. No.	Variant ID	Mutations	PMUT score	PMUT prediction	SusSpect Score	SusPect prediction	PhD-SNP score	PhD-SNP prediction	SNPs&GO Score	SNPs&GO prediction	MutPred score	MutPred prediction
20	rs1285236808	V194M	0.86	Disease	67	Disease	7	Disease	0.504	Disease	0.85	Disease
21	rs760161369	A195V	0.88	Disease	64	Disease	8	Disease	0.688	Disease	0.894	Disease
22	rs786204014	G196D	0.87	Disease	69	Disease	7	Disease	0.783	Disease	0.937	Disease
23	rs776901659	Y197D	0.93	Disease	64	Disease	8	Disease	0.768	Disease	0.958	Disease
24	rs1291640722	P198A		Disease	52	Disease	5	Disease	0.555	Disease	0.842	Disease
25	rs1302392264	D210H	0.85	Disease	90	Disease	6	Disease	0.632	Disease	0.678	Disease
26	rs144920629	A220V	0.85	Disease	68	Disease	7	Disease	0.501	Disease	0.849	Disease
27	rs144920629	G221R	0.87	Disease	84	Disease	8	Disease	0.917	Disease	0.954	Disease
28	rs748571395	T227M	0.93	Disease	96	Disease	8	Disease	0.862	Disease	0.896	Disease
29	rs1362436529	L229P	0.86	Disease	74	Disease	7	Disease	0.608	Disease	0.974	Disease
30	rs760971789	C243G	0.92	Disease	87	Disease	7	Disease	0.668	Disease	0.839	Disease
31	s774681018	P251S	0.92	Disease	67	Disease	2	Disease	0.533	Disease	0.869	Disease
32	rs786204017	P254S	0.92	Disease	89	Disease	4	Disease	0.646	Disease	0.897	Disease
33	rs775829502	G255R	0.93	Disease	90	Disease	7	Disease	0.73	Disease	0.951	Disease
34	rs786204018	G255V	0.93	Disease	85	Disease	7	Disease	0.686	Disease	0.954	Disease
35	rs373398993	I256N	0.93	Disease	91	Disease	8	Disease	0.888	Disease	0.951	Disease
36	rs1553186124	P258R	0.93	Disease	92	Disease	8	Disease	0.577	Disease	0.956	Disease
37	rs925312071	C306Y	0.92	Disease	75	Disease	7	Disease	0.665	Disease	0.917	Disease
38	rs200890679	G313V	0.92	Disease	46	Disease	9	Disease	0.65	Disease	0.85	Disease
39	rs761392430	L318H	0.92	Disease	90	Disease	6	Disease	0.699	Disease	0.94	Disease
40	rs774104819	H319Y	0.85	Disease	80	Disease	7	Disease	0.736	Disease	0.924	Disease

Sr. No.	Variant ID	Mutations	PMUT score	PMUT prediction	SusSpect Score	SusPect prediction	PhD-SNP score	PhD-SNP prediction	SNPs&GO Score	SNPs&GO prediction	MutPred score	MutPred prediction
41	rs121434297	L323P	0.9	Disease	59	Disease	9	Disease	0.866	Disease	0.968	Disease
42	rs778682219	R363C	0.85	Disease	58	Disease	6	Disease	0.761	Disease	0.813	Disease
43	rs121434296	R377C	0.88	Disease	71	Disease	9	Disease	0.905	Disease	0.886	Disease
44	rs750323424	R377H	0.87	Disease	65	Disease	9	Disease	0.784	Disease	0.8	Disease
45	rs1406297300	W381R	0.88	Disease	80	Disease	9	Disease	0.64	Disease	0.956	Disease
46	rs370007225	G387S	0.88	Disease	61	Disease	8	Disease	0.691	Disease	0.891	Disease
47	rs1430872491	G387D	0.89	Disease	79	Disease	7	Disease	0.794	Disease	0.936	Disease
48	rs200138092	R388C	0.9	Disease	77	Disease	7	Disease	0.905	Disease	0.915	Disease
49	rs769953411	R388H	0.89	Disease	50	Disease	7	Disease	0.784	Disease	0.861	Disease
50	rs759745583	G390V	0.88	Disease	79	Disease	7	Disease	0.828	Disease	0.931	Disease
51	rs754015864	F435S	0.88	Disease	91	Disease	9	Disease	0.893	Disease	0.922	Disease
52	rs1219122595	P454H	0.9	Disease	89	Disease	7	Disease	0.87	Disease	0.926	Disease
53	rs764455987	G478V	0.88	Disease	88	Disease	9	Disease	0.526	Disease	0.936	Disease
54	rs760349899	G490R	0.87	Disease	66	Disease	4	Disease	0.642	Disease	0.914	Disease
55	rs748104181	S493T	0.87	Disease	78	Disease	8	Disease	0.53	Disease	0.782	Disease
56	rs1357376759	W500G	0.88	Disease	94	Disease	8	Disease	0.806	Disease	0.954	Disease
57	rs786204026	Y506D	0.86	Disease	83	Disease	9	Disease	0.678	Disease	0.951	Disease
58	rs764650203	Y506C	0.86	Disease	81	Disease	8	Disease	0.773	Disease	0.927	Disease
59	rs1425929014	K510E	0.89	Disease	68	Disease	8	Disease	0.58	Disease	0.903	Disease
60	rs753049408	K510T	0.9	Disease	71	Disease	9	Disease	0.58	Disease	0.904	Disease
61	rs1464875135	Y512C	0.86	Disease	82	Disease	8	Disease	0.863	Disease	0.926	Disease

Sr. No.	Variant ID	Mutations	PMUT score	PMUT prediction	SusSpect Score	SusPect prediction	PhD-SNP score	PhD-SNP prediction	SNPs&GO Score	SNPs&GO prediction	MutPred score	MutPred prediction
62	rs995289143	Y538N	0.86	Disease	91	Disease	8	Disease	0.902	Disease	0.959	Disease
63	rs995289143	Y538H	0.86	Disease	86	Disease	6	Disease	0.848	Disease	0.946	Disease
64	rs1405669115	N550Y	0.86	Disease	59	Disease	9	Disease	0.858	Disease	0.93	Disease
65	rs1161759917	G562D	0.89	Disease	91	Disease	8	Disease	0.794	Disease	0.96	Disease
66	rs2274974	G566E	0.86	Disease	59	Disease	7	Disease	0.916	Disease	0.926	Disease
67	rs144508139	P572L	0.88	Disease	91	Disease	6	Disease	0.545	Disease	0.913	Disease
68	rs786204031	V575A	0.85	Disease	58	Disease	3	Disease	0.745	Disease	0.829	Disease
69	rs770471347	F580L	0.88	Disease	75	Disease	8	Disease	0.631	Disease	0.913	Disease
70	rs149278646	D585N	0.76	Disease	50	Disease	6	Disease	0.875	Disease	0.829	Disease
71	rs983672500	E586K	0.87	Disease	79	Disease	9	Disease	0.597	Disease	0.922	Disease
72	rs761226286	A587D	0.87	Disease	91	Disease	9	Disease	0.574	Disease	0.936	Disease
73	rs761226286	A587G	0.82	Disease	89	Disease	6	Disease	0.788	Disease	0.855	Disease
74	rs1030439905	F588S	0.85	Disease	92	Disease	8	Disease	0.708	Disease	0.941	Disease
75	rs786204034	L598P	0.76	Disease	80	Disease	6	Disease	0.818	Disease	0.946	Disease
76	rs758206023	S603C	0.85	Disease	76	Disease	6	Disease	0.732	Disease	0.817	Disease
77	rs773336859	N624D	0.85	Disease	82	Disease	0	Disease	0.807	Disease	0.928	Disease
78	rs747993832	F626L	0.85	Disease	87	Disease	8	Disease	0.897	Disease	0.925	Disease

Only those SNPs have been enlisted which are commonly predicted deleterious in all webservers

Figure 4. Bar chart representation of the Supervised Learning method based prediction of SNPs in different genes associated with meningioma; A: represents SNPs count for *NF2* gene (i.e., out of 347 missense SNPs, 72 in PhD-SNP, 142 in *SAAP*, 171 in Pmut, 141 in MutPred and 142 in SNAP2 were predicted damaging); B: represents SNPs count for *MTHFR* gene (i.e., out of 494 missense SNPs, 209 in SNAP2, 249 in Pmut, 174 in SuSPect, 243 in PhD-pro, 154 in SNPs&GO and 310 in MutPred were predicted damaging); C: representing SNPs count for *SMARCB1*, *AKT1*, *SMO*, *SUFU* genes (i.e., out of 12 missense SNPs, 8 in SNAP2, 9 in SuSPect, 6 in PMut, 6 in SNPs&GO, 6 in PhD-SNP and 8 in MutPred and 310 in SNAP2 were predicted damaging)

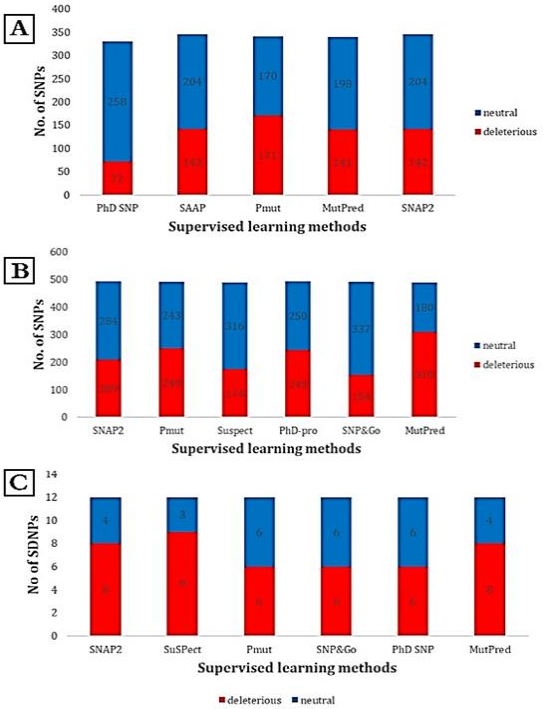


Table 6. S6: Supervised Learning Method based Predictions of Deleterious SNPs in *SMARCB1*, *AKT1*, *SUFU*, *SMO* Genes, using PMUT, SusPect, SNAP2, SNPs&GO, SAAP, PhD-SNP, MutPred2 Webservers

Gene	SMARCB1	SMARCB1	SMARCB1	AKT1	SMO	SUFU
Variant ID	rs387906812	rs387906811	rs267607072	rs121434592	rs121918347	rs202247756
Mutation	R377H	P48L	E31V	E17K	W535L	R123C

SNAP2 score	62	59	78	93	92	41
SNAP2 prediction	Effect	Effect	Effect	Effect	Effect	Effect
PMUT score	0.86	0.84	0.5	0.67	0.91	0.87
PMUT prediction	Disease	Disease	Disease	Disease	Disease	Disease
SusPect score	73	74	59	98	78	63
SusPect prediction	Disease	Disease	Disease	Disease	Disease	Disease
SNPs&GO score	0.58	0.87	0.9	0.61	0.68	0.88
SNPs&GO prediction	Disease	Disease	Disease	Disease	Disease	Disease
PhD-SNP score	3	7	4	4	10	8
PhD-SNP prediction	Disease	Disease	Disease	Disease	Disease	Disease
MutPred score	0.61	0.86	0.92	0.93	0.9	0.9
Mut Pred prediction	Disease	Disease	Disease	Disease	Disease	Disease
SAAP prediction	Disease	Disease	Disease	Disease	Disease	Disease

Only those SNPs have been enlisted which are commonly predicted deleterious in all webservers

Consensus-Based Methods

Webservers including Condel, Meta-SNP, and PredictSNP were applied to attain a consensus score. They worked in an integrated manner and transferred the results from one tool to the next. Hence, the obtained results culminated from the combined workings of all the applied tools. Condel assessed the results of five other computational tools, namely SIFT, PPH2, MA, MAPP, and Logre, to predict the effects of non-synonymous single nucleotide polymorphisms (nsSNPs) on the protein function by giving the mutations a weighted average of the normalized score (WAS). This score is generated by calculating the scoring probability [58]. Meta-SNP uses four computational tools named SNAP, SIFT, PANTHER, and PhD-SNP to make the final prediction based on the random forest approach. It predicts the mutated residue and generates frequencies of wild type and new residue. True positives show diseased variants while true negatives show

polymorphic variants [54]. Finally, the PredicSNP server was utilized since it is considered the most reliable tool for the analysis of nsSNPs due to its accuracy. These tools provide the confidence scores for each mutation given in the query depending upon their unique scale. The result ranges between 0 - 100%, with either positive or negative values. The score for a mutation in the range of < -1, 0> is considered 'neutral', while a value ranging between <0, +1> is considered 'deleterious' [53, 57]. A bar chart given in Figure 5 represents the number of SNPs predicted to be deleterious for each selected gene (s) in various tools used in this method. Hence, SNPs in *NF2*, *MHFR*, *AKTI*, *SMO*, *SUFU*, and *SMARCB1* genes are deleterious and may be linked to meningioma (Table S7-9).

Table 7. S7: Consensus Method based Predictions of Deleterious SNPs in *NF2* Gene, using Pred-SNP, Meta-SNP, and Condel Webservers

Variant ID	Mutation	Pred-SNP score	Pred-SNP prediction	Meta-SNP score	MetaSNP prediction	Condel score	Condel prediction
rs1383283673	W60R	76	Disease	0.82	Disease	0.72	Disease
rs780872661	W60C	87	Disease	0.82	Disease	0.68	Disease
rs1286915234	F61S	87	Disease	0.8	Disease	0.66	Disease
rs121434261	F62S	87	Disease	0.87	Disease	0.76	Disease
rs772274240	Y66N	87	Disease	0.81	Disease	0.74	Disease
rs559558673	D70Y	87	Disease	0.79	Disease	0.53	Disease
rs1060503668	A98D	87	Disease	0.808	Disease	0.67	Disease
rs781593146	E112K	61	Disease	0.58	Disease	0.68	Disease
rs1328289194	I113F	61	Disease	0.56	Disease	0.58	Disease
rs1388544922	A142V	87	Disease	0.54	Disease	0.63	Disease
rs1303879665	G161R	87	Disease	0.87	Disease	0.65	Disease
rs779353677	E166V	61	Disease	0.72	Disease	0.61	Disease
rs1555993319	Y192C	76	Disease	0.79	Disease	0.68	Disease
rs1487106309	R200G	72	Disease	0.7	Disease	0.62	Disease
rs776818377	G218S	87	Disease	0.81	Disease	0.7	Disease
rs776818377	G218C	87	Disease	0.83	Disease	0.67	Disease
rs746025177	Y221C	87	Disease	0.87	Disease	0.72	Disease
rs1298953582	I224M	87	Disease	0.51	Disease	0.63	Disease
rs1028670573	G229C	87	Disease	0.76	Disease	0.58	Disease
rs761195572	A238V	87	Disease	0.53	Disease	0.65	Disease
rs1432132718	L250Q	76	Disease	0.56	Disease	0.57	Disease
rs754093587	R291C	87	Disease	0.75	Disease	0.6	Disease
rs867836772	C300R	87	Disease	0.83	Disease	0.64	Disease
rs74315492	L360P	76	Disease	0.67	Disease	0.61	Disease

Variant ID	Mutation	Pred-SNP score	Pred-SNP prediction	Meta-SNP score	MetaSNP prediction	Condel score	Condel prediction
rs1280154812	L374R	76	Disease	0.55	Disease	0.62	Disease
rs74315493	L535P	87	Disease	0.73	Disease	0.56	Disease

Only those SNPs have been enlisted which are commonly predicted deleterious in all webservers

Table 8. S8: Consensus Method based Predictions of Deleterious SNPs in *MTHFR* Gene, using Pred-SNP, Meta-SNP, and Condel Webservers

Sr. No.	Variant ID	Mutations	Predict-SNP score	Predict-SNP prediction	Condel score	Condel prediction	Meta-SNP score	Meta-SNP prediction
1	rs774531580	E63K	87	Disease	0.71	Disease	0.85	Disease
2	rs796064512	P66L	87	Disease	0.74	Disease	0.85	Disease
3	rs763539350	R68G	87	Disease	0.68	Disease	0.75	Disease
4	rs770151367	T69A	87	Disease	0.67	Disease	0.51	Disease
5	rs746177570	T69I	87	Disease	0.73	Disease	0.73	Disease
6	rs786204009	R82W	87	Disease	0.69	Disease	0.76	Disease
7	rs1426036757	M83T	87	Disease	0.66	Disease	0.78	Disease
8	rs147257424	A113P	87	Disease	0.7	Disease	0.77	Disease
9	rs769381688	H127Y	87	Disease	0.68	Disease	0.81	Disease
10	rs786204012	C130R	87	Disease	0.74	Disease	0.907	Disease
11	rs761230732	L142P	87	Disease	0.72	Disease	0.82	Disease
12	rs761230732	L142R	87	Disease	0.72	Disease	0.8	Disease
13	rs1018189670	G149D	87	Disease	0.66	Disease	0.84	Disease
14	rs776195746	R157W	87	Disease	0.75	Disease	0.81	Disease
15	rs121434295	R157Q	87	Disease	0.72	Disease	0.9	Disease
16	rs772932189	G158E	87	Disease	0.75	Disease	0.91	Disease
17	rs138524217	D159N	87	Disease	0.73	Disease	0.81	Disease
18	rs1182635980	A175T	87	Disease	0.63	Disease	0.62	Disease
19	rs1172158928	D177H	87	Disease	0.73	Disease	0.62	Disease
20	rs1285236808	V194M	87	Disease	0.67	Disease	0.67	Disease
21	rs760161369	A195V	87	Disease	0.73	Disease	0.73	Disease
22	rs786204014	G196D	87	Disease	0.64	Disease	0.68	Disease
23	rs776901659	Y197D	87	Disease	0.71	Disease	0.78	Disease
24	rs1291640722	P198A	87	Disease	0.67	Disease	0.67	Disease
25	rs1302392264	D210H	87	Disease	0.75	Disease	0.7	Disease
26	rs144920629	A220V	87	Disease	0.7	Disease	0.68	Disease
27	rs144920629	G221R	87	Disease	0.72	Disease	0.91	Disease
28	rs748571395	T227M	87	Disease	0.74	Disease	0.88	Disease
29	rs1362436529	L229P	87	Disease	0.71	Disease	0.76	Disease
30	rs760971789	C243G	87	Disease	0.72	Disease	0.78	Disease
31	s774681018	P251S	87	Disease	0.75	Disease	0.8	Disease
32	rs786204017	P254S	87	Disease	0.74	Disease	0.76	Disease
33	rs775829502	G255R	87	Disease	0.75	Disease	0.91	Disease
34	rs786204018	G255V	87	Disease	0.69	Disease	0.91	Disease
35	rs373398993	I256N	87	Disease	0.73	Disease	0.82	Disease

Sr. No.	Variant ID	Mutations	Predict-SNP score	Predict-SNP prediction	Condel score	Condel prediction	Meta-SNP score	Meta-SNP prediction
36	rs1553186124	P258R	87	Disease	0.75	Disease	0.91	Disease
37	rs925312071	C306Y	87	Disease	0.72	Disease	0.8	Disease
38	rs200890679	G313V	87	Disease	0.71	Disease	0.61	Disease
39	rs761392430	L318H	87	Disease	0.7	Disease	0.75	Disease
40	rs774104819	H319Y	87	Disease	0.74	Disease	0.85	Disease
41	rs121434297	L323P	87	Disease	0.72	Disease	0.84	Disease
42	rs778682219	R363C	87	Disease	0.73	Disease	0.72	Disease
43	rs121434296	R377C	87	Disease	0.74	Disease	0.88	Disease
44	rs750323424	R377H	87	Disease	0.75	Disease	0.84	Disease
45	rs1406297300	W381R	87	Disease	0.74	Disease	0.83	Disease
46	rs370007225	G387S	87	Disease	0.71	Disease	0.77	Disease
47	rs1430872491	G387D	87	Disease	0.75	Disease	0.83	Disease
48	rs200138092	R388C	87	Disease	0.74	Disease	0.86	Disease
49	rs769953411	R388H	87	Disease	0.74	Disease	0.79	Disease
50	rs759745583	G390V	87	Disease	0.73	Disease	0.79	Disease
51	rs754015864	F435S	87	Disease	0.71	Disease	0.75	Disease
52	rs1219122595	P454H	87	Disease	0.72	Disease	0.76	Disease
53	rs764455987	G478V	87	Disease	0.72	Disease	0.78	Disease
54	rs760349899	G490R	87	Disease	0.69	Disease	0.73	Disease
55	rs748104181	S493T	87	Disease	0.72	Disease	0.78	Disease
56	rs1357376759	W500G	87	Disease	0.72	Disease	0.82	Disease
57	rs786204026	Y506D	87	Disease	0.69	Disease	0.87	Disease
58	rs764650203	Y506C	87	Disease	0.7	Disease	0.85	Disease
59	rs1425929014	K510E	87	Disease	0.73	Disease	0.85	Disease
60	rs753049408	K510T	87	Disease	0.73	Disease	0.85	Disease
61	rs1464875135	Y512C	87	Disease	0.7	Disease	0.82	Disease
62	rs995289143	Y538N	87	Disease	0.68	Disease	0.78	Disease
63	rs995289143	Y538H	87	Disease	0.68	Disease	0.78	Disease
64	rs1405669115	N550Y	87	Disease	0.67	Disease	0.78	Disease
65	rs1161759917	G562D	87	Disease	0.7	Disease	0.85	Disease
66	rs2274974	G566E	87	Disease	0.69	Disease	0.81	Disease
67	rs144508139	P572L	87	Disease	0.72	Disease	0.77	Disease
68	rs786204031	V575A	87	Disease	0.65	Disease	0.74	Disease
69	rs770471347	F580L	87	Disease	0.64	Disease	0.74	Disease
70	rs149278646	D585N	87	Disease	0.69	Disease	0.72	Disease
71	rs983672500	E586K	87	Disease	0.69	Disease	0.83	Disease
72	rs761226286	A587D	87	Disease	0.68	Disease	0.73	Disease
73	rs761226286	A587G	87	Disease	0.69	Disease	0.68	Disease
74	rs1030439905	F588S	87	Disease	0.67	Disease	0.73	Disease
75	rs786204034	L598P	87	Disease	0.62	Disease	0.76	Disease
76	rs758206023	S603C	87	Disease	0.72	Disease	0.72	Disease
77	rs773336859	N624D	87	Disease	0.68	Disease	0.75	Disease
78	rs747993832	F626L	87	Disease	0.67	Disease	0.79	Disease

Only those SNPs have been enlisted which are commonly predicted deleterious in all webservers

Figure 5. Bar Chart Representation of the Consensus-based Prediction of Snps in Different Genes Associated with Meningioma; A: Represents Snps Count for NF2 Gene (I.E., Out of 347 Missense Snps, 127 in Predict-SNP, 97 in Meta-SNP and 89 in Condel were Predicted Deleterious); B: Represents Snps Count for *MTHFR* Gene (I.E., Out Of 494 Missense, 107 Snps in Predict-SNP, 97 in Meta-SNP And 83 in Condel were Predicted Deleterious); C: Represents Snps Count for *SMARCB1*, *AKT1*, *SMO*, *SUFU* Genes (I.E., Out of 12 Missense Snps, 9 in Predict-SNP, 8 in Meta-SNP and 8 in Condel were Predicted Deleterious)

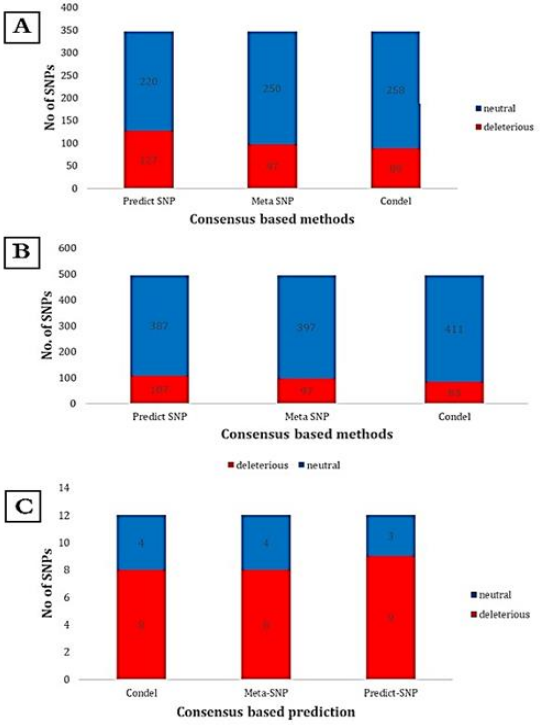


Table 9. S9: Consensus Method based Predictions of Deleterious SNPs in *SMARCB1, SUF'U, SMO, AKT1* Genes, using Pred-SNP, Meta-SNP, and Condel Webservers

Gene	Variant ID	Mutations	Condel score	Condel prediction	Meta-SNP score	Meta-SNP prediction	Predict-SNP score	Predict-SNP prediction
SMARCB1	rs387906812	R377H	0.60	Disease	0.70	Disease	76	Disease
SMARCB1	rs387906811	P48L	0.06	Disease	0.71	Disease	88	Disease

Gene	Variant ID	Mutations	Condel score	Condel prediction	Meta-SNP score	Meta-SNP prediction	Predict-SNP score	Predict-SNP prediction
SMARCB1	rs267607072	E31V	0.59	Disease	0.74	Disease	87	Disease
AKT1	rs121434592	E17K	0.55	Disease	0.69	Disease	76	Disease
SMO	rs121918347	W535L	0.67	Disease	0.82	Disease	87	Disease
SUFU	rs202247756	R123C	0.71	Disease	0.71	Disease	87	Disease

Only those SNPs have been enlisted which are commonly predicted deleterious in all webservers

Discussion

Meningioma accounts for 30 % of all brain tumours and does not require surgery due to its benign nature. This *in-silico* analysis highlighted the possible outcomes of the alterations, which can be either genetic or epigenetics both at structural and functional levels. Badgujar *et al* reported that Align GVGD, SIFT, SNAP2, PolyPhen-2, PANTHER, PhD-SNP, MuPro, PROVEAN, and iPTREE-STAB were used to conduct the *in-silico* analysis of variations in human CHK2. A total number of 79 mutations were selected and 7 SNPs (p.Arg188Trp, p.Arg160-Gly, p.Ile203Thr, p.Gly210Arg, p.Pro225His, p.Arg223Cys and p.Ser415Phe) were found to be most deleterious. These SNPs were not reported previously in any diseases, except for breast cancer. Single nucleotide polymorphisms can cause changes in the codons, which may result in the substitution or alteration of amino acids, leading to changes in the structure and function of proteins. The changes in amino acids can affect the bonding, synthesis, structure, function, and stability of proteins [59]. It is recommended to use a combination of computational tools for the study of SNPs, which would work in an integrated manner and produce more accurate results. For example, Meta-SNP, Condel, and Predict SNP, which were used in this research, predict more accurate results than individual tools.

The findings of our analysis showed that out of 694 missense SNPs in MTHFR gene, 78 were found to be unanimously deleterious by all the bioinformatics tools using homology, based methods, consensus-based methods, and structure-based methods. In the case of the NF2 gene, out of 347 missense SNPs, 27 were considered deleterious by all the methods using the aforementioned bioinformatics tools. For AKT1, SMO, SUFO, and SMARCB1, out of the 12 mutations reported to cause meningioma, only 6 mutations were found deleterious in our *in-silico* analysis. One of the

mutations studied in the SUFU gene is rs202247756 c.367C>T (p.Arg123Cys). It was studied in a family with 5 siblings who were suffering from meningioma, while 4 of them were also suffering from multiple tumosurs. This mutation was not reported in healthy individuals. Arginine is a positively charged amino acid residue and is important for the formation of hydrogen. It also has ionic interactions with amino acids His89, Asp182, Gln184, and Gln199. The loop structures in the subdomain of the N-terminal of the SUFU gene depends on these amino acids. If there is a change in Arg123, the tertiary formation of proteins is disturbed due to the increased flexibility of the loops [60]. Cysteine is comparatively smaller and more hydrophobic than wildtype arginine. It is also different in size and hydrophobicity and thus, affects the hydrogen bonding of the residues, creating a space at the core of the protein. The functions of the gene are influenced if the mutations are present in the exonic or gene regulatory regions since they have deleterious effects on the structure and function of proteins [61].

The rs121434592 (p.E17K) mutation in the AKT1 gene reduces the recurring time of cancer and is linked with mTOR and ERK1/2 activation [62]. The substitution of glutamic acid, a negatively charged amino acid with lysine, a positively charged amino acid at position 17 causes changes in the hydrogen bonding. This also causes repulsion within the protein molecules. Additionally, the large size of mutant residue causes bulging in the main functional domain of the protein. Hence, the binding of AKT increases with P13K, which increases cell proliferation. In a normal state, Lys14 interacts with Glu17, but the substitution of Lys17 causes a change in bonding, which increases the affinity of phosphoinositides with AKT1 [63].

The rs121918347 (p.W535L) mutation in SMO involves a wild-type residue of Tryptophan at position 535. Bacci et al., in his association study of Schwannomatosis associated with multiple meningiomas, reported that this residue is larger as compared to the mutant residue Leucine.[64]. According to Twigg *et al.*, this mutation affects the lipid membrane contact by disturbing the transmembrane domain of the smoothened protein [65]. In one of the mutations in SMARCB1 such as rs267607072 (p.E31V), the wild type residue glutamic acid at position 31 is negatively charged, and the

mutant residue valine is neutral. The wild-type residue forms a hydrogen bond with tyrosine at position 81, but the H-bonding is absent for the mutant residue. The mutant residue is smaller in size. The change in the charges leads to decreased external interaction affecting the function of the protein [66].

The PPI analysis of all selected genes predicted a network, which showed the possible interaction of these genes with each other. It was determined that all genes, namely NF2, AKT1, SMO, SUFU, SMARCB1, are interlinked to each other directly or indirectly. Furthermore, SUFU also showed an additional direct interaction with SMARCB1 (Figure 6). Conversely, MTHFR showed no involvement with any of these genes. This network can predict possible genomic targets of many drugs, which can be used to treat meningioma. This *in-silico* study determined that several above enlisted deleterious SNPs in NF2, MTHFR, SMARCB1, AKT1, SMO, and SUFU genes can be used as diagnostic biomarkers and molecular targets for the diagnosis and treatment of meningioma. It should be noted that further genome-wide association studies are still required to confirm their association with meningioma in the Pakistani population as well as in other ethnic groups.

Conclusion

Single nucleotide polymorphisms (SNPs) are highly associated with genetic and other metabolic diseases. They change the properties of proteins to show strong genetic and phenotypic effects. Our study ascertained that many missense SNPs induce structural and functional effects/changes upon/in proteins. After the analysis carried out using various computational tools, the reported SNPs in SMARCB1, AKT1, SMO, SUFU, and SNPs in NF2 and MTHFR obtained from the Ensemble were predicted to be highly deleterious. These SNPs were conserved in an evolutionary pedigree and were found to be involved in the high instability of proteins. SDM tool confirmed that the stability of proteins was reduced. Moreover, their structure was occasionally reported to be denatured. PPI analysis also revealed a coherent interaction of all the selected SNPs with each other except MTHFR These interactions can be used to predict the combined effect of these genes and their SNPs to develop a diseased phenotype. SNPs are the primary cause of meningioma, which is why they can be used for

diagnostic purposes as molecular markers and in drug designing by therapeutic targeting.

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Author's Contribution
AJ participated in the design, interpretation, and writing of this manuscript; BW designed and performed the analysis; RA and SS supervised the entire study and provided valuable suggestions. All the authors have read the manuscript and agree with the submitted version of this article.

Declaration of Conflict of Interest
All the author (s) declare no potential conflict of interest concerning the research, authorship, and publication of this article.

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References

[1] Apra C, Peyre M, Kalamarides M. Current treatment options for meningioma. *Expert Rev Neurother.* 2018;18(3):241-9. https://doi.org/10.1080/14737175.2018.1429920

[2] Fathi A-R, Roelcke U. Meningioma. *Curr Neurol Neurosci Rep.* 2013;13(4):337. https://doi.org/10.1007/s11910-013-0337-4

[3] Kotecha RS, Pascoe EM, Rushing EJ, et al. Meningiomas in children and adolescents: a meta-analysis of individual patient data. *Lancet Oncol.* 2011;12(13):1229-39. https://doi.org/10.1016/S1470-2045(11)70275-3

[4] Ganjeifar B, Abouei Mehrizi MA. Multiple Intracranial Meningioma in a Woman with Headache. *Rev Clin Med*. 2018;5(2):72-5.

[5] Jamal S, Mammon N, Mushtaq S, Luqman M. Pattern of central nervous system (CNS) tumor: a study of 430 cases. *Pak J Pathol*. 2005;16(4):106-9.

[6] Saraf S, McCarthy BJ, Villano JL. Update on meningiomas. *Oncologist*. 2011;16(11):1604. https://doi.org/10.1634/theoncologist.2011-0193

[7] Wiemels J, Wrensch M, Claus EB. Epidemiology and etiology of meningioma. *J Neurooncol.* 2010;99(3):307-14. https://doi.org/10.1007/s11060-010-0386-3

[8] Moreau JT, Hankinson TC, Baillet S, Dudley RW. Individual-Patient prediction of meningioma malignancy and survival using the surveillance, epidemiology, and end results database. *NPJ Digit Med.* 2020;3(1):1-10. https://doi.org/10.1038/s41746-020-0219-5

[9] Ostrom QT, Cioffi G, Gittleman H, et al. CBTRUS statistical report: primary brain and other central nervous system tumors diagnosed in the United States in 2012–2016. *Neuro-Oncol*. 2019;21 (Supplement_5):v1-v100. https://doi.org/10.1093/neuonc/noz150

[10] Sung H, Ferlay J, Siegel RL, et al. Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA: Cancer J Clin.* 2021;71(3):209-49. https://doi.org/10.3322/caac.21660

[11] Ostrom QT, Truitt G, Gittleman H, et al. Relative survival after diagnosis with a primary brain or other central nervous system tumor in the National Program of Cancer Registries, 2004 to 2014. *Neuro-Oncol Pract.* 2020;7(3):306-12. https://doi.org/10.1093/nop/npz059

[12] Bowers DC, Moskowitz CS, Chou JF, et al. Morbidity and mortality associated with meningioma after cranial radiotherapy: a report from the childhood cancer survivor study. *J Clin Oncol.* 2017;35(14):1570. https://doi.org/10.1200/JCO.2016.70.1896

[13] Zeidman LA, Ankenbrandt WJ, Du H, Paleologos N, Vick NA. Growth rate of non-operated meningiomas. *J Neurol.* 2008;255(6):891-5. https://doi.org/10.1007/s00415-008-0801-2

[14] Louis DN, Perry A, Reifenberger G, et al. The 2016 World Health Organization classification of tumors of the central nervous system: a summary. *Acta Neuropathol.* 2016;131(6):803-20. https://doi.org/10.1007/s00401-016-1545-1

[15] Sahm F, Schrimpf D, Stichel D, et al. DNA methylation-based classification and grading system for meningioma: a multicentre, retrospective analysis. *Lancet Oncol.* 2017; 18(5):682-94. https://doi.org/10.1016/S1470-2045(17)30155-9

[16] Vernooij MW, Ikram MA, Tanghe HL, et al. Incidental findings on brain MRI in the general population. *N Engl J Med.* 2007; 357(18):1821-8. https://doi.org/10.1056/NEJMoa070972

[17] Hill CI, Nixon CS, Ruehmeier JL, Wolf LM. Brain tumors. *Phys Ther*. 2002;82(5):496-502. https://doi.org/10.1093/ptj/82.5.496

[18] Goldbrunner R, Minniti G, Preusser M, et al. EANO guidelines for the diagnosis and treatment of meningiomas. *Lancet Oncol*. 2016;17(9):e383-e91. https://doi.org/10.1016/S1470-2045(16)30321-7

[19] Preusser M, Brastianos PK, Mawrin C. Advances in meningioma genetics: novel therapeuticopportunities. *Nat Rev Neurol.* 2018;14(2):106. https://doi.org/10.1038/nrneurol.2017.168

[20] Mirian C, Duun-Henriksen AK, Juratli T, et al. Poor prognosis associated with TERT gene alterations in meningioma is independent of the WHO classification: an individual patient data meta-analysis. *J Neurol Neurosurg Psychiatry. 2*020;91(4):378-87. https://doi.org/10.1136/jnnp-2019-322257

[21] Paramasivam N, Hübschmann D, Toprak UH, et al. Mutational patterns and regulatory networks in epigenetic subgroups of meningioma. *Acta Neuropathol*. 2019;138(2):295-308. https://doi.org/10.1007/s00401-019-02008-w

[22] Prager BC, Vasudevan HN, Dixit D, et al. The Meningioma Enhancer Landscape Delineates Novel Subgroups and Drives Druggable Dependencies. *Cancer Discov*. 2020;10(11):1722-41. https://doi.org/10.1158/2159-8290.CD-20-0160

[23] AlSahlawi A, Aljelaify R, Magrashi A, et al. New insights into the genomic landscape of meningiomas identified FGFR3 in a subset of patients with favorable prognoses. *Oncotarget*. 2019;10(53):5549. https://doi.org/10.18632/oncotarget.27178

[24] Oya S, Kim S-H, Sade B, Lee JH. The natural history of intracranial meningiomas. *J Neurosurg*. 2011;114(5):1250-6. https://doi.org/10.3171/2010.12.JNS101623

[25] Gazzeri R, Galarza M, Gazzeri G. Growth of a meningioma in a transsexual patient after estrogen–progestin therapy. *N Engl J Med*. 2007;357(23):2411-2. https://doi.org/10.1056/nejmc071938

[26] Carlberg M, Hardell L. Pooled analysis of Swedish case-control studies during 1997-2003 and 2007-2009 on meningioma risk associated with the use of mobile and cordless phones. *Oncol. Rep.* 2015;33(6):3093-8. https://doi.org/10.3892/or.2015.3930

[27] Hosking F, Feldman D, Bruchim R, et al. Search for inherited susceptibility to radiation-associated meningioma by genomewide SNP linkage disequilibrium mapping. *Br J Cancer.* 2011;104(6):1049-54. https://doi.org/10.1038/bjc.2011.61

[28] Lee S, Karas PJ, Hadley CC, et al. The role of merlin/NF2 loss in meningioma biology. *Cancers.* 2019;11(11):1633. https://doi.org/10.3390/cancers11111633

[29] Evans DR. Neurofibromatosis type 2 (NF2): a clinical and molecular review. *Orphanet J. Rare Dis.* 2009;4(1):1-11. https://doi.org/10.1186/1750-1172-4-16

[30] Rouleau GA, Merel P, Lutchman M, et al. Alteration in a new gene encoding a putative membrane-organizing protein causes neuro-fibromatosis type 2. *Nature*. 1993;363(6429):515-21. https://doi.org/10.1038/363515a0

[31] Ruttledge MH, Sarrazin J, Rangaratnam S, et al. Evidence for the complete inactivation of the NF2 gene in the majority of sporadic meningiomas. *Nat Genet*. 1994;6(2):180-4. https://doi.org/10.1038/ng0294-180

[32] Clark VE, Erson-Omay EZ, Serin A, et al. Genomic analysis of non-NF2 meningiomas reveals mutations in TRAF7, KLF4, AKT1, and SMO. *Sci.* 2013;339(6123):1077-80. https://doi.org/10.1126/science.1233009

[33] Youngblood MW, Duran D, Montejo JD, et al. Correlations between genomic subgroup and clinical features in a cohort of more than 3000 meningiomas. *J Neurosurg.* 2019;133(5):1345-54. https://doi.org/10.3171/2019.8.JNS191266

[34] Harada T, Irving RM, Xuereb JH, et al. Molecular genetic investigation of the neurofibromatosis type 2 tumor suppressor gene in sporadic meningioma. *J Neurosurg.* 1996;84(5):847-51. https://doi.org/10.3171/jns.1996.84.5.0847

[35] Birzu C, Peyre M, Sahm F. Molecular alterations in meningioma: prognostic and therapeutic perspectives. *Curr Opin Oncol.* 2020;32(6):613-22. https://doi.org/10.1097/CCO.0000000000000687

[36] Benson D, Lipman DJ, Ostell J. GenBank. *Nucleic Acids Res.* 1993;21(13):2963-5. https://doi.org/10.1093/nar/21.13.2963

[37] Berman HM, Westbrook J, Feng Z, et al. The Protein Data Bank. *Nucleic Acids Res.* 2000;28(1):235-42. https://doi.org/10.1093/nar/28.1.235

[38] Howe KL, Achuthan P, Allen J, et al. Ensembl 2021. *Nucleic Acids Res.* 2021;49(D1):D884-D91. https://doi.org/10.1093/nar/gkaa942

[39] Sim N-L, Kumar P, Hu J, Henikoff S, Schneider G, Ng PC. SIFT web server: predicting effects of amino acid substitutions on proteins. *Nucleic Acids Res.* 2012;40(W1):W452-W7. https://doi.org/10.1093/nar/gks539

[40] Reva B, Antipin Y, Sander C. Predicting the functional impact of protein mutations: application to cancer genomics. *Nucleic Acids Res.* 2011;39(17):e118-e. https://doi.org/10.1093/nar/gkr407

[41] Choi Y, Chan AP. PROVEAN web server: a tool to predict the functional effect of amino acid substitutions and indels. *Bioinform.* 2015;31(16):2745-7. https://doi.org/10.1093/bioinformatics/btv195

[42] Breiman L. Random forests. *Mach Learn*. 2001;45(1):5-32. https://doi.org/10.1023/A:1010933404324

[43] Yang ZR. Neural networks. *Methods Mol Biol.* 2010;609:197-222.

[44] Cortes C, Vapnik V. Support-vector networks. *Mach Learn*. 1995;20(3):273-97. https://doi.org/10.1007/BF00994018

[45] Hecht M, Bromberg Y, Rost B. Better prediction of functional effects for sequence variants. *BMC Genom.* 2015;16(8):S1. https://doi.org/10.1186/1471-2164-16-S8-S1

[46] Al-Numair NS, Martin ACR. The SAAP pipeline and database: tools to analyze the impact and predict the pathogenicity of mutations. *BMC Genom*. 2013;14(3):S4. https://doi.org/10.1186/1471-2164-14-S3-S4

[47] Yates CM, Filippis I, Kelley LA, Sternberg MJ. SuSPect: enhanced prediction of single amino acid variant (SAV) phenotype using network features. *J Mol Biol.* 2014;426(14):2692-701. https://doi.org/10.1016/j.jmb.2014.04.026

[48] Capriotti E, Calabrese R, Fariselli P, Martelli PL, Altman RB, Casadio R. WS-SNPs&GO: a web server for predicting the deleterious effect of human protein variants using functional annotation. *BMC Genom*. 2013;14(3):1-7. https://doi.org/10.1186/1471-2164-14-S3-S6

[49] Ferrer-Costa C, Gelpí JL, Zamakola L, Parraga I, de la Cruz X, Orozco M. PMUT: a web-based tool for the annotation of pathological mutations on proteins. *Bioinformatics*. 2005;21(14):3176-8. https://doi.org/10.1093/bioinformatics/bti486

[50] Pejaver V, Urresti J, Lugo-Martinez J, et al. Inferring the molecular and phenotypic impact of amino acid variants with MutPred2. *Nat Commun.* 2020;11(1):5918. https://doi.org/10.1038/s41467-020-19669-x

[51] Capriotti E, Fariselli P. PhD-SNPg: a webserver and lightweight tool for scoring single nucleotide variants. *Nucleic Acids Res.* 2017;45(W1):W247-w52. https://doi.org/10.1093/nar/gkx369

[52] González-Pérez A, López-Bigas N. Improving the assessment of the outcome of nonsynonymous SNVs with a consensus deleteriousness

score, Condel. *Am J Hum Genet.* 2011;88(4):440-9. https://doi.org/10.1016/j.ajhg.2011.03.004

[53] Bendl J, Stourac J, Salanda O, et al. PredictSNP: robust and accurate consensus classifier for prediction of disease-related mutations. *PLoS Comput Biol.* 2014;10(1):e1003440. https://doi.org/10.1371/journal.pcbi.1003440

[54] Capriotti E, Altman RB, Bromberg Y. Collective judgment predicts disease-associated single nucleotide variants. *BMC Genom.* 2013;14(3):1-9. https://doi.org/10.1186/1471-2164-14-S3-S2

[55] Franceschini A, Szklarczyk D, Frankild S, et al. STRING v9. 1: protein-protein interaction networks, with increased coverage and integration. *Nucleic Acids Res.* 2012;41(D1):D808-D15. https://doi.org/10.1093/nar/gks1094

[56] Ng PC, Henikoff S. SIFT: Predicting amino acid changes that affect protein function. *Nucleic Acids Res.* 2003;31(13):3812-4. https://doi.org/10.1093/nar/gkg509

[57] Bromberg Y, Rost B. SNAP: predict effect of non-synonymous polymorphisms on function. *Nucleic Acids Res.* 2007;35(11):3823-35. https://doi.org/10.1093/nar/gkm238

[58] Frousios K, Iliopoulos CS, Schlitt T, Simpson MA. Predicting the functional consequences of non-synonymous DNA sequence variants—evaluation of bioinformatics tools and development of a consensus strategy. *Genomics.* 2013;102(4):223-8. https://doi.org/10.1016/j.ygeno.2013.06.005

[59] Arshad M, Bhatti A, John P. Identification and in silico analysis of functional SNPs of human TAGAP protein: A comprehensive study. *PloS One.* 2018;13(1):e0188143. https://doi.org/10.1371/journal.pone.0188143

[60] Aavikko M, Li S-P, Saarinen S, et al. Loss of SUFU function in familial multiple meningioma. *Am J Hum Genet.* 2012;91(3):520-6. https://doi.org/10.1016/j.ajhg.2012.07.015

[61] Kono H, Yuasa T, Nishiue S, Yura K. coliSNP database server mapping nsSNPs on protein structures. *Nucleic Acids Res.* 2007;36(suppl_1):D409-D13. https://doi.org/10.1093/nar/gkm801

[62] Yesilöz Ü, Kirches E, Hartmann C, et al. Frequent AKT1 E17K mutations in skull base meningiomas are associated with mTOR and ERK1/2 activation and reduced time to tumor recurrence. *Neuro-Oncol.* 2017;19(8):1088-96. https://doi.org/10.1093/neuonc/nox018

[63] Carpten JD, Faber AL, Horn C, et al. A transforming mutation in the pleckstrin homology domain of AKT1 in cancer. *Nature.* 2007;448(7152):439-44. https://doi.org/10.1038/nature05933

[64] Bacci C, Sestini R, Provenzano A, et al. Schwannomatosis associated with multiple meningiomas due to a familial SMARCB1 mutation. *Neurogenetics*. 2010;11(1):73-80. https://doi.org/10.1007/s10048-009-0204-2

[65] Twigg SR, Hufnagel RB, Miller KA, et al. A recurrent mosaic mutation in SMO, encoding the hedgehog signal transducer smoothened, is the major cause of Curry-Jones syndrome. *Am J Hum Genet*. 2016;98(6):1256-65. https://doi.org/10.1016/j.ajhg.2016.04.007

[66] Venselaar H, Te Beek TA, Kuipers RK, Hekkelman ML, Vriend G. Protein structure analysis of mutations causing inheritable diseases. An e-Science approach with life scientist friendly interfaces. *BMC Bioinform.* 2010;11(1):1-10. https://doi.org/10.1186/1471-2105-11-548