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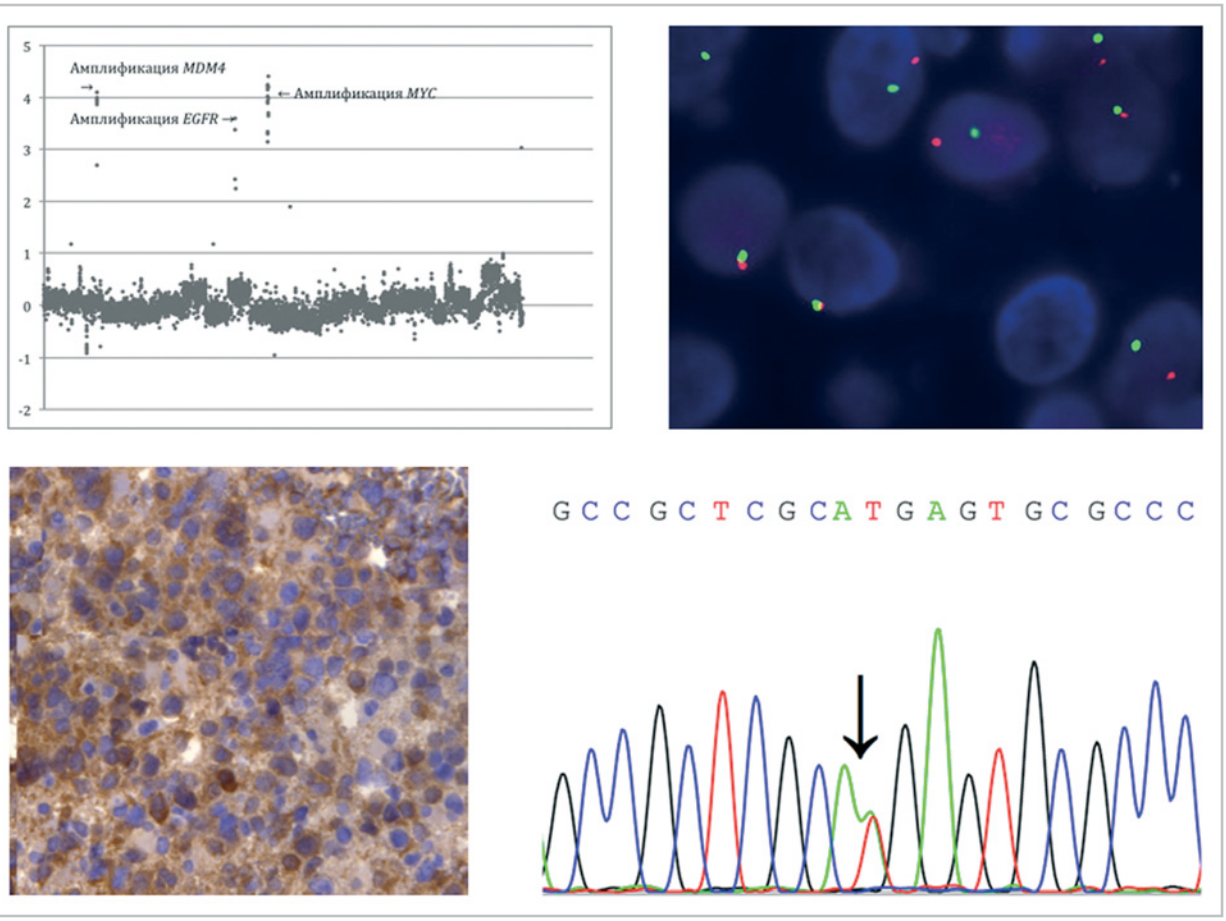

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PRACTICAL NOTES

In accordance with the resolution of the Higher Attestation Commission of the Ministry of Education and Science of the Russian Federation, the N.N. Burdenko Journal of Neurosurgery was included in the List of Leading Peer-Reviewed Journals and Periodicals issued in the Russian Federation where the main results of Candidate and Doctor Theses are recommended to be published.

Comparative Characteristics of Genetic Aberrations in Glioblastomas in Children and Adults

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Glioblastomas in children and adults are a heterogeneous group of tumors that can be divided into at least three different subgroups: pediatric glioblastomas, IDH1 mutant adult glioblastomas (a subtype with the most favorable prognosis), and IDH1 wild-type adult glioblastomas. The frequency of observed cytogenetic aberrations (amplification of MYC/MYCN, EGFR and PDGRFA oncogenes, homozygous deletion of CDKN2A and PTEN deletion) reveals that pediatric glioblastomas display similarities to the IDH1 mutant adult glioblastoma subtype.

Keywords: *pediatric glioblastoma, IDH1 mutant adult glioblastoma*

Glioblastomas are highly malignant brain tumors with predominantly astrocytic differentiation, which affect mostly adults with peak incidence between 45 and 75 years of age [14].

The genome of glioblastomas contains numerous aberrations: amplifications of *EGFR, PDGFRA, MDM2, CDK4* and *CDK6* oncogenes, homozygous deletion of *CDKN2A*, *PTEN* deletion, and *TP53* mutation. Most of these aberrant genes are involved in regulatory processes of cell cycle, cell proliferation, invasion and migration [16, 22]. The pathogenesis of glioblastomas also involves numerous interplaying signaling pathways with *EGFR / RAS / NF1 / PTEN / PI3K; TP53 / MDM2/MDM4/p14ARF* and *p16INK4a/CDK4/RB1* [18] being the major ones.

Glioblastomas in children are less common and amount to ~15% of all brain tumors [1, 3]. It may explain why glioblastomas in children and adults were for a long time considered together. Indeed, histologically indistinguishable tumors in children and adults have equally unfavorable prognosis.

The main difference lies in pathogenesis of these tumors; patients from the two age groups display markedly different sets of cytogenetic aberrations, including *MYC*, *MYCN*, *EGFR* and *PDGRFA* amplifications, homozygous deletion of *CDKN2A* and *PTEN* deletion, which indicates that different molecular pathways are involved in the development of tumors in children and adults [3, 15, 17, 21].

Epigenetic events that contribute to glioblastoma development include DNA methylation, post-translational histone modification and post-transcriptional regulation of microRNA gene expression [6, 8].

The discovery of *IDH1* [20] and *H3F3A* [23] mutations was the pivotal moment in the studies of the glioblastoma genome and elaboration of their molecular classification.

Isocitrate dehydrogenase (IDH) is an enzyme catalyzing the oxidative decarboxylation of isocitrate, producing ketoglutarate and CO_2. The *IDH1* gene is located on the long arm of chromosome 2 (2q33.3) and encodes NADP+-dependent IDH. In glioblastomas, point mutations of the *IDH1* gene occur in codon 123 and are heterozygous and somatic; they involve replacement of guanine by adenine, leading to the replacement of an arginine with a histidine at amino acid residue 132 (R132H) [2]. The *IDH1* mutant glioblastomas are associated with a more favorable prognosis [7, 10].

Two versions of heterozygous *H3F3A* mutations are described for pediatric glioblastomas (including a project with our participation [23]): change of a lysine to methionine (K27M) or change of a glycine to either arginine or valine (G34R/V). Both mutations occur at the position close to the N-terminus, which undergoes important post-translational modifications, involving either suppression (K27) or activation (K36) of the transcription process. The *H3F3A* mutations are pathognomonic for glioblastomas and according to E. Je et al. [11], who have examined 1351 tumors, do not occur in any other type of tumors, including malignant meningiomas, sarcomas and carcinomas of various origins.

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while chromosome 10q deletion was found in 54 and 60% of the glioblastomas, respectively.

The major cytogenetic aberrations in glioblastomas revealed by the comparative genomic hybridization and confirmed by fluorescence *in situ* hybridization (FISH) are shown at **Fig. 1—8**.

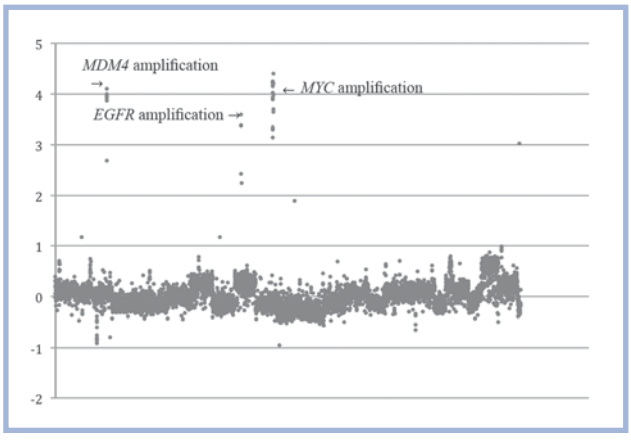

Fig. 1. **Cytogenetic profile of a glioblastoma with numerous amplifications of the *MDM4* (locus 1q32.1), *EGFR* (locus 7p11.12) and *MYC* (locus 8q24.13) genes.**

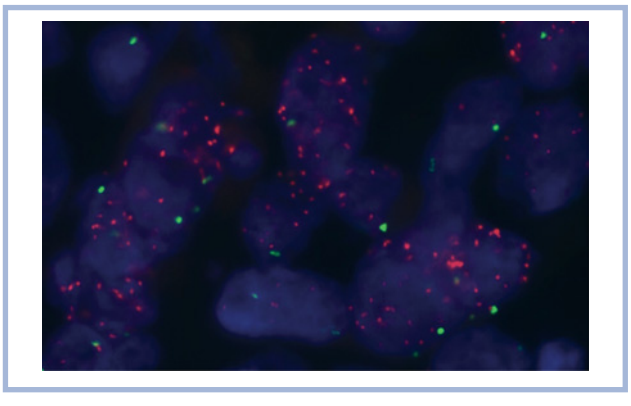

Fig. 2. **Amplification (increased number of copies) of the *EGFR* gene (locus 7p11.12) in glioblastomas.**

EGFR (locus 7p11.12) is shown in red, chromosome 7 centromere CEP 7 (loci 7p11.1-q11.1) is shown in green.

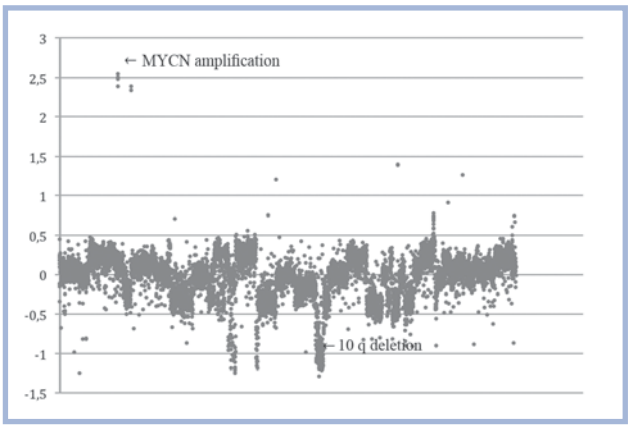

Fig. 3. **Citogenetic profile of glioblastoma with *MYCN* amplification (locus 2p24.3) and 10q deletion.**

Analysis of mutations in the *IDH1*, *TP53* and *H3F3A* genes

By employing direct sequencing analysis we found *IDH1* mutation (R132H) in 29 (38%) adult glioblastomas and 4 (8%) pediatric glioblastomas **(Figs. 9, 10)**; *TP53*

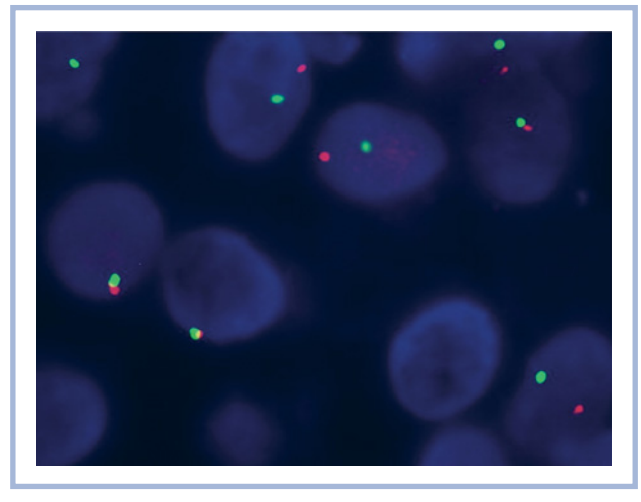

Fig. 4. **Monosomy of chromosome 10q in glioblastoma.**

PTEN (locus 10q23) is shown in red, chromosome 10 centromere CEP 10 (loci 10p11.1-q11.1) is shown in green.

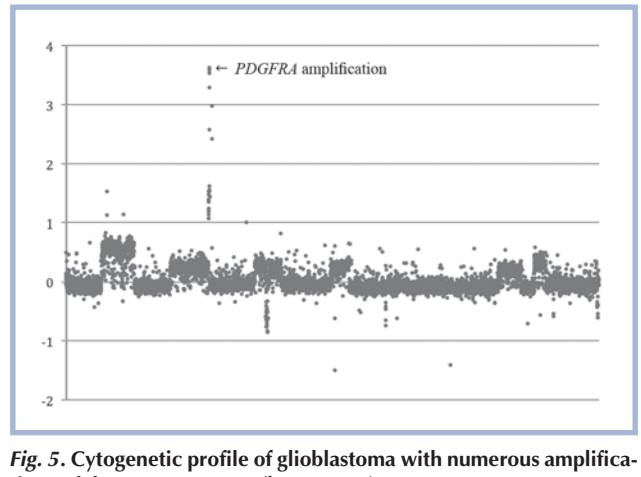

Fig. 5. **Cytogenetic profile of glioblastoma with numerous amplifications of the *PDGFRA* gene (locus 4q12).**

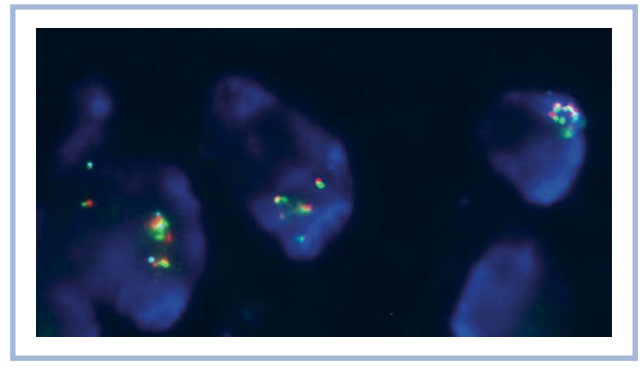

Fig. 6. **Amplification (increased number of copies) of the *PDGFRA* gene (locus 4q12) in glioblastoma.**

LNX (locus 4q12) is shown in red, *SCFD2* (locus 4q12) is shown in green, *PDGFRA* (locus 4q12) is shown in blue.

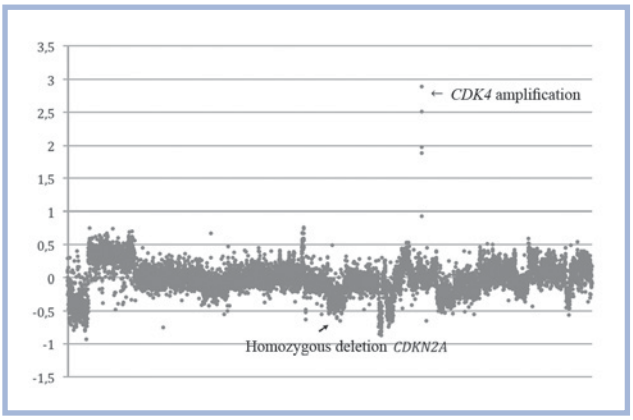

***Fig. 7*. Cytogenetic profile of glioblastoma with amplification of the *CDK4* gene (locus 12q14.1) and homozygous deletion of *CDKN2A* (locus 9p21).**

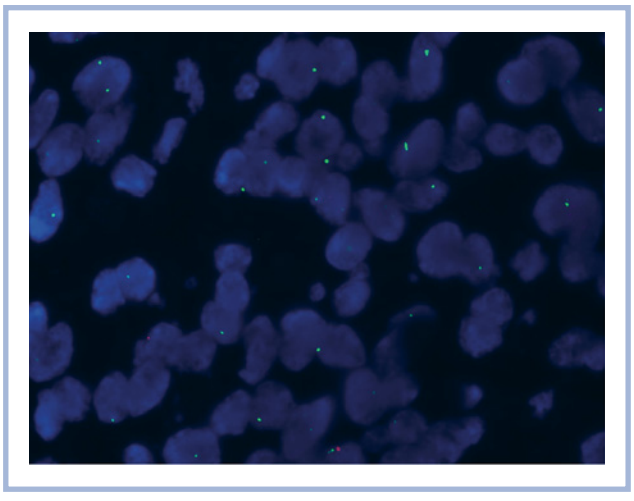

***Fig. 8*. Homozygous deletion of *CDKN2A* (*p16*) (locus 9p21) and monosomy of chromosome 9q in glioblastoma.**

CDKN2A (locus 9p21) is shown in red (singular signals), chromosome 9 centromere CEP 9 (loci 9p11.1-q11.1) is shown in green.

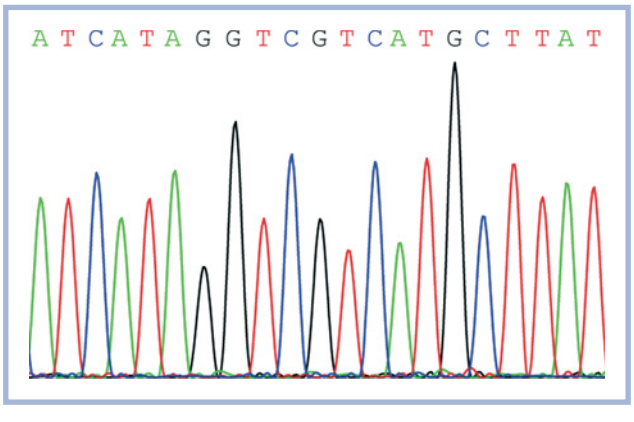

***Fig. 9*. Electrophoregram of studying *IDH1* gene mutation by direct sequencing: no mutations in *IDH1*.**

Here and in Fig. 10–14: the product of direct gene sequencing was isolated by capillary gene electrophoresis. For this purpose nucleotides were marked by four different fluorochrome dyes: adenine (green), guanine (black), cytosine (blue) and thymine (red). Mutation involves replacement of the base pair, leading to the replacement of one amino acid with another .

mutation, in 33 (43%) and 24 (50%) of cases, respectively, and *H3F3A* mutations in 17 (35%) and 1 (1%). Two variants of *H3F3A* gene mutations were identified: *K27M* **(Figs. 11, 12)** and *G34R/V* **(Figs. 13, 14)**. *K27M* mutation was identified in 11 (23%) cases for children and in 1 (1%) case for adults, whereas G34R/V mutation was observed in 6 (12%) cases in children.

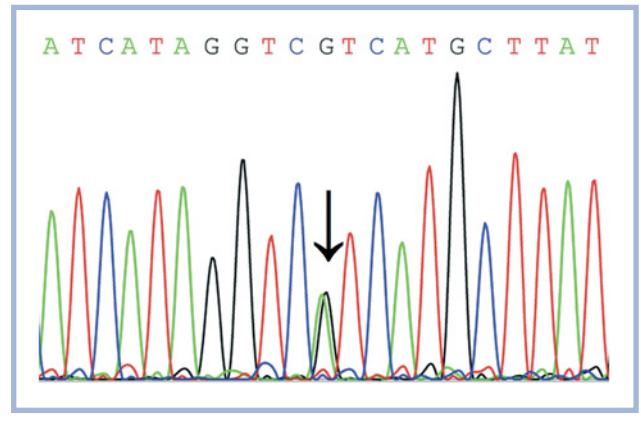

***Fig. 10*. Electrophoregram of studying *IDH1* gene mutation by direct sequensing.**

Mutation involves replacement of guanine to adenine, leading to the replacement of arginine amino acid with histidine (indicated by an arrow ↓).

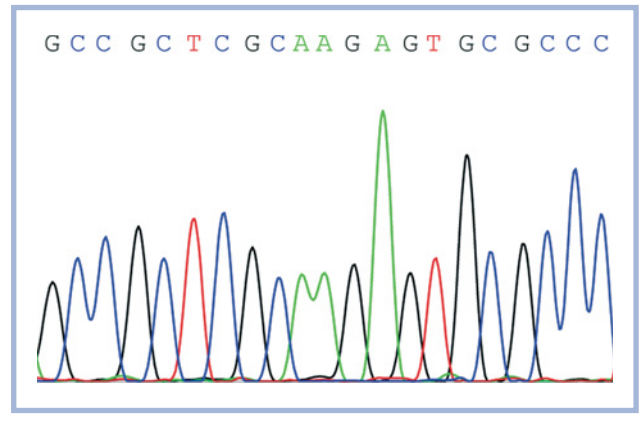

***Fig. 11*. Electrophoregram of studying *H3F3A K27M* mutation (no mutation).**

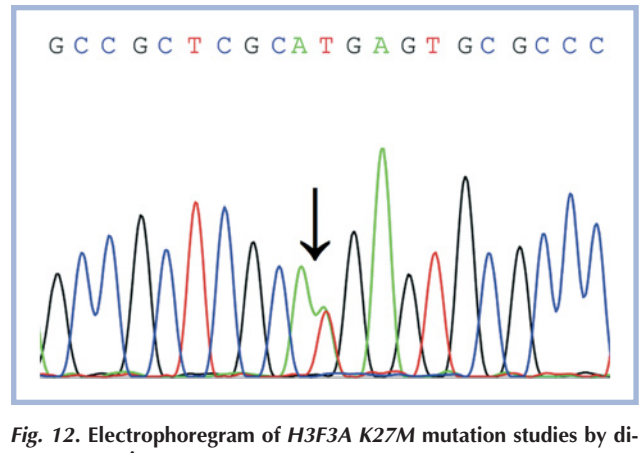

***Fig. 12*. Electrophoregram of *H3F3A K27M* mutation studies by direct sequencing.**

The mutation involves replacement of adenine to thymine, leading to the replacement of lysine amino acid with methionine (K27M, indicated by an arrow ↓).

Immunohistochemical identification of the mutant IDH1 protein

This approach was used to identify the mutant IDH1 R132H protein in parallel with direct sequencing of the genes. Marked cytoplasmic expression of IDH1 **(Fig. 15)** was observed in 29 (38%) adult glioblastomas and in 4

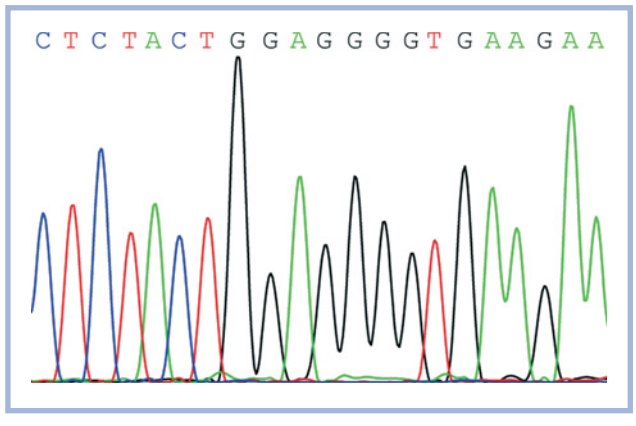

***Fig. 13*. Electrophoregram of studying *H3F3A G34R* mutation (no mutation).**

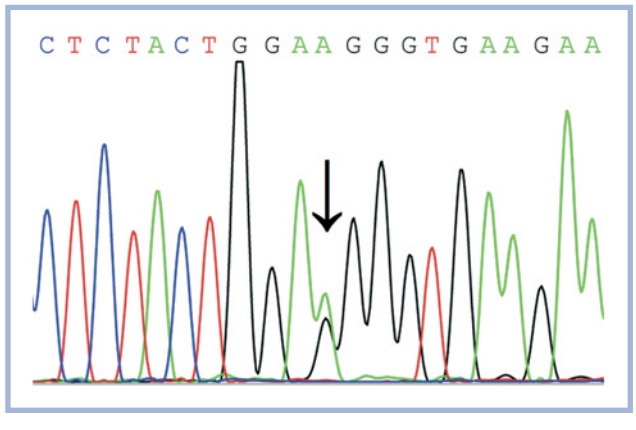

***Fig. 14*. Electrophoregram of studying *H3F3A G34R* mutation by direct sequencing.**

Mutation involves replacement of guanine to adenine, leading to the replacement of glycine amino acid with either arginine (G34R) or valine (G34V, indicated by an arrow ↓).

(8%) pediatric glioblastomas; with the ratios being identical to those of *IDH1* mutation revealed by direct sequencing. No cytoplasmic expression of IDH1 was observed for 48 adult glioblastomas and for 44 pediatric glioblastomas. Therefore, the data of the direct gene sequencing and of the immunohistological studies aimed at identifying the mutant IDH1 protein were in complete agreement.

Glioblastoma subgroups

The glioblastomas in the study were divided in three subgroups based on patients' age and presence or absence of mutations of the *IDH1* and *H3F3A* genes, which are considered to be crucial for glioblastoma pathogenesis.

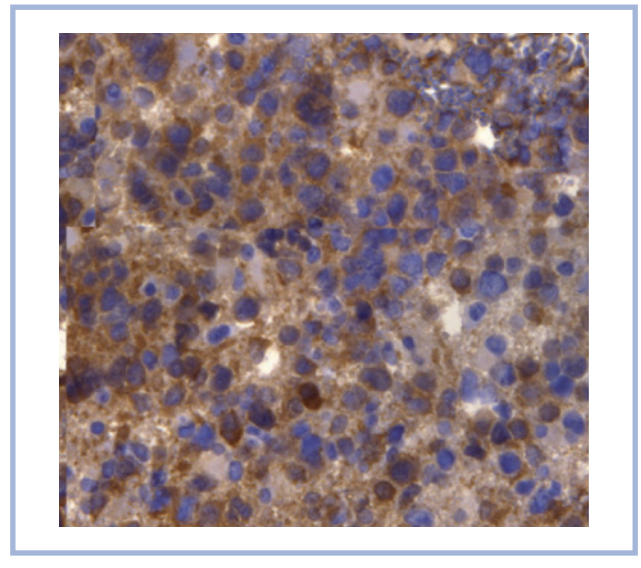

***Fig. 15*. Positive expression of IDH1 in glioblastoma with *IDH1* mutation.**

Immunohistochemical analysis with anti-human IDH1 R132H Dianova antibody.

The distribution of the observed chromosome aberrations and gene mutations across these three subgroups is shown in **Fig. 16**.

MYC/MYCN amplification was observed in pediatric glioblastomas and *IDH1* mutant adult glioblastomas at approximately equal ratios of 19 and 17%, respectively, whereas amplification of *MYC/MYCN* gene in *IDH1* wild-type adult glioblastomas occurred only in 4% of cases.

EGFR amplification was a distinctive feature of *IDH1* wild-type adult glioblastomas; in this subgroup this cytogenetic aberration was observed in 42% of cases, whereas *EGFR* amplification in pediatric glioblastomas was observed only in 12% of cases and in *IDH1* mutant adult glioblastomas, only in 6%.

CDK4 amplification was observed predominantly in adult glioblastomas, irrespective of presence or absence of *IDH1* mutation.

Homozygous deletion of *CDKN2A* and chromosome 10q deletion were observed in all three glioblastoma subgroups, with a minor increase in prevalence in the adult glioblastomas.

TP53 mutations were observed in *IDH1* mutant adult glioblastomas and in pediatric glioblastomas.

We have also examined the overall survival of patients with glioblastomas in the three aforementioned subgroups **(Fig. 17)**.

An analysis of the overall survival reliably showed that the *IDH1* mutant adult glioblastoma subgroup has the most favorable prognosis with the 5-year survival rate of approximately 80%. No reliable difference was observed between the overall survival in the pediatric glioblastoma and the *IDH1* wild-type adult glioblastoma subgroups. The 5-year survival rates in these subgroups are approximately 8 and 0%, respectively.

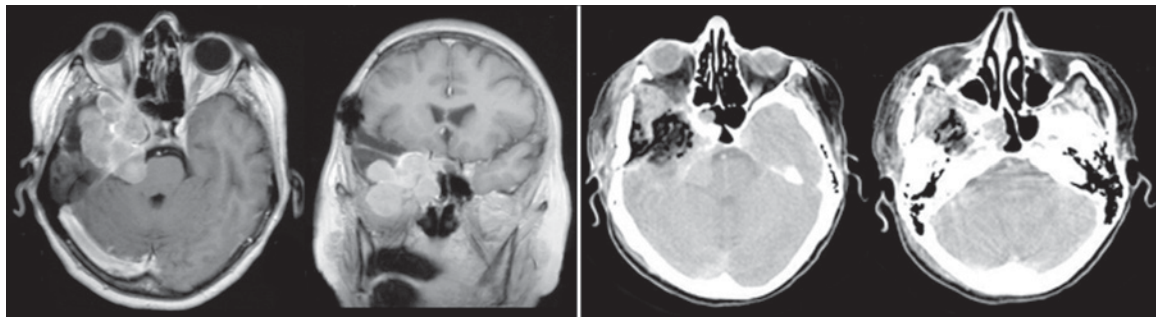

***Fig. 5*. Orbitosphenopetroclival meningioma.**

On the left — view before operation; on the right — after operation. The lateral portion of the tumor was dissected from the middle cranial and infratemporal fossae from the right; the remained medial portion of meningioma is to be treated by stereotactic radiotherapy.

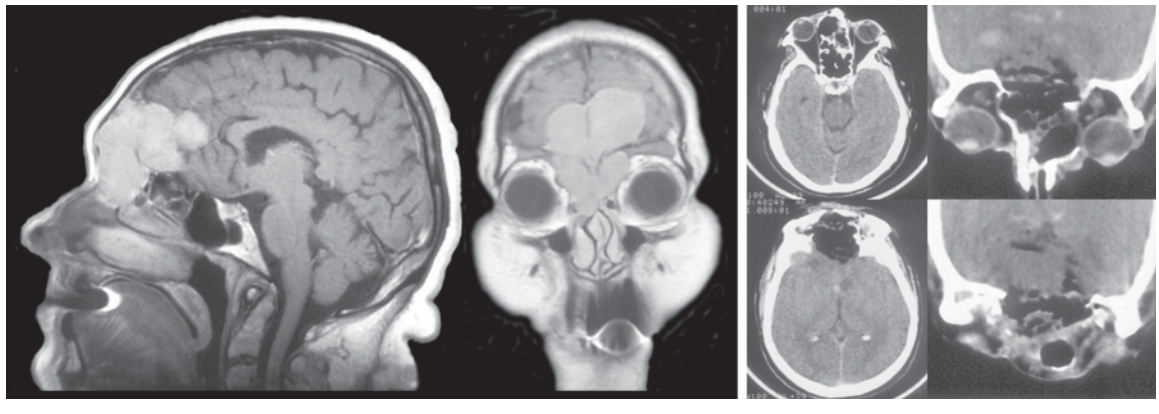

***Fig. 6*. Midline craniofacial meningioma with primarily intracranial extension.**

On the left – MRI before operation: tumor occupies the anterior cranial fossa, frontal and ethmoidal sinuses and invades the nasal cavity. On the right — CT after transcranial resection of tumor through the frontal sinus.

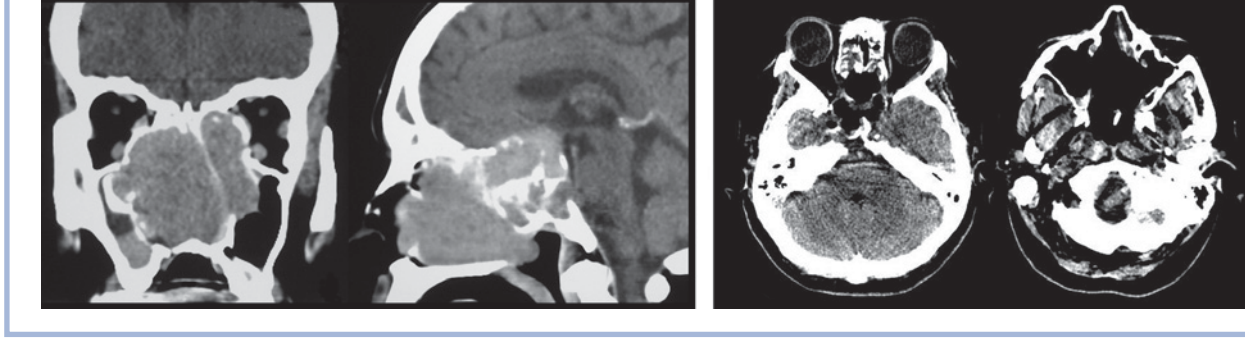

***Fig. 7*. Medially located meningioma with primarily extracranial extention.**

On the left – SCT before operation. Tumor fully infests the nasal cavity, ethmoidal labyrinth, sphenoid sinus and extends into the orbits and maxillary sinuses, causing compression of both optic nerves and chiasm. The intracranial portion is represented by the suprasellar mass. On the right — CT after endoscopic endonasal resection of extracranial portion of tumor with decompression of the orbits and optic canals.

by significant volume and profound lateral extension **(Fig. 8)**, it is recommended that the orbitozygomatic approach with endoscopic assistance or combination of that with endoscopic endonasal approach is used [4, 9]. When total excision of tumor is impossible or there is a risk of reoccurrence, stereotactic radiotherapy or radiosurgery is administrated [14]. Juvenile angiofibromas are androgen-dependent and occur only in prepubescent and pubescent boys, so tumor remnants typically involute after the age of 20.

Malignant craniofacial tumors (n=51) represent a difficult interdisciplinary problem as surgical treatment in such cases requires mutual efforts of oncologists, neurosurgeons and plastic surgeons [12]. All operations can be classified into the radical and palliative ones; conditions for radical surgery depend on particular features of

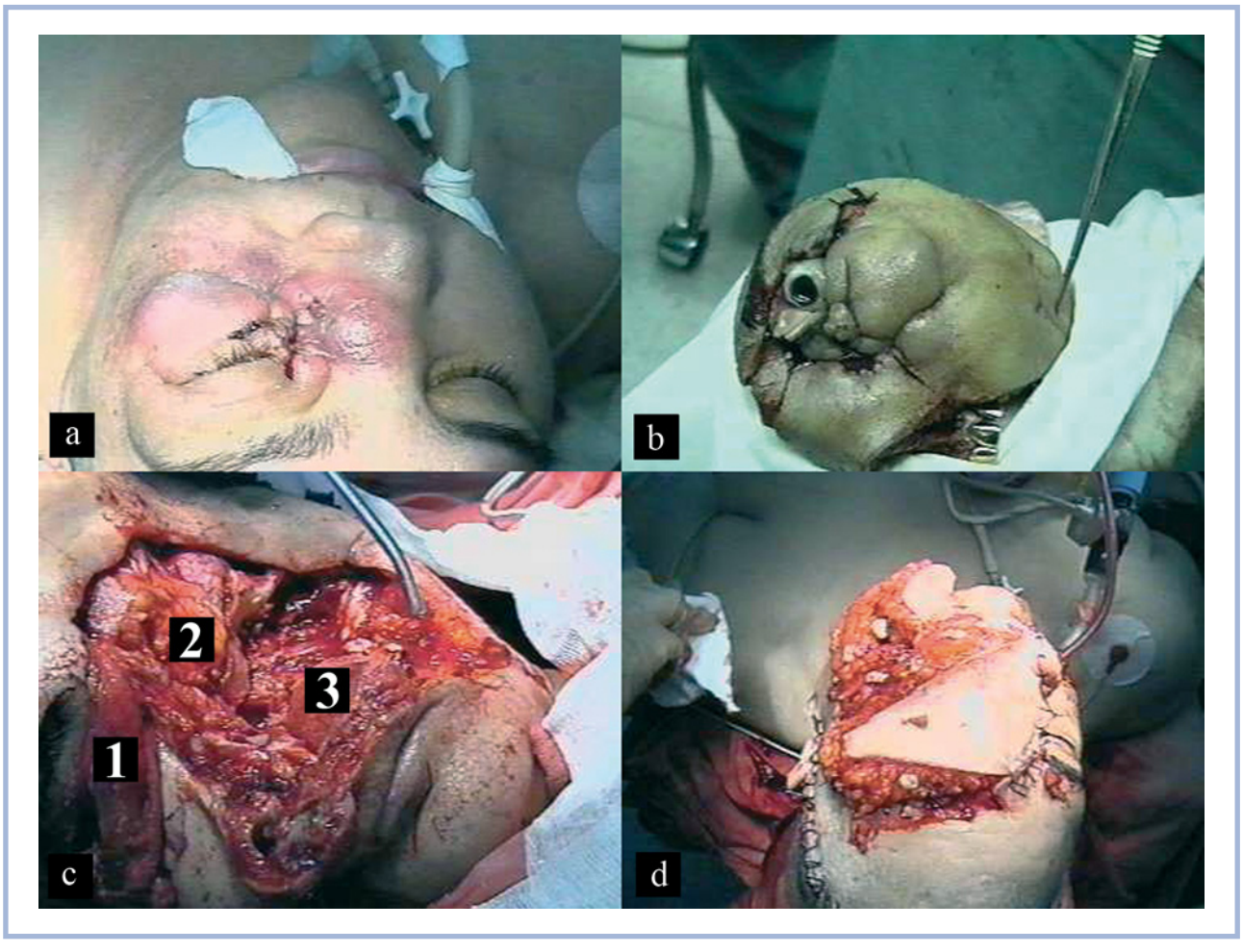

***Fig. 10*. Craniofacial en bloc resection — steps of operation (intraoperative photograms).**

a — view before operation; b – excised mass containing the tumor; c — the first step of scull base defect reconstruction (1 — calvarial periosteum flap; 2 — free anterior abdominal wall fat; 3 — tongue in the oral cavity). We used osteomyocutaneous graft containing fragment of the rectus abdominis muscle and costal arch to reconstruct face defects; d — view after operation.

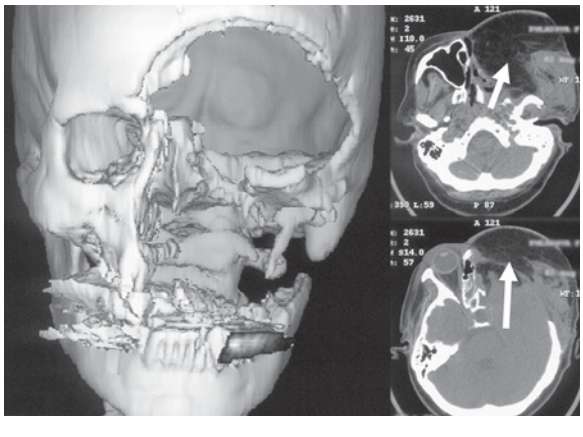

***Fig. 11*. CT one month after the operation.**

On the left — 3D-reconstruction of scull base defect; on the right — plastic material (indicated by an arrow) in the defect's projection.

The most problematic group of osteogenic tumors are those of the ***chondroid type*** (n=18) that can be benign (chondroma, chondromyxoid fibroma, chondroblastoma) and malignant (chondrosarcoma) [2, 10]. Craniofacial chondroid tumors are mainly located medially (the sellar area, the cribriform bone). Chondrosarcoma is a rare tumor located craniofacially in 10% of cases. Most of chondrosarcomas are primary malignant, rarely they result from chondroma malignant transformation. They are characterized by aggressive growth, bone degradation and moderate vascularization. Chondromas are typically slow-progressing and less invasive than other types. As far as radiological parameters are concerned, benign and malignant tumors do not significantly differ; they look like heterogeneous extensive formations containing petrifacts, contrast-enhancing and not causing peritumoral edema **(Fig. 13)**. Therapeutic approach in such cases directly depends on the degree of anaplasia. The optimal way of treating benign tumors is total resection. Even after incomplete resection, radiotherapy is not recommended as it may induce malignant transformation. Chondrosarcoma requires treatment combining surgical removal of the tumor with subsequent high-dose radiotherapy. Nevertheless, recurrent tumors are observed quite often, where the extent of intradural infiltra-

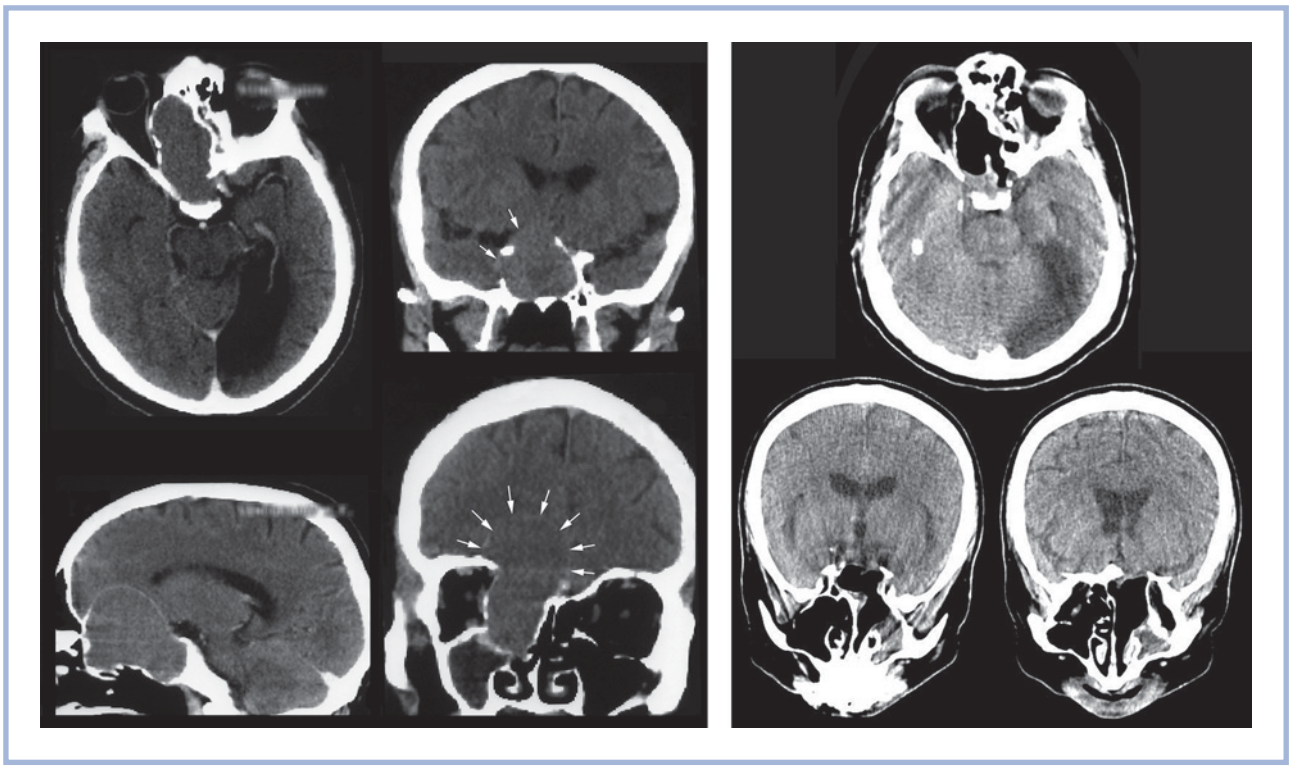

***Fig. 14*. Considerable pyocele of the base of the anterior and middle cranial fossae on the right side was a reason for vision and oculomotor impairment that regressed after endoscopic endonasal drainage.**
On the left — SCT before operation; on the right — 2 months after operation.

of refining diagnosis (biopsy); in some cases decompression of the orbit and/or the optic canal is needed. Current less traumatic treatment methods using the transnasal approach with endoscopic assistance or ophtalmological approaches are the best option for most patients with craniofacial pseudotumors **(Fig. 14)**.

Plasmacytoma is a ***lymphoproliferative lesion*** (n=3) characterized by monoclonal proliferation of plasmocytes secreting immunoglobulins. This malignant tumor may infest both the calvaria and scull base, though the cranio-orbital location is among the most frequent ones. Plasmacytomas can be of the solitary (bones or soft tissue) and multiple types. Multiple plasmacytoma prognosis is much worse than that of solitary plasmacytoma. Tumor causes degradation of the entire bone thickness and uniformly absorbs contrast media **(Fig. 15)**. Venosity formed as a result of exaggerated branches of the external carotid artery is typical of this tumor type. Plasmacytomas are successfully treated by radiotherapy; another therapeutic option is total surgical excision with adjuvant therapy [1].

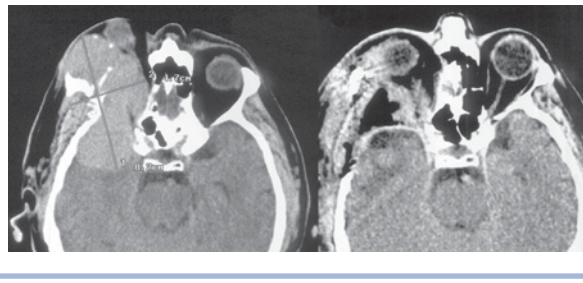

***Fig. 15*. Cranio-orbital plasmacytoma.**
On the left — CT before the operation, on the right — after the operation.

Conclusion

Craniofacial tumors are a very diverse group of lesions having absolutely different origins, growing patterns, clinical signs and, consequently, requiring various means of treatment. That is why there are no standard ways of treatment; one only can speak of the common approach to treating the most frequent pathologies such as cranio-orbital meningiomas. Current trends in the surgery of scull base tumors extending into the orbit, nasal cavity, paranasal sinuses, infratemporal and pterygopalatine fossae, can be summed up in two – making the approach less traumatic and surgical manipulations more invasive by means of intraoperative control techniques (such as neuronavigation, endoscopy, neuromonitoring, etc.). Here, endoscopy may serve either as assistant means or as the main means of removing the tumor (at transnasal approach). It should be mentioned once again that diagnosis and treatment of craniofacial tumors should be carried out on an interdisciplinary basis. Despite the fact that microsurgical and endoscopic equipment are advancing more and more, nowadays decision against inappropriate and risky radical interference in

many cases lets one to keep a considerably high level of the patient's life quality at benign and malignant processes. It has become possible due to evolution of contemporary methods of highly precise stereotactic radiotherapy that has recently been growing into an essential component of the complex treatment of scull base neoplastic tumors. The results of treating various tumors will be reported in our further publications.

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