Histology and Histopathology

From Cell Biology to Tissue Engineering

Next generation sequencing identifies novel potential actionable mutations for grade I meningioma treatment

Francesco Pepe[1]*, Pasquale Pisapia[1]*, Maria Laura Del Basso de Caro[2], Floriana Conticelli[1], Umberto Malapelle[1], Giancarlo Troncone[1] and Juan Carlos Martinez[3]

[1]Department of Public Health, [2]Department of Advanced Biomedical Sciences, University of Naples "Federico II", Naples, Italy and [3]Department of Pathology, Gregorio Marañon University Hospital, Madrid, Spain

*These authors contributed equally

Summary. Meningiomas are common brain tumors that arise from the meningeal membranes that envelope the brain and spinal cord. The World Health Organization classifies these tumors into three histopathological grades. Because of tumor recurrence, treating meningiomas may be challenging even in well-differentiated grade I (GI) neoplasms. Indeed, around 5% of completely resected GI meningiomas relapse within 5 years. Therefore, identifying driver mutations in GI meningiomas through next generation sequencing (NGS) assays is paramount. The aim of this study was to validate the use of the 50-gene AmpliSeq Hotspot Cancer Panel v2 to identify the mutational status of 23 GI meningioma, namely, 12 non recurrent and 11 recurrent. In 18 out of the 23 GI meningiomas analyzed, we identified at least one gene mutation (78.2%). The most frequently mutated genes were *c-kit* (39.1%), *ATM* (26.1%), *TP53* (26.1%), *EGFR* (26.1%), *STK11* (21.7%), *NRAS* (17.4%), *SMAD4* (13%), *FGFR3* (13%), and *PTPN11* (13%); less frequent mutations were *SMARCB1* (8.7%), *FLT3* (8.7%), *KRAS* (8.7%), *FBWX7* (8.7%), *ABL1* (8.7%), *ERBB2* (8.7%), *IDH1* (8.7%), *BRAF* (8.7%), *MET* (8.7%), *HRAS* (4.3%), *RB1* (4.3%), *CTNNB1* (4.3%), *PIK3CA* (4.3%), *VHL* (4.3%), *KDR* (4.3%), *APC* (4.3%), *NOTCH1* (4.3%), *JAK3* (4.3%), and *SRC* (4.3%). To our knowledge, mutations in all of these genes, except for *TP53, STK11, SMARCB1, PIK3CA, VHL,* and *BRAF*, have never been described before in meningiomas. Hence, these findings demonstrate the viability of NGS to detect new genetic alterations in GI meningiomas. Equally important, this technology enabled us to detect possible novel actionable mutations not previously associated with GI and for which selective inhibitors already exist.

Key words: NGS, Biomarkers, Meningiomas

Introduction

Meningiomas, which are the most frequent tumors arising from the meningeal linings of the brain and spinal cord, account for about 36% of all primary central nervous system (CNS) tumors (Ostrom et al., 2016). The World Health Organization (WHO) classifies them into three histological grades: grade I (benign), grade II (atypical), and grade III (anaplastic) (Louis et al., 2007). Approximately 80% of these tumors belong to WHO grade I (GI), 15-20% to WHO grade II (GII), and 1-2 % to WHO grade III (GIII) (Mawrin and Perry, 2010). Typically, complete resection is considered the gold standard treatment for all three types of meningiomas. However, full resection does not safeguard against tumor recurrence. Indeed, within 5 years of surgery, relapse occurs in about 5% of GI, 40% of GII, and 50-80% of GIII meningiomas (Perry et al., 1999; Riemenschneider et al., 2006; Yang et al., 2008). Accordingly, in GIII meningiomas, surgical resection followed by radiotherapy is inevitable. To date, even chemotherapy has yielded poor outcomes (Chamberlain and Bhumenthal, 2004). Therefore, preventing recurrence

and choosing targeted treatments for incomplete resected meningiomas remain major challenges.

In the past, meningiomas were commonly associated with mutations in the neurofibromatosis 2 (*NF2*) tumor suppressor gene (TSG), accounting for up to 50% of cases (Fontaine et al., 1991; Ruttledge et al., 1994). Nowadays, however, the advent of cutting-edge sequencing platforms, namely next geneneration sequencing (NGS) technologies, has expanded the mutational landscape of cancer research by enabling simultaneous identification of multiple gene mutations in a single specimen (Malapelle et al., 2015). Indeed, NGS has led to the discovery of new gene mutations in human meningiomas including *TRAF7, AKT1, KLF4, SMO, PIK3CA, NOTCH2, SMARCB1, CHEK, SMARCE1,* and *POLR2A* (Brastianos et al., 2013; Clark et al., 2013; Smith et al., 2013). However, in a study by Clark et al. (2013) on 300 meningiomas, but restricted to only 5 genes (*NF2, TRAF7, AKT1, KLF4* and *SMO*), *TRAF7, AKT1, KLF4,* and *SMO* were found mutually exclusive of *NF2* and present mainly in GI meningiomas.

Generally, a fully resected benign tumor is not a matter of major concern. Oddly, though, in few cases, certain GI meningioma patients are prone to relapse despite having undergone complete resection. Accordingly, over the years, major attempts have been made to identify possible markers that could help predict relapse in such tumors, but to no avail. Thus, to gain some insight into the early development of meningiomas, we endeavored to identify gene mutations associated with low-grade meningiomas. Then, we investigated whether some of these mutations could be ascribable to tumor relapse and, therefore, serve as predictors of different biological behaviors. Finally, after having identified the molecular profile of patients' GI meningiomas, we addressed the possibility of developing customized therapies by targeting those specific gene alterations. As opposed to previous studies, we investigated a small rather than a large representative cohort of GI meningiomas and focused on a large rather than a small number of genes. For these analyses, we employed the Ion Torrent Cancer Hotspot Panel v2 (CHPv2)-a 50-gene NGS assay highly suitable for clinical testing (Tsongalis et al., 2014; Bellevicine et al., 2016).

Materials and methods

Samples, DNA extraction, and NGS analysis

Documented cases of GI meningiomas, previously diagnosed at the Servicio de Anatomía Patológica, Hospital Universitario Gregorio Marañón, Madrid, Spain, were reviewed. A group of 23 GI meningiomas (12 non recurrent and 11 recurrent) was chosen among the most common histopathological types: meningothelial (n=7), fibrous (n=4) and transitional (n=12); all patients had been followed up clinically for

10-19 years (Table 1). For each sample, representative H&E-stained slides, indicating the tissue areas with the highest density of neoplastic cells, were reviewed by an experienced neuropathologist (JCM) (Fig. 1A,B). After annotation of the percentage of neoplastic cells, DNA was extracted with the QIAamp DNA Mini Kit (Qiagen, Crawley, West Sussex, UK) from three 10-µm-thick serial sections, according to the manufacturer's instructions. DNA was first suspended in 30 µL of molecular biology water; then it was quantitatively and qualitatively evaluated with the Qubit photometer (Life Technologies, Carlsbad, CA) and the Qubit dsDNA High Sensitivity Assay Kit, according to the manufacturer's instructions.

The NGS library for each sample was prepared by applying the Ion AmpliSeq Library 96LV Kit 2.0 (Life Technologies) and the Ion AmpliSeq Hot-Spot Cancer Panelv2 (Life Technologies). As recommended by the manufacture's protocol, each sample contained at least 10 ng of DNA. This sequencing panel provides 207 amplicons covering 2,800 mutational hotspot regions in 50 genes, thereby yielding at least 500x sequence coverage for eight samples on a single Ion 316 chip. For samples yielding less than 10 ng DNA input, additional cycling conditions were used for the library preparation, as recommended by the manufacturer. Each library was barcoded with the Ion Xpress Barcode Adapters 1-16 Kit (Life Technologies). Barcoded libraries were combined with a final concentration of 100 pM. Template preparation by emulsion PCR (emPCR) was performed on the Ion OneTouch 2 system (Life Technologies). Library quality control was performed with the Ion Sphere Quality Control Kit. As recommended in the instruction manual, 10-30% of template positive Ion Sphere particles (ISPs) were targeted in the emPCR reaction. Sequencing primers and polymerases were added to the final enriched ISPs prior to loading onto 316 (100 Mb output) chips. Sequencing was carried out on a PGM (Life Technologies). Data analysis was carried out with Torrent Suite Software V.3.2 (Life Technologies). After alignment of the sequence reads to

Table 1. Patient characteristics.

Characteristics	Value
Patients, n	23
Male	9
Female	14
Age Range	21-80
Median	61
Non recurrent Meningiomas GI	12
Recurrent Meningiomas GI	11
Meningioma menigothelial	7
Meningioma fibrous	4
Meningioma transitional	12

GI, grade I.

the hg19 human reference genome, the Variant Caller plug-in was applied by using the Hotspot Cancer Panel file as reference (version 5.0.3.5). The Ion Reporter suite (Life Technologies) was used to filter polymorphic variants. In addition, all nucleotide variations with less than 1% variant frequency were filtered. All detected variants were manually reviewed with the Integrative Genomics Viewer (IGV V.2.1, Broad Institute, Cambridge, Massachusetts, USA).

Ethical approval

Ethical approval was obtained in compliance with current European laws and regulations for human tissues. All information regarding human material was managed using anonymous numerical codes, and all samples were handled in compliance with the Helsinki Declaration (http://www.wma.net/en/30publications/10policies/b3/).

Results

All GI meningioma samples (n=23) yielded satisfactory analytical parameters. In particular, one or more gene mutations were detected in 18 (78.2%) of the 23 GI meningiomas analyzed, and in 28 of the 50 panel genes (Table 2). A single gene mutation was observed in 3 patients (13.0%), double gene mutations in 4 patients (17.4%), and 3 or more gene mutations in 11 patients (47.8%). All the detected mutations and their respective gene function are reported in Table 2. No correlation was found between the mutations and the different histopathological types.

Interestingly, we identified several candidate mutated genes that, to the best of our knowledge, have never been associated with meningiomas. Among these were mutated genes from different cancer-related gene families including receptor tyrosine kinases [RTKs] (*c-KIT, FGFR3, EGFR, STK11, FLT3, ERBB2, KDR,* and
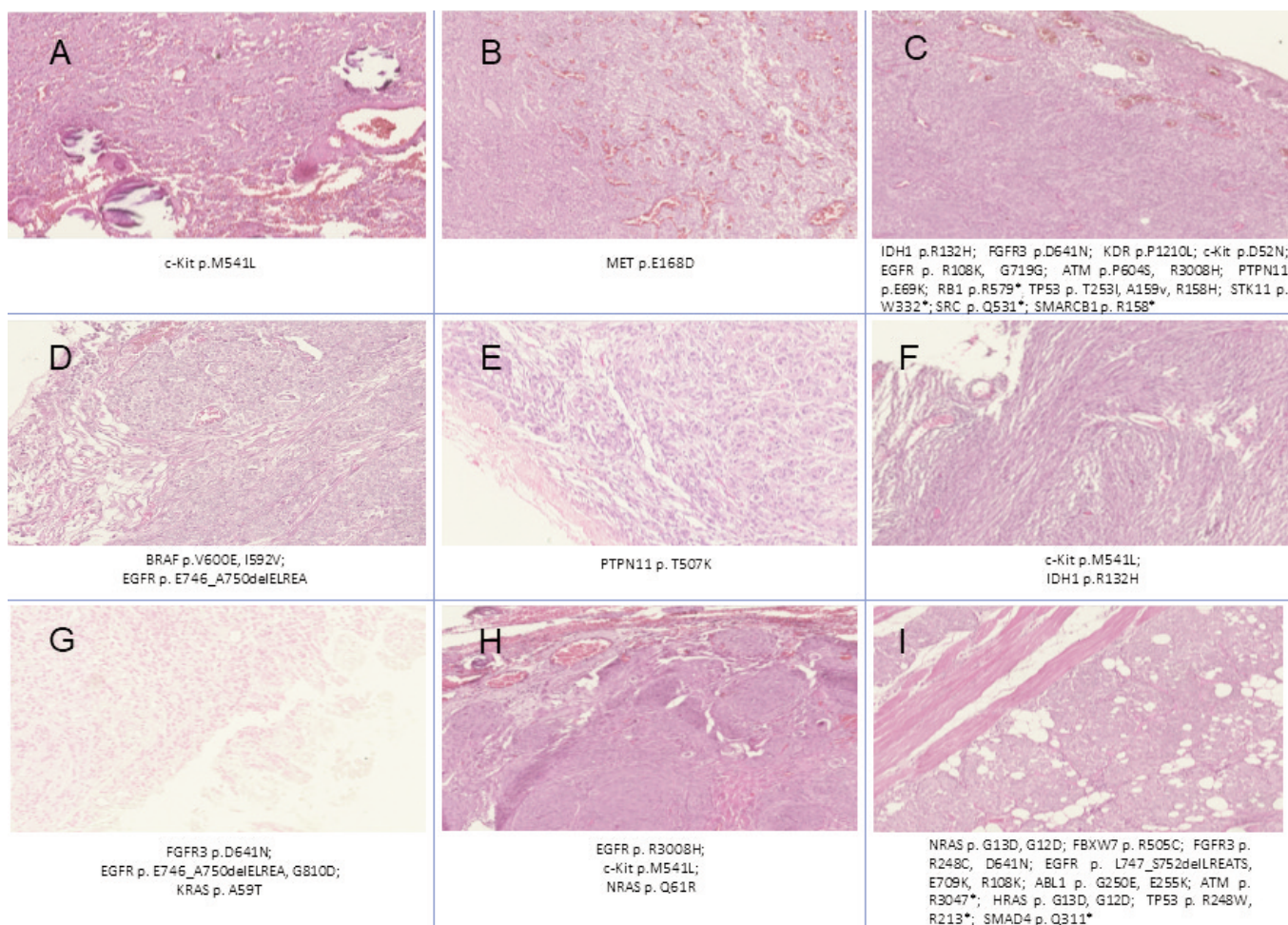
Fig. 1. A. H&E representative fields **(A-I)** of the n=18 mutated GI meningiomas with the concurrent mutations, identified by using the Ion AmpliSeq Hot-Spot Cancer Panel v2.

JAK3), tyrosine kinases [TKs] (*ABL, MET,* and *SRC)*, G proteins (*NRAS, KRAS,* and *HRAS)*, DNA repair genes (*TP53, ATM*), TGF-β signal transduction (*SMAD4*), protein kinases (*BRAF, PIK3CA*), protein tyrosine phosphatase [PTP] (*PTPN11*), oxidative decarboxylation 1 (*IDH1*), ubiquitin ligases (*FBXW7* and *VHL*), tumor suppressor genes [TSGs] (*RB, APC, CTNNB1,* and *NOTCH1*). Notably, these mutations, except for *TP53, SMARCB1, PIK3CA, VHL,* and *BRAF,* have never been reported in meningiomas, as evidenced by the COSMIC v85 Catalogue of Somatic Mutations in Cancer (https://cancer.sanger.ac.uk/cosmic).
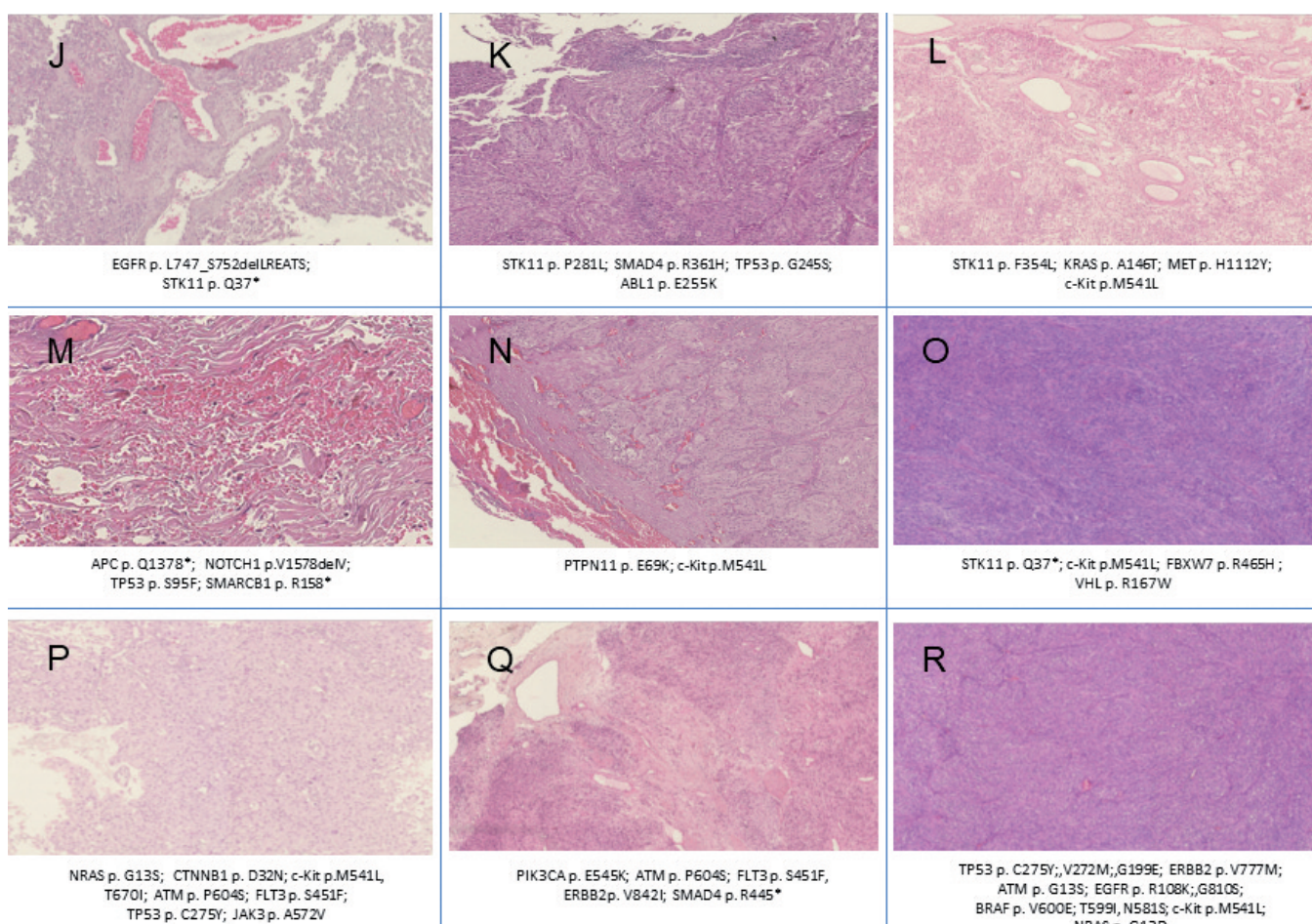
Discussion

Scientists studying the molecular drivers in meningiomas have long focused on one particular gene, namely, Neurofibromin 2 (merlin, *NF2*), whose loss of function occurs in 40% to 60% of patients (Riemenschneider et al., 2006). However, the advent of

NGS has enabled scientists to identify several other driver mutations in human meningiomas. For instance, a recent mutational analysis of a series of GI, GII, and GIII non-NF2 mutated meningiomas has detected four new mutations in *TRAF7, KLF4, AKT1*, and *SMO* (Clark et al., 2013).

In this context, we set out to identify new gene mutations in a small representative cohort of GI human meningiomas by using a NGS cancer-related gene panel (Ion AmpliSeq Hot-Spot Cancer Panel v2).

The *c-KIT* oncogene was the most frequently mutated gene. It was detected in 9 of the 23 meningiomas profiled in the study (39.1% cases). In particular, the encoded protein of this oncogene is a member of the receptor tyrosine kinase family. Alterations in *c-KIT* have been reported in other cancers, such as lung cancer, acute myeloid leukemia (AML), breast cancer, and astrocytomas (Zhao et al., 2014). In line with our results, previous studies have reported either overexpression or downregulation of c-kit protein

EGFR p. L747_S752delLREATS;
STK11 p. Q37*

STK11 p. P281L; SMAD4 p. R361H; TP53 p. G245S;
ABL1 p. E255K

STK11 p. F354L; KRAS p. A146T; MET p. H1112Y;
c-Kit p.M541L

APC p. Q1378*; NOTCH1 p.V1578delV;
TP53 p. S95F; SMARCB1 p. R158*

PTPN11 p. E69K; c-Kit p.M541L

STK11 p. Q37*; c-Kit p.M541L; FBXW7 p. R465H ;
VHL p. R167W

NRAS p. G13S; CTNNB1 p. D32N; c-Kit p.M541L,
T670I; ATM p. P604S; FLT3 p. S451F;
TP53 p. C275Y; JAK3 p. A572V

PIK3CA p. E545K; ATM p. P604S; FLT3 p. S451F,
ERBB2p. V842I; SMAD4 p. R445*

TP53 p. C275Y;,V272M;,G199E; ERBB2 p. V777M;
ATM p. G13S; EGFR p. R108K;,G810S;
BRAF p. V600E; T599I, N581S; c-Kit p.M541L;
NRAS p. G13D

Fig. 1. B. H&E representative fields **(J-R)** of the n=18 mutated GI meningiomas with the concurrent mutations, identified by using the Ion AmpliSeq Hot-Spot Cancer Panel v2.

expression in human meningiomas (Mawrin and Evert, 2007; Saini et al., 2012). Moreover, activating mutations in *c-kit* or *PDGFRA* genes are also involved in in gastrointestinal stromal tumors (GISTs), the most common mesenchymal tumor of the digestive tract (Heinrich et al., 2003; Lasota and Miettinen, 2006). Most of these tumors are sensitive to imatinib (Demetri et al., 2002; Heinrich et al., 2013), whose therapeutic effect depends on the presence and type of kit mutation, i.e., highest with exon 11 mutations and lowest with a wild-type phenotype (Hornick and Fletcher, 2007). In our series of GI meningiomas, 8 of the 9 mutated cases displayed p.M541L mutation in exon 10. Interestingly, patients with chronic eosinophilic leukemia harboring this mutation seem to respond to low doses of imatinib (Iurlo et al., 2014).

To the best of our knowledge and according to the COSMIC v85 Catalogue of Somatic Mutations in Cancer, our study reports for the first time *c-KIT* mutations in GI meningiomas. This finding may be clinically significant especially when the location of the tumor hampers full resection, thereby increasing the chances of tumor relapse. However, given the low number of patients involved in our study, larger studies are warranted to estimate the frequency of this mutation and to test the potential therapeutic effect of imatinib on GI meningioma patients.

The *ATM* gene was mutated in 6 out of 23 cases (26.1%): c.1810C>T (substitution, position 1810, C→T). *ATM* is a nuclear protein that plays an essential role in the recognition of double-strand DNA breaks and repair, thereby allowing the stability of genetic information. Alterations in this gene have been associated with high sensitivity to PARP inhibitors in different human cancers (Bouwman and Jonkers, 2012).

Mutations in the *TP53* gene (one of the most frequently mutated genes in cancer) were detected in 6 out of 23 cases (26.1%). Like the ATM protein, the p53 protein is essential for recognition and repair of damaged DNA (Lane, 1992). We hypothesize that the deleterious effect of ATM and p53 inactivation on DNA repair may allow accumulation of genetic alterations that, in turn, may contribute to meningioma pathogenesis and progression.

Mutations in the epidermal growth factor receptor (*EGFR*) gene were identified in 6 out of 23 cases (26.1%). This is consistent with earlier findings showing EGFR protein overexpression in human meningiomas, evidence that substantiates the involvement of *EGFR* in meningioma tumorigenesis (Arnli et al., 2017).

A previous study by Bujko et al. on a series of menigiomas revealed no mutations in *EGFR, KRAS*, and

Table 2. Mutation detected in n=23 meningiomas by using Cancer Hot Spot NGS Panel v2.

Gene	Mutated cases	Mutations (%)	Non Recurrent	Recurrent	Function
c-kit	9/23	39.1%	5	4	RTK
ATM	6/23	26.1%	2	4	DNA repair
TP53	6/23	26.1%	2	4	DNA repair
EGFR	6/23	26.1%	1	5	RTK
STK11	5/23	21.7%	3	2	RTK
NRAS	4/23	17.4%	1	3	G protein (signal transduction)
SMAD4	3/23	13.0 %	1	2	TGF-B (signal transduction)
FGFR3	3/23	13.0 %	1	2	RTK
PTPN11	3/23	13.0 %	2	1	PTP
SMARCB1	2/23	8.7%	1	1	TSG
FLT3	2/23	8.7%	0	2	RTK
KRAS	2/23	8.7%	1	1	G protein (signal transduction)
FBXW7	2/23	8.7%	0	2	F-box protein (Ubiquitin ligase)
ABL1	2/23	8.7%	1	1	TK
ERBB2	2/23	8.7%	0	2	RTK
IDH1	2/23	8.7%	2	0	Prevents oxidative damage
BRAF	2/23	8.7%	0	2	PK
MET	2/23	8.7%	2	0	TK
HRAS	1/23	4.3%	0	1	G protein (signal transduction)
RB1	1/23	4.3%	1	0	TSG
CTNNB1	1/23	4.3%	0	1	PTP
PIK3CA	1/23	4.3%	0	1	PK
VHL	1/23	4.3%	0	1	TSG
KDR	1/23	4.3%	1	0	RTK
APC	1/23	4.3%	0	1	TSG
NOTCH1	1/23	4.3%	0	1	TSG
JAK3	1/23	4.3%	0	1	RTK
SRC	1/23	4.3%	1	0	TK

PK, Protein Kinase; PP, Protein SMARCB1Phosphatase; PTP, Protein Tyrosine Phosphatase; RTK, Receptor Tyrosine Kinase; TK, Tyrosine Kinase; TSG, Tumor Suppressor Gene.

BRAF (Bujko et al., 2014). On the contrary, our NGS analysis detected the following EGFR alterations: p.R108K in exon 3, p.G719D in exon 18, p.G810S and p.G810D in exon 20; deletion mutation of p.E746_A750delELREA, p.L747_S752delLREATS, and p.L747_S752delLREATS was detected in exon 19. Remarkably, this is the first study reporting *EGFR* mutations in GI human meningiomas. A possible explanation for these discrepancies is that Bujko et al adopted Sanger sequencing, a less advanced and sensitive technique than the more recent NGS used in our study. Indeed, NGS has enabled scientists to uncover new driver mutations not previously revealed by the Sanger technique, thereby expanding the genomic landscape of human tumors and opening the possibility of more efficient genomic-guided personalized treatments.

Luckily, *EGFR* can be inhibited by specific drugs. Accordingly, an ongoing active search for *EGFR* inhibitors is showing promising results. For example, it has recently led to the development of first, second, and third generation *EGFR* inhibitors for the treatment of lung cancer patients (Mancini et al., 2018). In our study, *EGFR* mutations were present in 5 recurrent meningiomas and in 1 non recurrent. Although this finding suggests that the EGFR mutation is associated with a recurrent phenotype, the small number of cases in this series does not allow us to draw significant conclusions. Consequently, large-scale studies addressing whether this mutation is related to recurrent behavior and whether *EGFR* may be a potential candidate for targeted therapy against GI meningiomas are strongly warranted.

LKB1/STK11, a well known tumor suppressor gene, was found mutated in 5 of the 23 cases studied (21.7%). *LKB1* mutation in lung cancer can lead to COX-2 up-regulation, resulting in apoptosis resistance, angiogenesis, invasion, and metastasis (Taketo, 1998; Wolff et al., 1998; Brown and Dubois, 2004). COX-2, which is involved in prostaglandin biosynthesis, is overexpressed in breast, prostate, lung, colorectal, and ovarian cancers (Cianchi et al., 2001; Gupta et al., 2003; Kulp et al., 2004; Wang and Dubois, 2004). Interestingly, clinical studies have demonstrated that nimesulide, a COX-2 inhibitor, has antitumor activity against non–small cell lung cancer cell lines (Hida et al., 2000). The potential therapeutic application to meningiomas with LKB1 mutations deserves further research.

NRAS, KRAS, and *HRAS* were mutated in 4 out of 23 cases (17.4%), in 2 out of 23 cases (8.7%), and 1 out of 23 cases (4.3%), respectively. These findings corroborate previous research indicating that RAS overexpression in GI meningioma is linked to its pathogenesis (Jiang et al., 2017). Deregulation of the RAS/RAF/MEK pathway plays a central role in cancer cell proliferation and frequently occurs in many human tumors. For this reason, the identification of RAS inhibitors has been a very active field of research.

Initially, although farnesyl transferase inhibitors showed interesting *in vitro* results, they failed at the clinical level. Nowadays, the inhibition of RAS downstream kinases RAF and MEK seems a more promising strategy (Rusconi et al., 2012).

The *SMAD4* gene was mutated in 3 out of 23 cases (13%). The *SMAD4* protein is a signal transductor of the transforming growth factor beta (*TGF-β*) signaling pathway. SMAD proteins are located in the cytoplasm and, upon activation, form a protein complex which is translocated to the nucleus where it acts as a transcription factor and a tumor suppressor (McCarthy and Chetty, 2018). Accordingly, alterations in the *SMAD4* pathway increase proliferation by inhibiting apoptosis, a phenomenon that might contribute to meningioma development (Werner et al., 2017). Consistently, our results suggest that *SMAD4* alteration might have a role in meningioma pathogenesis.

Mutations in the *FGFR3* gene, encoding another RTK protein, were found in 3 of the 23 cases (13%) analyzed. This result is in line with previous studies indicating that *FGFR3* is expressed not only in human leptomeninges but also in GI and GII meningiomas (Johnson et al., 2010). In particular, *FGFR3* expression is associated with p-STAT3, p-Akt, and p-p44/42 MAPK expression, ultimately suggesting that *FGFR3* activation stimulates meningioma proliferation by activating the phosphoinositide 3 kinase-Akt-PRAS40-mTOR and STAT3 pathways (Johnson et al., 2010). Thus, our results further expand the possibility that *FGFR3* alterations may have clinical relevance in meningioma development.

The *PTPN11* gene was mutated in 3 out of 23 cases (13%). The *PTPN11* gene encodes for the SHP-2 protein tyrosine phosphatase, which regulates the RAS/MAPK signaling pathway involved in proliferation, migration, and apoptosis. Studies have shown that when this gene is mutated, it acquires a tumorigenic potential (Roccograndi et al., 2017). Accordingly, developing PTP inhibitors capable of reducing SHP2 activity has been a major challenge for many years. A promising breakthrough in this field has been the recent discovery of the allosteric inhibitor SHP099 (Chen et al., 2016). The presence of PTPN11 gene mutation in 3 out of 23 samples, suggests the possibility of using SHP2 inhibitors in targeted therapies against some types meningiomas.

Mutations in the *SMARCB1* gene were observed in 2 out of 23 cases (8.7%). The *SMARCB1* gene regulates gene expression through chromatin remodeling. This gene was previously found mutated in 4 out of 126 meningiomas, suggesting that SMARCB1 is associated with meningioma pathogenesis (Schmitz et al., 2001). Interestingly, a later study reports mutations in the *SMARCB1* gene of family members with multiple schwannomas and meningiomas, arguing that meningiomas may also be part of the schwannomatosis tumor spectrum (Bacci et al., 2010). Our NGS analysis is in line with these studies by revealing mutated

SMARCB1 in 8.7% of our cases.

Another gene of interest emerging from our study was *FLT3*. Its protein is a receptor tyrosine kinase, sensitive to drugs already available on the market, including linifanib (Shi et al., 2018). In our study, we found, for the first time, mutated forms of this gene in 2 out of 23 cases (8.7%).

BRAF mutations were identified in 2 patients (8.7%) with recurrence both recurrent cases. In a previous study, Mordechai et al. reported a *BRAF* mutation in a child with metastatic high grade rhabdoid meningioma. The *BRAF* inhibitor dabrafenib yielded positive treatment outcomes (Mordechai et al., 2015).

Isocitrate dehydrogenase 1 (*IDH1*) mutation was identified in 2 non-recurrent cases (8.7%). In brief, *IDH1* is an enzyme involved in cellular metabolism, epigenetic regulation, redox states, DNA repair, and oxidative damage mitigation (Molenaar et al., 2014). Although originally observed in brain gliomas, *IDH1* mutations have been later documented in other cancer types, including sarcomas, hematologic malignancies, colon cancer, and thyroid cancer (Raineri and Mellor, 2018). To the best of our knowledge, this is the first description of *IDH1* mutations in human GI meningiomas. Clinical trials based on inhibitors of mutant-*IDH* enzymes are ongoing. Indeed, phase I clinical trials of IDH inhibitors in adults with hematologic malignancies are showing promising results (Mondesir et al., 2016).

Less frequently, we observed mutations in several other genes, including *FBXW7, ABL1, ERBB2, MET, RB1, CTNNB1, PIK3CA, VHL, KDR, APC, NOTCH1, JAK3*, and *SRC*.

In conclusion, to the best of our knowledge, this is the first study reporting mutations in 22 cancer genes that have never been associated with human GI meningiomas. Although most mutations were found in both non recurrent and recurrent cases, we did see a molecular pattern associated with GI melanoma recurrence. For instance, *EGFR* was mutated in 5 recurrent cases vs 1 non mutated case. *ERBB2, FLT3, and BRAF* were mutated in 2 recurrent cases and not in non recurrent cases. On the contrary, the *IDH1* mutation was absent in all recurrent cases but present in 2 non recurrent cases. This evidence suggests that some mutations can indeed be associated with recurrence.

Altogether, our study highlights that the complex genomic landscape of GI human meningiomas may include potentially actionable genetic alterations. In this setting, NGS plays an essential role in the identification of meningioma driver mutations in humans. Indeed, thanks to this sequencing tool, we were able to identify a number of mutations that had never been identified in GI meningiomas. Notably, some of these mutations were found in patients with recurrence. Moreover, the fact that some existing drugs have proven effective in treating different types of tumors sharing similar oncogenetic drivers may have a promising impact on the way GI meningioma is studied and treated today. In fact, despite advances in surgery, radiation therapy, and stereotactic radiosurgery, a subset of patients remains refractory to these types of treatments. Therefore, the implementation of NGS technologies to detect actionable genetic alterations could be highly useful in clinical practice not only to predict meningioma recurrence but also to develop targeted treatments based on existing drugs.

References

Arnli M.B., Backer-Grøndahl T., Ytterhus B., Granli U.S., Lydersen S., Gulati S. and Torp S.H. (2017). Expression and clinical value of EGFR in human meningiomas. PeerJ. 5, e3140.

Bacci C., Sestini R., Provenzano A., Paganini I., Mancini I., Porfirio B., Vivarelli R., Genuardi M. and Papi L. (2010). Schwannomatosis associated with multiple meningiomas due to a familial SMARCB1 mutation. Neurogenetics 11, 73-80.

Bellevicine C., Sgariglia R., Malapelle U., Vigliar E., Nacchio M., Ciancia G., Eszlinger M., Paschke R. and Troncone G. (2016). Young investigator challenge: Can the Ion AmpliSeq Cancer Hotspot Panel v2 be used for next-generation sequencing of thyroid FNA samples?. Cancer Cytopathol. 124, 776-784.

Bouwman P and Jonkers J. (2012). The effects of deregulated DNA damage signalling on cancer chemotherapy response and resistance. Nat. Rev. Cancer. 12, 587-598.

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., Raza A., Sunkavalli A., Macconaill L.E., Stemmer-Rachamimov A.O., Louis D.N., Hahn W.C., Dunn I.F. and Beroukhim R. (2013). Genomic sequencing of meningiomas identifies oncogenic SMO and AKT1 mutations. Nat. Genet. 45, 285-289.

Brown J.R. and DuBois R.N. (2004). Cyclooxygenase as a target in lung cancer. Clin. Cancer Res. 10, 4266s-4269s.

Bujko M., Kober P., Tysarowski A., Matyja E., Mandat T., Bonicki W. and Siedlecki J.A. (2014). EGFR, PIK3CA, KRAS and BRAF mutations in meningiomas. Oncol. Lett. 7, 2019-2022.

Chamberlain M.C. and Blumenthal D.T. (2004). Intracranial meningiomas: diagnosis and treatment. Expert Rev. Neurother. 4, 641-648.

Chen Y.N., LaMarche M.J., Chan H.M., Fekkes P., Garcia-Fortanet J., Acker M.G., Antonakos B., Chen C.H., Chen Z., Cooke V.G., Dobson J.R., Deng Z., Fei F., Firestone B., Fodor M., Fridrich C., Gao H., Grunenfelder D., Hao H.X., Jacob J., Ho S., Hsiao K., Kang Z.B., Karki R., Kato M., Larrow J., La Bonte L.R., Lenoir F., Liu G., Liu S., Majumdar D., Meyer M.J., Palermo M., Perez L., Pu M., Price

E., Quinn C., Shakya S., Shultz M.D., Slisz J., Venkatesan K., Wang P., Warmuth M., Williams S., Yang G., Yuan J., Zhang J.H., Zhu P., Ramsey T., Keen N.J., Sellers W.R., Stams T. and Fortin P.D. (2016). Allosteric inhibition of SHP2 phosphatase inhibits cancers driven by receptor tyrosine kinases. Nature. 535, 148-152.

Cianchi F., Cortesini C., Bechi P., Fantappiè O., Messerini L., Vannacci A, Sardi I., Baroni G., Boddi V., Mazzanti R. and Masini E. (2001). Up-regulation of cyclooxygenase 2 gene expression correlates with tumor angiogenesis in human colorectal cancer. Gastroenterology 121, 1339-1347.

Clark V.E., Erson-Omay E.Z., Serin A., Yin J., Cotney J., Ozduman K., Avşar T., Li J., Murray P.B., Henegariu O., Yilmaz S., Günel J.M., Carrión-Grant G., Yilmaz B., Grady C., Tanrikulu B., Bakircioğlu M., Kaymakçalan H., Caglayan A.O., Sencar L., Ceyhun E., Atik A.F., Bayri Y., Bai H., Kolb L.E., Hebert R.M., Omay S.B., Mishra-Gorur K., Choi M., Overton J.D., Holland E.C., Mane S., State M.W., Bilgüvar K., Baehring J.M., Gutin P.H., Piepmeier J.M., Vortmeyer A., Brennan C.W., Pamir M.N., Kiliç T., Lifton R.P., Noonan J.P., Yasuno K. and Günel M. (2013). Genomic analysis of non-NF2 meningiomas reveals mutations in TRAF7, KLF4, AKT1, and SMO. Science 339, 1077-1080.

Demetri G.D., von Mehren M., Blanke C.D., Van den Abbeele A.D., Eisenberg B., Roberts P.J., Heinrich M.C., Tuveson D.A., Singer S., Janicek M., Fletcher J.A., Silverman S.G., Silberman S.L., Capdeville R., Kiese B., Peng B., Dimitrijevic S., Druker B.J., Corless C., Fletcher C.D. and Joensuu H. (2002). Efficacy and safety of imatinib mesylate in advanced gastrointestinal stromal tumors. N. Engl. J. Med. 347, 472-480.

Fontaine B., Rouleau G.A., Seizinger B.R., Menon A.G., Jewell A.F., Martuza R.L. and Gusella J.F. (1991). Molecular genetics of neurofibromatosis 2 and related tumors (acoustic neuroma and meningioma). Ann. NY Acad. Sci. 615, 338-343.

Gupta R.A., Tejada L.V., Tong B.J., Das S.K., Morrow J.D., Dey S.K. and DuBois R.N. (2003). Cyclooxygenase-1 is overexpressed and promotes angiogenic growth factor production in ovarian cancer. Cancer Res. 63, 906-911.

Heinrich M.C., Corless C.L., Duensing A., McGreevey L., Chen C.J., Joseph N., Singer S., Griffith D.J., Haley A., Town A., Demetri G.D., Fletcher C.D. and Fletcher J.A. (2003). PDGFRA activating mutations in gastrointestinal stromal tumors. Science 299, 708-710.

Hida T., Kozaki K., Muramatsu H., Masuda A., Shimizu S., Mitsudomi T., Sugiura T., Ogawa M. and Takahashi T. (2000). Cyclooxygenase-2 inhibitor induces apoptosis and enhances cytotoxicity of various anticancer agents in non-small cell lung cancer cell lines. Clin. Cancer Res. 6, 2006-2011.

Hornick J.L. and Fletcher C.D. (2007). The role of KIT in the management of patients with gastrointestinal stromal tumors. Hum. Pathol. 38, 679-687.

Iurlo A., Gianelli U., Beghini A., Spinelli O., Orofino N., Lazzaroni F., Cambiaghi S., Intermesoli T., Rambaldi A. and Cortelezzi A. (2014). Identification of kit(M541L) somatic mutation in chronic eosinophilic leukemia, not otherwise specified and its implication in low-dose imatinib response. Oncotarget 5, 4665-4670.

Kulp S.K., Yang Y.T., Hung C.C., Chen K.F., Lai J.P., Tseng P.H., Fowble J.W., Ward P.J. and Chen C.S. (2004). 3-phosphoinositide-dependent protein kinase-1/Akt signaling represents a major cyclooxygenase-2-independent target for celecoxib in prostate cancer cells. Cancer Res. 64, 1444-1451.

Jiang C., Song T., Li J., Ao F., Gong X., Lu Y., Zhang C., Chen L., Liu

Y., He H. and Huang O. (2017). RAS Promotes Proliferation and Resistances to Apoptosis in Meningioma. Mol. Neurobiol. 54, 779-787.

Johnson M.D., O'Connell M.J., Pilcher W. and Reeder J.E. (2010). Fibroblast growth factor receptor-3 expression in meningiomas with stimulation of proliferation by the phosphoinositide 3 kinase-Akt pathway. J. Neurosurg. 112, 934-939.

Lane D.P. (1992). Cancer p53, guardian of the genome. Nature. 358, 15-16.

Lasota J. and Miettinen M. (2006). KIT and PDGFRA mutations in gastrointestinal stromal tumors (GISTs). Semin. Diagn. Pathol. 23, 91-102.

Louis D.N., Ohgaki H., Wiestler O.D., Cavenee W.K., Burger P.C., Jouvet A., Scheithauer B.W. and Kleihues P. (2007). The 2007 WHO classification of tumours of the central nervous system. Acta Neuropathol. 114, 97-109.

Malapelle U., Vigliar E., Sgariglia R., Bellevicine C., Colarossi L., Vitale D., Pallante P. and Troncone G. (2015). Ion Torrent next-generation sequencing for routine identification of clinically relevant mutations in colorectal cancer patients. J. Clin. Pathol. 68, 64-68

Mancini M., Gal H., Gaborit N., Mazzeo L., Romaniello D., Salame T.M., Lindzen M., Mahlknecht G., Enuka Y., Burton D.G., Roth L., Noronha A., Marrocco I., Adreka D., Altstadter R.E., Bousquet E., Downward J., Maraver A., Krizhanovsky V. and Yarden Y. (2018). An oligoclonal antibody durably overcomes resistance of lung cancer to third-generation EGFR inhibitors. EMBO Mol. Med. 10, 294-308.

Mawrin C. and Evert M. (2007). Meningiomas do not express CD117 (KIT). Histopathology 51, 426-427.

Mawrin C. and Perry A. (2010). Pathological classification and molecular genetics of meningiomas. J. Neurooncol. 99, 379-391.

McCarthy A.J. and Chetty R. (2018). Smad4/DPC4. J. Clin. Pathol. 71, 661-664.

Molenaar R.J., Radivoyevitch T., Maciejewski J.P., van Noorden C.J. and Bleeker F.E. (2014). The driver and passenger effects of isocitrate dehydrogenase 1 and 2 mutations in oncogenesis and survival prolongation. Biochim. Biophys Acta 1846, 326-341.

Mondesir J., Willekens C., Touat M. and de Botton S. (2016). IDH1 and IDH2 mutations as novel therapeutic targets: current perspectives. J. Blood Med. 7, 171-180.

Mordechai O., Postovsky S., Vlodavsky E., Eran A., Constantini S., Dotan E., Cagnano E. and Weyl-Ben-Arush M. (2015). Metastatic rhabdoid meningioma with BRAF V600E mutation and good response to personalized therapy: case report and review of the literature. Pediatr. Hematol. Oncol. 32, 207-211.

Ostrom Q.T., Gittleman H., Xu J., Kromer C., Wolinsky Y., Kruchko C. and Barnholtz-Sloan J.S. (2016). CBTRUS statistical report: primary brain and central nervous system tumors diagnosed in the United States; 2009-2013. Neuro. Oncol. 18, (suppl 5), v1-v75.

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 85, 2046-2056.

Raineri S. and Mellor J. (2018). IDH1: Linking Metabolism and Epigenetics. Front Genet. 9, 493.

Riemenschneider M.J., Perry A. and Reifenberger G. (2006). Histological classification and molecular genetics of meningiomas. Lancet Neurol. 5, 1045-1054.

Roccograndi L., Binder Z.A., Zhang L., Aceto N., Zhang Z., Bentires-Alj M., Nakano I., Dahmane N. and O'Rourke D.M. (2017). SHP2

regulates proliferation and tumorigenicity of glioma stem cells. J. Neurooncol. 135, 487-496.

Rusconi P., Caiola E. and Broggini M. (2012). RAS/RAF/MEK inhibitors in oncology. Curr. Med. Chem. 19, 1164-1176.

Ruttledge M.H., Sarrazin J., Rangaratnam S., Phelan C.M., Twist E., Merel P., Delattre O., Thomas G., Nordenskjöld M. and Collins V.P. (1994). Evidence for the complete inactivation of the NF2 gene in the majority of sporadic meningiomas. Nat. Genet. 6, 180-184.

Saini M., Jha A.N., Abrari A. and Ali S. (2012). Expression of proto-oncogene KIT is up-regulated in subset of human meningiomas. BMC Cancer 12, 212.

Schmitz U., Mueller W., Weber M., Sévenet N., Delattre O. and von Deimling A. (2001). INI1 mutations in meningiomas at a potential hotspot in exon 9. Br. J. Cancer 84, 199-201.

Smith M.J., O'Sullivan J., Bhaskar S.S., Hadfield K.D., Poke G., Caird J., Sharif S., Eccles D., Fitzpatrick D., Rawluk D., du Plessis D., Newman W.G. and Evans D.G. (2013). Loss-of-function mutations in SMARCE1 cause an inherited disorder of multiple spinal meningiomas. Nat. Genet. 45, 295-298.

Shi Z.H., Liu F.T., Tian H.Z., Zhang Y.M., Li N.G. and Lu T. (2018). Design, synthesis and structure-activity relationship of diarylureas with novel isoxazol [3,4-b]pyridine-3-amino-structure as multi-target inhibitors against receptor tyrosine kinase. Bioorg. Med. Chem. 26, 4735-4744.

Taketo M.M. (1998). Cyclooxygenase-2 inhibitors in tumorigenesis (part I). J. Natl. Cancer Inst. 90, 1529-1536.

Tsongalis G.J., Peterson J.D., de Abreu F.B., Tunkey C.D., Gallagher T.L., Strausbaugh L.D., Wells W.A. and Amos C.I. (2014). Routine use of the Ion Torrent AmpliSeq™ Cancer Hotspot Panel for identification of clinically actionable somatic mutations. Clin. Chem. Lab. Med. 52, 707-714.

Wang D. and Dubois R.N. (2004). Cyclooxygenase-2: a potential target in breast cancer. Semin Oncol. 31, 64-73.

Werner T.V., Hart M., Nickels R., Kim Y.J., Menger M.D., Bohle R.M., Keller A., Ludwig N. and Meese E. (2017). MiR-34a-3p alters proliferation and apoptosis of meningioma cells *in vitro* and is directly targeting SMAD4, FRAT1 and BCL2. Aging (Albany NY). 9, 932-954.

Wolff H., Saukkonen K., Anttila S., Karjalainen A., Vainio H. and Ristimäki A. (1998). Expression of cyclooxygenase-2 in human lung carcinoma. Cancer Res. 58, 4997-5001.

Yang S.Y., Park C.K., Park S.H., Kim D.G., Chung Y.S. and Jung H.W. (2008). Atypical and anaplastic meningiomas: prognostic implications of clinicopathological features. J. Neurol. Neurosurg. Psychiatry 79, 574-580.

Zhao F., Chen Y., Wu Q., Wang Z. and Lu J. (2014). Prognostic value of CD117 in cancer: a meta-analysis. Int. J. Clin. Exp. Pathol. 15, 1012-1021.