Medulloblastoma Variants: Age-Dependent Occurrence and Relation to Gorlin Syndrome—A New Clinical Perspective

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Abstract

Purpose: We aimed to test the hypothesis that medulloblastoma (MB) variants show a different age distribution and clinical behavior reflecting their specific biology, and that MB occurring at very young age is associated with cancer predisposition syndromes such as Gorlin syndrome (GS).

Experimental Design: We investigated the frequency, age distribution, location, response to treatment, outcome, and association with familial cancer predisposition syndromes in a series of 82 cases of MB in patients ages <14 years diagnosed at the Giannina Gaslini Children's Hospital, Genoa, between 1987 and 2004.

Results: Desmoplastic MB and MB with extensive nodularity (MBEN), were present in 22 of 82 cases (27%) and were more frequent in children ages ≤3 years (13 of 25; 52%). In this age group, MBEN was significantly more frequent than desmoplastic MB and classic MB ($P < 0.001$) and had a good prognosis. MBEN was associated with GS in 5 of 12 cases. Overall, 8 cases occurred in the context of familial tumor predisposition syndromes (5 GS, 1 each NF1, Li-Fraumeni, and Fragile X) and 7 of these patients were ages ≤3 years at diagnosis. Desmoplastic histology and a more intensive treatment represented independent favorable prognostic factors in multivariate analysis ($P = 0.003$ and $P = 0.0139$, respectively). Metastasis was a predictor of bad outcome ($P = 0.0001$).

Conclusions: Our data indicate that biologically different MB entities warrant risk-adapted treatment and that MBEN is strongly associated with GS. Patients, ages ≤3 years, with MB and their families should be investigated for tumor predisposition syndromes such as GS.

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Medulloblastoma (MB) with extensive nodularity (MBEN) occurring in young children is a rare but well-defined entity (1–3). It is believed to be related to, but distinct from, desmoplastic MB (DMB). However, many aspects of this peculiar entity are still unclear, including its frequency, why it occurs at a very young age, why its prognosis is good, and its association with Gorlin syndrome (GS) or nevoid basal cell carcinoma syndrome. Most of the largest cooperative trials that have been published on MB in infants do not report on the relative frequency of this distinct entity compared with other variants and/or comment on the effect of the diagnosis of this variant on subsequent follow-up and treatment strategies (4–6). A large prospective study by Eberhart reported that 73 of 330 (22%) cases had various degrees of desmoplasia and 14 of 330 cases were MBEN (4.2%; ref. 7). A more recent, extensive neuropathogical review focused on the morphologic features and biological behavior of 2 large contemporaneous cohorts of patients with MB and showed that DMB represents 5% of all MB and up to 57% of cases in children ages <3 years (3). A recent prospective, cooperative trial reported that desmoplastic histologic variants have a clearly favorable effect on prognosis in infants with MB who are treated with chemotherapy (CT) as first-line therapy. However, it did not mention the frequency of

Translational Relevance

This study shows a strong association between specific histologic variants of medulloblastoma and age at diagnosis. Medulloblastoma with extensive nodularity (MBEN), which occurs with increased frequency in young children, is commonly associated with Gorlin syndrome (GS) caused by germ line *PTCH* mutations. The link between MBEN and GS leads us to recommend close clinical follow-up, genetic counseling and investigations, and to avoid radiotherapy in patients affected by GS and or MBEN.

Our findings also suggest that the better prognosis described for MBEN might be related to several factors including decreased propensity to metastasis and a higher sensitivity to chemotherapy and radiotherapy than other histologic variants of medulloblastoma. This implies that the early identification of this entity at the time of first diagnosis of a posterior fossa tumor in very young children by histology and imaging criteria is necessary for an optimal treatment strategy.

MBEN versus DMB cases within the subgroup of tumors with a desmoplastic component (8), and whether there was an association with GS. GS is an autosomal dominant disorder associated with a large number of phenotypic abnormalities. MB is one of the features of GS included among the minor criteria for clinical diagnosis (Walter AW. Gorlin Syndrome[17]; refs. 9, 10). Incidence of GS in MB cohorts reported by Evans was 1% to 2% at all ages but higher at age <5 years (4.5%; ref. 11).

In a most recent series, Amlashi (12) reported a frequency of 3 of 76 cases (4%) but a higher frequency (20%) in patients with desmoplastic histology. It is well-known that GS-associated MBs, as well as sporadic DMBs, are related to *Patched 1* (*PTCH1*) mutations (1). Recently, mutations of *SUFU* have also been identified in individual MB cases (13). MBs associated with GS show desmoplastic histology, although the current literature does not specify the frequency of the MBEN variant (12, 14). Only Aliani et al. (15) suggested the association of this variant to Gorlin's syndrome in a single case. Because our Institution contributed to the first large published MBEN series (2), we started recognizing MBEN early on, and subsequently began investigating activation markers of the hedgehog/patched signaling pathway in this subset of tumors (16).[18] A retrospective evaluation of our series revealed that MBEN was over-represented in patients of very young age and in patients with GS. To test this hypothesis, we decided to review all consecutive patients with MB diagnosed and treated at the Giannina Gaslini Children's Hospital, Genoa, between 1987 and 2004. Our aim was to study the various MB variants with respect to frequency, age distribution, prognosis, and response to treatment, and then to address the question of the real frequency of MBEN as a favorable variant, as well as its association with GS.

[17] http://www.emedicine.com/ped/topic890.htm

[18] Unpublished data, T. Pietsch, A. Raso, V. Capra, et al.

Materials and Methods

All the consecutive cases diagnosed as MB and treated at Giannina Gaslini Children's Hospital between 1987 and 2004 were eligible for the study. Patients recruited at our institution only at relapse, or those without pathology specimens available for review, were excluded.

Demographic data of each eligible patient were retrieved from the clinical records. Two categories of age at diagnosis were defined using age 3 y as the cut off; also, the period of diagnosis was stratified in two groups using the 1997 as cut-off year when high-dose chemotherapies regimens were administered more systematically.

Histologic slides of all eligible cases were reviewed [with special emphasis on the different MB variants: classic MB (CMB), DMB, MBEN, and anaplastic/large cell variants] by a panel of pathologists and classified according to the revised 2007 WHO classification of brain tumors. Standard histologic methods including H&E, reticulum stains, and immunohistochemical reactions were used (1). Differentiation was assessed by immunostaining for neuron-specific enolase, synaptophysin, NeuN, GFAP, p75 low-affinity neutrophin receptor, and Ki-67 (MIB-1), as well as for myogenic markers. In accordance with the 2007 WHO classification and one of our previous publications, we defined MBEN as an MB in which there is (>50% of the examined tissue) histology of predominant nodularity, with intranodular nuclear uniformity within a desmoplastic background (1, 2).

Clinical features and follow-up data of study patients were also retrieved from clinical records. At diagnosis, all patients were carefully examined to identify possible signs of comorbid conditions as stigmata of cancer predisposition syndromes (CPS); family history was periodically updated. At follow-up, special attention was paid to search for skin lesions, and bone or dental cysts as for intracranial calcifications. All available X-rays and brain computer tomography scans were reviewed looking for GS signs.

GS was diagnosed in patients with two major or one major and two minor criteria (9, 10, 12). All cases with GS were investigated for constitutional and somatic *PTCH1* mutations. Tumor location (hemispheres or vermis) was evaluated reviewing imaging of all cases.

Standard of care during the period. During the study period, the neurosurgical approach and staging procedures had remained basically unchanged since magnetic resonance imaging (MRI) had been adopted in 1988. The first few cases enrolled before the MRI era were staged by preoperative and postoperative computed tomography scans as well as by myelography. Staging procedures included cerebrospinal fluid cytologic examination and postoperative cranial and spinal MRI for detection of metastasis. Surgical removal was defined as complete or partial using the 1.5 cm^2 size of the residual tumor as cutoff between the groups. Response to treatment was evaluated mainly by MRI after every CT course or early during salvage radiotherapy (RT) in patients with measurable disease. Patients without residual tumor (T) and/or metastasis (M) were defined as T0/M0. Children age ≤3 y were most often treated with up-front CT to avoid or delay irradiation. The main protocols used for children in this age group (infants) were the UK9204 (17) and the AIEOP SNC 9501 (18); after 1997, a more intensive protocol using high-dose CT (HDCT) was started (18). With all these protocols, RT was only administered in metastatic cases or when residual disease was still present at the end of CT. As for children ages >3 y, the Packer regimen (19) was adopted in most of cases as postsurgical treatment protocol; however, the AIEOP MB national studies (SNC85, SNC91, SNC99 where adopted in 6 cases; ref 20). After 1997, patients age >3 y considered at high risk were treated with an Institutional protocol using HDCT followed by craniospinal irradiation (CSI; ref. 21).

For purposes of this study, postsurgical treatment categories were pooled in three groups: (*a*) CT or RT only; (*b*) HDCT plus RT; (*c*) standard dose CT plus RT.

Statistical analysis. Descriptive statistics were reported in terms of absolute frequencies and percentages for qualitative data, and the

Table 1. OS of 82 children with MB, by different risk factors

	n (%)	No deaths	5-y survival (95% CI)	*P*
Gender				0.9622
Male	49 (60)	15	0.73 (0.57-0.83)	
Female	33 (40)	10	0.72 (0.53-0.85)	
Age at diagnosis (y)				0.4146
≤3	25 (30)	9	0.63 (0.41-0.79)	
>3	57 (70)	16	0.76 (0.63-0.86)	
Year of diagnosis				0.1079
1987-1996	39 (48)	16	0.61 (0.44-0.75)	
1997-2004	43 (52)	9	0.79 (0.63-0.89)	
Histology				0.0247
CMB	55 (67)	20	0.66 (0.52-0.77)	
MBEN	12 (15)	1	0.92 (0.54-0.99)	
DMB	10 (12)	1	0.90 (0.47-0.98)	
Anapl./large cell	5 (6)	3	0.60 (0.12-0.88)	
Location				0.4923
Cerebellar hemisphere	9 (11)	2	0.89 (0.43-0.98)	
Vermis*	73 (89)	23	0.70 (0.58-0.79)	
Surgical resection				0.2421
Complete (<1.5 cm^2 residual tumor)	59 (72)	16	0.75 (0.62-0.85)	
Partial (>1.5 cm^2 residual tumor)	23 (28)	9	0.65 (0.42-0.81)	
Metastasis †				<0.0001
No	62 (77)	11	0.85 (0.73-0.92)	
Yes	19 (23)	13	0.34 (0.14-0.56)	
Treatment †				0.2571
CT or RT only	17 (21)	7	0.58 (0.32-0.78)	
HDCT+RT	20 (24)	3	0.84 (0.58-0.94)	
Standard CT+ RT	44 (55)	14	0.74 (0.59-0.85)	
Total	82 (100)	25	0.72 (0.61-0.81)	

*One patient with vermis+hemisphere.
† For one patient data were not available.

Pearson's χ^2 test or Fisher's exact test, if appropriate, were applied to compare proportions. Due to their non-normal (Gaussian) distribution and to the lower number of observations, quantitative data were described in terms of median values and range. Accordingly, comparisons between groups were done by the nonparametric Mann-Whitney *U* test, or by the Kruskall-Wallis test when more than two groups had to be compared.

Follow-up was censored at the end of 2006 and overall survival (OS) was evaluated by the Kaplan-Meier method (22), and differences between groups were assessed by the log-rank test (23). The Cox proportional hazard model (24) was used to model for the combined effect of the various prognostic factors. For this analysis, the desmoplastic variants (DMB and MBEN) were pooled, thus, allowing for only three histologic types. The hazard ratio with the 95% confidence interval (95% CI) was calculated to measure the effect of each predictor compared with the reference value. The likelihood ratio test was used to assess the effect of predictors. Proportional hazard assumption was tested using scaled Schoenfeld residuals against log of time.

All tests were two tailed and a *P* value of ≤0.05 was considered as statistically significant. The statistical software "Statistica'" (release 6.0, Stat Soft Corporation) and the software "Stata'" (release 7.0, StataCorp 2001) were used.

Results

Of the 90 consecutive patients treated at our institution during the study period, 8 children where excluded from final analysis because pathology specimens were not available (3 cases) or arrived at Giannina Gaslini Children's Hospital at relapse (5 cases); Clinical characteristics of the 82 patients eligible and valuable for analysis are summarized in Table 1. There was a prevalence of males (*n* = 49; 60%) with an M/F ratio 1.48, and of children ages >3 years at diagnosis (*n* = 57; 70%). The histologic review confirmed CMB as the most frequent variant (*n* = 55; 67%), followed by MBEN (*n* = 12; 15%) and DMB (*n* = 10; 12%); anaplastic or large cell variants represented only 6% of our series (*n* = 5).

Significant differences in age at diagnosis were observed if the 4 histologic variants were considered; although CMB represented the most frequent tumor type in children ages >3 years (47 of 57; 82%), it represented only 32% (8 of 25) of cases diagnosed in children ≤3 years. In this age group, desmoplastic variants (DMB/MBEN) represented 52% (13 of 25) of cases; $P < 0.001$. This difference remained statistically significant ($P < 0.001$, Kruskall-Wallis test) also if age was considered as a continuous variable (Fig. 1).

As for *tumor site*, the vermis was the most frequent site (*n* = 73; 89%), although cerebella hemispheres were involved in only 9 children (11% of cases). If also *histology* was considered, the vermis remained as the most frequent tumor site for most of the variants (100% for CMB, 83% for MBEN, and 80% for other variables) with the exception for DMB tumors where 6 of 10 tumors (60%) originated from the hemispheres. Seven of 12 cases (58%) with MBEN showed the typical MRI appearance described as "grape like" by Giangaspero et al. (2); and reported by other authors (15, 25).

Complete tumor removal was possible in 59 children (72%; Table 1), and no differences in tumor removal frequency were observed among the different histologic variants (67%, 80%,

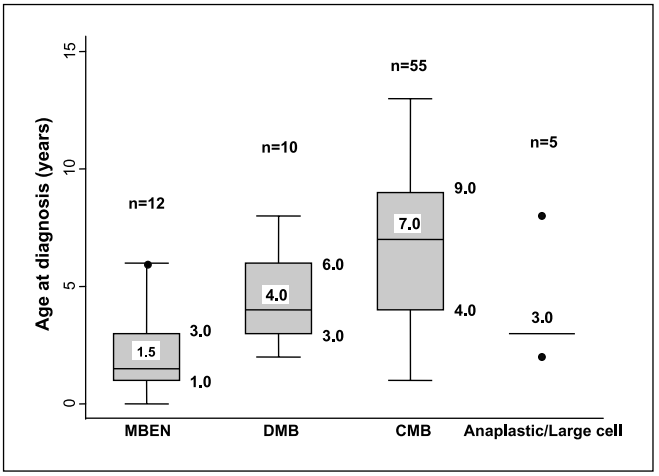

Fig. 1. Distribution of histologic variants and age.

83%, 80% for CMB, DMB, MBEN, and other variants, respectively; $P = 0.732$). Also, if tumor location was considered, no differences in frequency of complete resection were observed between children with tumor originating from the vermis (70%) versus those with a hemispheric location (89%; $P = 0.433$).

Data regarding the presence of metastasis and the postsurgical front line therapy were not available for one patient with a large cell MB who died shortly after surgery. Of the 81 children with available information, 19 (23%) had metastases at diagnosis (i.e., 6 in the cerebrospinal fluid, 4 in spine, 8 both spine and supratentorial site, and 1 in the bone marrow). If the histologic variant was considered, only 5% of children with DMB/MBEN (1 of 22) had metastases, whereas the corresponding figure for children with CMB and other variants were 29% (16 of 55) and 50% (2 of 4), respectively ($P = 0.016$).

The case with metastasis in the DMB/MBEN group was age <3 years and eventually died. Fourteen of the 16 children with metastases in the CMB group were ages >3 years, and 9 of them (64%) died of disease; 2 other patients were ages ≤3 years at diagnosis: both died. The two cases with anaplastic/large cell tumor and metastasis were also infants (one of whom died). Postsurgical front line therapy was based on only CT or RT in 17 cases (21%; Table 1). Of these, 15 received CT only (14 of them were infants), whereas the other 2 cases received RT only: 1 case, a T0/M0 7-year-old girl who could not undergo CT because of poor clinical conditions, received 35 Gy CSI + posterior fossa boost at 54 Gy; the other child was an infant with Fragile X Syndrome and severe multiple gastrointestinal malformations who received posterior fossa irradiation alone (50 Gy). Twenty cases (24%) with high-risk features were treated (after 1997) with our institutional protocol using HDCT followed by standard CSI (20). Finally, 44 patients (55%) underwent conventional CT and standard CSI.

Considering in more detail the treatment of the infants cases, 14 of 24 (58%) assessable cases received upfront CT; HDCT was delivered to 15 patients (62.5%) of whom 12 treated with this modality upfront and 3 at relapse; 15 of 24 (62.5%) received RT (9 as part of initial treatment and 6 at relapse); irradiation was limited to posterior fossa in 5 of 15 (33%) cases.

Survival. Length of follow-up ranged between 1 month and 20 years (median, 4.9 years), and during that period, 25 children died. The estimated 5-year OS of the entire cohort was 72% (95% CI, 61-81): Only 1 further death occurred after the 5th year and the projected 10-year OS was 67% (95% CI, 54-76). Table 1 reports on the 5-year survival estimates stratified by the different risk factors. From this analysis, only the presence of metastases and the histologic variant were found to significantly affect the probability of survival. In more detail, children with metastases at diagnosis had a 34% probability of survival versus 85% of those without metastases ($P < 0.001$). As for histologic variants, children with DMB/MBEN had a 90% and 92% probability of OS, respectively, whereas children with CMB had a 66% OS and those with anaplastic/large cell tumors a 60% OS ($P = 0.0247$; see Fig. 2).

In the Cox proportional hazard model that considered the combined effect of the different risk factors, the presence of metastases and histology remained as independent risk factors (Table 2). In this analysis, also the type of treatment seemed to have an effect. In more detail, children with metastases at diagnosis had an 8.84-fold (95% CI, 2.89-26.99) increased risk of dying compared with children without metastases. If histologic variants were considered, and children with CMB were taken as the reference group, children with desmoplastic variants had a 95% reduced risk of death (hazard ratio, 0.05). Finally, combined therapy (CT plus RT) has confirmed to significantly improve probability of survival (Table 2). In our model, in fact, compared with children who received either CT alone or RT alone, those who were treated with high dose CT plus RT had a 92% (hazard ratio, 0.08) reduced risk of death, whereas those treated with standard dose CT plus RT had a 35% (hazard ratio, 0.65) reduced risk of death.

Family history and genetic predisposition. Evaluation of the family history, together with a thorough clinical and genetic investigation identified 8 tumors (9.7%), which had occurred within the context of a CPS. Of these, five were associated with Gorlin's syndrome (Table 3A) and one with FXS and multiple malformations; all these cases had a MBEN variant. The remaining two cases, one with neurofibromatosis type 1 and one with the Li-Fraumeni syndrome, had CMB. Seven of the 8 cases with a familiar predisposition syndrome were ages

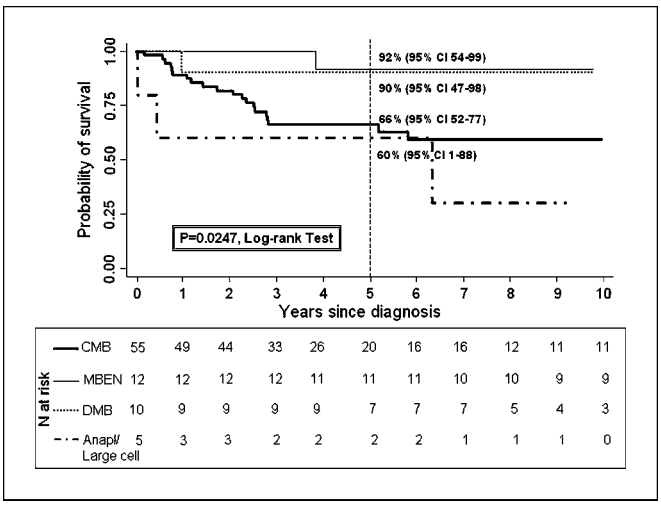

Fig. 2. Survival estimates by histologic variants.

Table 2. Multivariate analysis on the effect of different risk factors on the hazard function in 81 children with MB

Factor	Hazard ratio	95% CI	LRT	*P*
Gender	—	—	1.12	0.290
Male	1.00	—	—	—
Female	1.71	0.64-4.59	—	—
Age at diagnosis (y)	—	—	2.40	0.121
≤3	1.00	—	—	—
>3	0.23	0.33-1.56	—	—
Year of diagnosis	—	—	0.30	0.585
1987-1996	1.00	—	—	—
1997-2004	1.43	0.40-5.07	—	—
Histology	—	—	11.60	0.003
CMB	1.00	—	—	—
Desmoplastic variants	0.05	0.01-0.43	—	—
Anapl./large cell	0.61	0.08-4.85	—	—
Location	—	—	0.95	0.329
Cerebellar hemisphere	1.00	—	—	—
Vermis	0.33	0.03-3.09	—	—
Surgical resection	—	—	2.63	0.1046
Complete (<1.5 cm^2 residual tumor)	1.00	—	—	—
Partial (>1.5 cm^2 residual tumor)	2.16	0.87-5.37	—	—
Metastasis	—	—	16.44	0.0001
No	1.00	—	—	—
Yes	8.84	2.89-26.99	—	—
Treatment	—	—	8.56	0.0139
CT or RT only	1.00	—	—	—
HDCT+RT	0.08	0.01-0.59	—	—
Standard CT+ RT	0.65	0.09-4.62	—	—

NOTE: Likelihood of ratio of model, 44.39; $P < 0.0001$; desmoplastic variants, DMB + MBEN.
Abbreviations: LRT, likelihood of ratio; anpl., anaplastic.

≤3 years, leading to a 28% frequency (7 of 25) of tumor predisposing syndrome in this age group.

The MBEN variant. The 12 children with the MBEN variant have been further evaluated for their clinical characteristics and outcome. Their main data are reported in Table 3B.

In more detail, 3 MBEN cases relapsed and were all in the group of the 7 patients treated with up-front conventional CT to delay or avoid irradiation (3 of 7, 42.8% relapse rate). Two of these cases were successfully rescued with RT (#76) or HDCT+ RT (#52); the third case (#42) achieved two complete remission with HDCT followed by RT but subsequently relapsed again and died of tumor progression. Out of the four patients treated with upfront CT+RT, none of them relapsed, although one (#18), a GS syndrome patient, developed multiple nevoid basal cell carcinomas within the irradiation field. Patient #33 developed thyroid dysfunction and patient #80 developed a SMN (parotid gland adenocarcinoma) 9 years after RT.

Interestingly, in all the 4 cases treated either at diagnosis (#55) or at relapse (#42, #52, #76) while having measurable tumor, the tumor response was marked and prompt (>50% tumor volume reduction after only 4 weeks since beginning of therapy). Figure 3 shows the impressive response observed in case #52. All these patients were ages ≤3 years at diagnosis. As a comparison group, we selected seven age-matched children with CMB and measurable tumor who were also treated in a similar fashion. Only two of these children achieved partial or complete response and their overall prognosis was much poorer (five of seven died) compared with the four MBEN patients where only one death was observed. The difference in tumor response between the 2 groups failed significance but may indicate a clear trend ($P = 0.061$).

Discussion

During the last 15 years, treatment of MB in children has undergone several important improvements thanks to the widely adopted interdisciplinary approach to the disease and to national or international cooperative trials. The availability of more precise imaging and irradiation techniques, the development of microsurgical techniques, and the introduction of more intensive CT in infants and high-risk patients have also contributed to these improvements (8, 21, 25–27).

MBs are now subclassified into distinct entities with different histopathologic, genetic, biological, and clinical characteristics. Currently recognized subtypes include CMB, DMB, MBEN, anaplastic, and large cell variants (1).

At the same time, genetic and biological studies uncovered important genetic alterations involved in the molecular pathogenesis of MB. These alterations involve developmental pathways (*Hedgehog, Wnt,* and *Notch*) that are crucial for normal cerebellar development and which are pathologically activated in MBs (28–31).

Candidate biological factors indicating good prognosis include *TrkC* expression and β-catenin nuclear accumulation, whereas *Myc* gene amplification is associated with poor prognosis (1, 32, 33). In the near future, these biological markers may be combined with classic clinical prognosticators (age, presence of metastasis, extent of resection) and histologic prognosticators for more precise patient stratification.

Table 3. Main features of cases with GS(A) and with MBEN (B)

A. Main clinical features of the 5 cases with GS

#	Age(mo) /gender	MB variant	Other features for Gorlin S.	Family history
76	20/F	MBEN	Macrocrania, frontal bossing, NBCC, falx calcifications	Negative
42	16/M	MBEN	Odontogenic keratocysts, multiple basal cell carcinomas	Positive
55	15/M	MBEN	Macrocrania	Positive
37	11/M	MBEN	Macrocrania. mental retardation/ delay, multiple basal cell carcinomas palmar pits, odontogenic keratocysts	Positive
18	46/M	MBEN	Mental retardation/delay, coarse face, hypertelorism, falx calcifications multiple basal cell carcinomas	Negative

B. Main clinical features, treatment and outcome of 12 cases with MBEN

#	Age at diagnosis (mo)/gender	Inherited genetic syndromes	Grape-like aspect at MRI	Tumor removal/ metastasis
76	20/F	Gorlin	Yes	Total/no
74	24/F	—	Yes	Total/no
42	16/M	Gorlin	Yes	Total/no
52	5/F	—	Yes	biopsy/no
37	11/M	Gorlin	No	Total/no
55	15/M	Gorlin	Yes	Partial/no
70	18/M	—	Yes	Total/no
12	18/M	Fragile-X Syndrome	Yes	Total/no
22	36/M	—	No	Total/no
33	36/M	—	No	Total/no
18	46/M	Gorlin	No	Total/no
80	72/M	—	No	Total/no

Abbreviations: NBCC, nevoid basal cell carcinomas; FU, follow-up; ANED: alive, no evidence of disease; DOD: dead of disease.

The data from our study were obtained from a large monoinstitutional series of MB patients adopting modern diagnostic and treatment strategies. It adds novel information regarding the most important clinical prognostic factors (age, histology, metastasis, response to treatment) as well as features that may potentially be correlated with the biology of this tumor (GS, site of tumor origin).

Age. A cutoff age of 3 years has been used in most trials that subgrouped malignant tumors to identify a cohort of patients with peculiar features in terms of disease behavior, prognosis, and with increased risk of radiation toxicity. However, this cutoff is not based on clear scientific data (4, 26).

The suggestion that MB may show distinct biology and clinical behavior based on age is not new because initial studies reported worse prognosis in infants and better prognosis in adult MB. The difference between adult MB and childhood MB regarding site and histology (because hemispheric and DMB is more frequent in adults) is well-known, and recent articles reported the likelihood of adult and childhood MB having different biological markers (1, 34, 35). However, the reason for such differences has not yet been fully explained. Furthermore, because trials on children above age 3 years with MB were run separately from the ones on infants, we may have lost the "overall view" that might allow us to identify both the common and the distinct features. At our Institute, we had the opportunity to look at the spectrum of cases in a large, well-characterized, single-center series that included all the pediatric age groups. This allowed us to identify a very clear pattern of age-related distribution of particular histologic variants with a statistically significant difference in age at diagnosis between MBEN-DMB on the one hand and CMB on the other. This suggests that there is an age-related susceptibility to the development of these tumors, and that each variant has its peculiar interval of appearance.

Furthermore, with regards to age, in our study, we did not observe that age below 3 years is not per se an adverse prognostic factor as reported in the literature: this may be due to the fact that there was a prevalence of favorable histology and of a more effective treatment adopted because 1997.

Histologic variants. The prognostic value of histologic subtyping of MB has been controversial (27, 30). As a matter of fact, with the exception of anaplastic and large cell variants treated more aggressively, none of the national or international cooperative protocols stratifies patients according to histology. MB variants have been better defined, and presently, some of them present clearly different aggressiveness. At one end of the spectrum, there are the anaplastic and large cell variants showing aggressive behavior (1, 36), whereas at the other end, MBEN has been identified as occurring mainly in infants and having a favorable outcome (2). Histologically, MBEN contains large nodules with low proliferative activity and advanced neurocytic differentiation, as well as smaller areas with

Table 3. (Cont'd)

A. Main clinical features of the 5 cases with GS

Constitutional *PTCH1* mutation	Time of GS dx (mo from MB dx)			
—	120	—	—	—
Present	1	—	—	—
—	1	—	—	—
Present	72	—	—	—
—	192	—	—	—

B. Main clinical features, treatment and outcome of 12 cases with MBEN

Up-front treatment	Protocol	Relapse pattern	Treatment at relapse	Outcome (y of FU)
Standard CT	UK9204	Local	CSI	ANED (13.4)
Standard CT	AIEOP-SNC9501	—	—	ANED (10.9)
Standard CT	AIEOP-SNC9501	Local	HDCT/RT	DOD (3.8)
Standard CT	AIEOP-SNC9501	Local	HDCT/RT	ANED (10.3)
Standard CT	AIEOP-SNC9501	—	—	ANED (11.1)
HDCT	AIEOPinfants high risk	—	—	ANED (6.2)
HDCT	AIEOPinfants high risk	—	—	ANED (12.8)
RT	Posterior Fossa,50 Gy	—	—	ANED (12.4)
Standard CT + CSI	AIEOP SNC85	—		ANED (18.2)
Standard CT + CSI	AIEOP SNC85	—	—	ANED (13.5)
Standard CT + CSI	Packer	—	—	ANED (19)
Standard CT + CSI	Packer	—	—	ANED (8.65)

proliferative cells that are embedded in a dense reticulum fiber network. The latter areas resemble the more frequent DMBs, which are believed to arise from granule cell progenitors of the cerebellum and that frequently carry activating mutations of genes encoding for components of the *Hedgehog-Patched* signaling pathway (14, 37). Similar to a previous study (8), our study confirms in greater detail that desmoplastic variants are associated to better prognosis in infants. However, in that series, the authors did not separate DMB from MBEN. Not only did we find better prognosis of both variants in infants, but the same was also found in older children. This observation warrants confirmation in larger prospective studies. In this study, MBEN cases represented up to 50% of all cases with desmoplastic features. MBEN has a peculiar pattern of age distribution because it is clearly more frequent at a young age. In addition, it is often associated with GS. This strong link between GS and MBEN had not yet been reported in a large and well-documented series of patients treated in the modern era, but, if confirmed in prospective larger studies, it will have a major effect on the management of these patients and their families. Further investigation are necessary on distinctive features of DMB and MBEN and particularly if they represent the same biological phenotype with two different, age-dependant histologic appearances.

Location. An analysis of tumor location showed that it had no effect on prognosis. However, analyzing this aspect led us

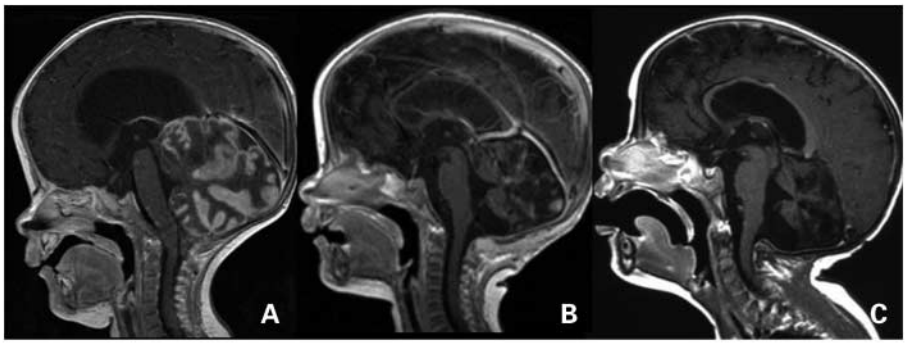

Fig. 3. MRI at diagnosis in a 5-mo-old child with MBEN; *A,* gadolinium-enhanced sagittal brain MRI showing the uniform enhancement of the tumor assuming a nodular and gyriform pattern. *B,* gadolinium-enhanced sagittal image showing marked reduction of all areas of pathologic enhancement after two courses (Cisplatinum and VP16) of CT according to protocol AIEOP SNC9501; *C,* almost complete disappearance of all the lesions after 9 mo of standard CT.

to observe that CMB almost always had a midline (vermis) location, whereas DMB frequently presented as hemispheric tumors. Surprisingly, MBEN, which is believed to be more closely related to DMB than to CMB, had a more frequent vermian (midline) location. However, due to the presence of large masses, it was not always possible to define the tumor origin. This was also true in one MBEN case (Fig. 3), who showed the gyriform presentation described by Agrawal et al. (38). The reasons of such a difference in location between these histologic variants of MB remain to be elucidated.

Metastasis. Our analysis showed a clear difference with regards to the frequency of metastasis present at diagnosis between CMB and desmoplastic variants. Presence of metastasis is the most important adverse prognostic factor in CMB and in the other nondesmoplastic variants (1, 27). Analysis of our series showed that this subgroup of cases with metastasis at diagnosis had the worst prognosis, regardless of histology or age.

Treatment and prognosis. It is well-known that prognostic factors can be influenced by treatment; some may loose relevance as more intensive and effective therapies are introduced. Despite the advent of more intensive therapies adopted since 1997 at our institution, some features, such as histology and presence of metastasis at diagnosis, maintain their power as independent prognostic factors. The use of HDCT followed by irradiation is also associated in our series with a statistically significantly improved outcome, but this observation deserves a longer period of observation and a larger series of cases. The use of a more intensive CT adopted in most of infants cases in conjunction with prevalence of MBEN in this age group, may explain why age less ≤3 years lost its relevance as prognostic factor.

DMB and MBEN are associated with better prognosis than other MB variants in our series as well as in some previous reports, but the basis of this difference is still unknown (2, 8). In our series, MBEN showed a high frequency of complete removal and a significantly lower frequency of metastasis. It also showed a prompt response to CT, which we have not observed in CMB. Of course, our data are based on a small sample size (11 patients) and need to be confirmed in a larger series. However, it is also clear that favorable prognosis does not mean that MBEN and DMB represent "benign tumors" or that they can be cured without postoperative therapy. In fact, our MBEN patients were all treated with intensive postsurgical CT, combined with CSI in some cases. Grill and al. (6), using a less intensive CT approach for infants with MB, did not observe a difference in OS between desmoplastic versus non-DMBs in T0/M0 patients. However, in another series of 45 MB patients age <3 years prospectively treated with intensive systemic CT and intrathecal methotrexate, a significant percentage of these patients, most of whom had desmoplastic variants of MB and were T0/M0, could be treated and cured without RT (8). This certainly suggests that for desmoplastic variants of MB, intensive CT alone could eventually replace current combined CT/RT and this should be tested in future clinical trials. Reducing or eliminating RT in this subset of patients is particularly important, given the association between desmoplastic variants of MB (especially MBEN) and tumor predisposition syndromes, in particular GS (see below).

Association with cancer predisposing syndromes. Recent research has contributed much information on the molecular genetic defects underlying CPS increasing the risk of developing certain brain tumors such as DMB in GS or CMB and glioblastomas in Turcot syndrome, or choroid plexus carcinomas, gliomas, and MB in Li-Fraumeni syndrome (1, 39–41).

Our series provides significant data on frequency and type of CPS in MB. In our series, a frequency of CPS in 28% in MB patients ages ≤3 years at diagnosis was found. GS is the most frequent MB-associated CPS, occurring in 5.8% of cases over all, and in 22.7% of patients diagnosed with desmoplastic variants and 41% of patients in the MBEN group. This represents a higher frequency than reported in the literature. Regarding age, 20% of cases ages ≤3 years had GS, which is a higher frequency than reported by Evans (11) and lower than reported by Amilashi (12) as the 3 syndromic cases (GS) they observed were also the only patients under ages 2 years in their series of 76 MB recruited from 1970 to 2000.

Irradiation, especially with craniospinal extension, is a mainstay of curative therapy in MB but carries a significant risk of long-term sequelae (neurocognitive, growth impairment, and endocrine dysfunctions). Radiation-induced tumors are a major concern and occur after a median delay of 17 years posttreatment (42–44). When MB is associated with GS, nevoid basal cell carcinomas and radiationinduced meningiomas may appear early after irradiation (11, 45, 46). Nevoid basal cell carcinomas may be more difficult to diagnose and treat in early childhood (Walter AW. Gorlin Syndrome).[17] In the series by Amlashi et al. (12), two of three patients with GS-associated MB developed a second tumor, and one of them (anaplastic astrocytoma) subsequently died. On the other hand, MB relapse carries a poor prognosis, thus making treatment decisions for GS-associated MB difficult. Furthermore, the effect of novel treatment modalities (high doses of alkylating agents) on the risk of developing secondary tumors is presently unknown. Current literature suggests that irradiation in GS patients should be avoided whenever possible, and skin sparing techniques or reduced volumes should be applied when necessary (12).

Due to limitations (detection rate is 50-85%) in identification of mutations in *PTCH1*, clinical and radiological examination remains important diagnostic tools for GS. However, several features of GS that are present at a young age may be misinterpreted as changes caused by the tumor (macrocrania, delayed mental development; refs. 11, 12). In addition, skin lesions and intracranial calcifications usually occur only later in life, so this syndrome can be difficult to identify at the time of tumor presentation (11, 47, 48). On the basis of our observations, patients with MBEN are at high risk of GS. Therefore, they should be carefully examined at diagnosis (family history, serial dermatologic examinations, X-ray, genetic counseling, identification of germ-line *PTCH* mutations), to confirm or rule out GS (12, 13, 47, 48).

To date, none of published series of MB in infants have identified this subset of patients or discussed this subgroup in detail (5–8). Our data highlight the need to introduce precise diagnostic and treatment guidelines for GS-related MB patients. In the future, national or international cooperative trials will have to take this genetic predisposing condition into consideration prospectively and, thus, follow guidelines for an optimal diagnostic and therapeutic approach (11, 47, 48). The presence of MBEN should become a major criteria of diagnosis for GS because it is one of its earliest sign together with bone alterations.

In our opinion, genetic investigation and counseling should be offered to MB patients and their families in all cases presenting as MBEN or if patients are age ≤3 years.

Conclusions

Our data show a clear clinical-pathologic definition of MB subgroups. Variants with desmoplastic histology, namely DMB and MBEN, are associated with better outcome. We believe that our data from a large monoinstitutional cohort should be confirmed in larger prospective trials and should be taken into consideration in future patient stratification for risk-adapted treatment.

Furthermore, we showed that MB patients age ≤3 years frequently carry a cancer predisposition condition (e.g., GS) and that patients with MBEN often have GS. These latter patients particularly should be considered candidates for a specific risk-adapted treatment, and genetic counseling.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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