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ORIGINAL REPORT

Subgroup-Specific Prognostic Implications of *TP53* Mutation in Medulloblastoma

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ABSTRACT

Purpose
Reports detailing the prognostic impact of *TP53* mutations in medulloblastoma offer conflicting conclusions. We resolve this issue through the inclusion of molecular subgroup profiles.

Patients and Methods
We determined subgroup affiliation, *TP53* mutation status, and clinical outcome in a discovery cohort of 397 medulloblastomas. We subsequently validated our results on an independent cohort of 156 medulloblastomas.

Results
TP53 mutations are enriched in wingless (WNT; 16%) and sonic hedgehog (SHH; 21%) medulloblastomas and are virtually absent in subgroups 3 and 4 tumors ($P < .001$). Patients with SHH/*TP53* mutant tumors are almost exclusively between ages 5 and 18 years, dramatically different from the general SHH distribution ($P < .001$). Children with SHH/*TP53* mutant tumors harbor 56% germline *TP53* mutations, which are not observed in children with WNT/*TP53* mutant tumors. Five-year overall survival (OS; ± SE) was 41% ± 9% and 81% ± 5% for patients with SHH medulloblastomas with and without *TP53* mutations, respectively ($P < .001$). Furthermore, *TP53* mutations accounted for 72% of deaths in children older than 5 years with SHH medulloblastomas. In contrast, 5-year OS rates were 90% ± 9% and 97% ± 3% for patients with WNT tumors with and without *TP53* mutations ($P = .21$). Multivariate analysis revealed that *TP53* status was the most important risk factor for SHH medulloblastoma. Survival rates in the validation cohort mimicked the discovery results, revealing that poor survival of *TP53* mutations is restricted to patients with SHH medulloblastomas ($P = .012$) and not WNT tumors.

Conclusion
Subgroup-specific analysis reconciles prior conflicting publications and confirms that *TP53* mutations are enriched among SHH medulloblastomas, in which they portend poor outcome and account for a large proportion of treatment failures in these patients.

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INTRODUCTION

Medulloblastoma is a malignant small-cell embryonal neoplasm of the cerebellum. It is the most common malignant brain tumor of childhood and remains a major cause of morbidity and mortality in this age group. Currently, medulloblastomas are mostly stratified based on histologic and clinical and radiologic criteria, which include age of onset, metastatic spread, and residual tumor after surgery. Stratification and management according to clinical risk criteria and development of large cooperative clinical trials resulted in improved survival for these individuals.[1-3] However, aggressive multimodal treatment protocols carry high morbidity; thus differentiation between patients with favorable and poor outcomes would be highly desirable. The difficulty to predict tumor recurrence based on clinical

criteria only has resulted in recent extensive efforts using integrative genomics to allow for genetic and molecular stratification of the disease.[4-6] As of today, none of these molecular markers are routinely used in the clinic or as part of clinical trials. We have reported previously, using the Toronto cohort, that survival is dismal for children with medulloblastoma harboring *TP53* mutations.[7] Subsequently, a similar analysis of patients from the Heidelberg cohort did not confirm the unfavorable prognosis of *TP53*-mutated tumors.[8] Moreover, additional reports from English and German cohorts revealed uncertainty with regard to the role of *TP53* alterations in risk stratification of medulloblastoma.[9,10] Recently, several groups were able to demonstrate that although morphologically similar, medulloblastomas could be divided into several subgroups on the basis of expression profiling.[11-13] A consensus meeting resulted in the current molecular subclassification of medulloblastoma into four subgroups: wingless (WNT), sonic hedgehog (SHH), group 3, and group 4.[14] It is hoped that, in the near future, this subclassification will be used to select targeted therapies and improve understanding of the behavior of this disease. However, other than the WNT group, which is consistently associated with excellent survival,[15,16] SHH tumors, group 3 tumors, and group 4 tumors show heterogeneous outcomes. Recent meta-analysis revealed lack of significant difference in the overall survival (OS) between the latter three groups.[17] Because our group was recently able to demonstrate a unique association of certain genetic catastrophic events (chromothripsis) specifically in patients with SHH/*TP53* mutant medulloblastomas,[18] we hypothesized that this unique association might explain both the controversy regarding the prognostic role of *TP53* mutations in medulloblastoma and the heterogeneous outcome of patients with SHH medulloblastomas. We therefore compiled both clinical and molecular data from multiple centers to generate a large discovery cohort of patient and tumors with known *TP53* status. We then performed subgroup combined with outcome analysis for the first time (Appendix Table A1, online only). We then sequenced and characterized a second, large independent validation cohort from the Medulloblastoma Advanced Genomics International Consortium (MAGIC), for which high-quality clinical data and sufficient tissue were available, thereby allowing us to determine the association of *TP53* mutations, molecular groups, and survival in medulloblastoma.

PATIENTS AND METHODS

Patients

We assembled the clinical and biologic data of patients included in our previous studies for which subgroups have now been assigned, along with additional new samples from all centers (n = 397, Table 1). These included tumors and patient data from the Hospital for Sick Children (HSC) in Toronto, Canada, the Heidelberg database (DKFZ), and the British (Newcastle)[9] and German cohorts.[10] All patient samples were procured in accordance with the research ethics board of their corresponding institution. For 373 patients (92%), clinical and survival data were available for analysis. An independent validation cohort of SHH and WNT tumors with available outcome data and sufficient DNA for sequencing (n = 156) was obtained through MAGIC.[19] Further clinical and demographic data from both cohorts are detailed in Table 1. For progression and OS analysis, time was defined as months from initial diagnosis.

Samples from all centers were either obtained as frozen tissue or formalin-fixed paraffin-embedded biopsies, and nucleic acids from the frozen tissue were extracted as previously described.[19] Nucleic acids were extracted from frozen and formalin-fixed paraffin-embedded samples as previously described.[8]

Table 1. Patient Characteristics by *TP53* Mutational Status

	Discovery Cohort					Validation Cohort				
	TP53 Mutant		*TP53* Wild Type			*TP53* Mutant		*TP53* Wild Type		
Variable	No.	%	No.	%	*P*	No.	%	No.	%	*P*
No.	41		356			22		134		
Age, years										
Median	11.7		10.65			10.7		12.1		
Range	1-45		0-52			1.8-33		0-56		
Age < 3	2	4.9	60	16.8	.04	1	4.5	43	32.1	.009
Adults > 18	4	9.8	60	16.9	.4	4	18.2	30	22.4	.8
Male sex	18	43.9	213	60	.07	10	47.6	73	55.3	.7
Histology					< .001					.002
LCA	17	42.5	43	12.3		8	44.4	14	10.7	
Classic	22	55.0	254	72.8		8	44.4	68	59.8	
Desmoplastic	1	2.5	52	14.9		2	11.1	33	29.5	
M+ disease	4	10.5	110	31.9	.005	5	25	21	18.3	.5
Recurrence	24	58.5	114	32.0	.003	NA		NA		
Dead	18	43.9	82	23.0	.011	9	40.9	27	20.1	.03
Subgroup					< .001					.28
WNT	11	26.8	55	15.4		7	31.8	28	20.9	
SHH	28	68.3	105	29.5		15	68.2	106	79.1	
Group 3	0		72	20.3						
Group 4	1	2.4	121	34						
Missing	1		3	0.8						

NOTE. Histology was available for 389 cases in the discovery and 130 cases in the validation cohorts. Metastatic status at diagnosis was available in 269 cases in the discovery and 135 cases in the validation cohorts.
Abbreviations: LCA, large cell/anaplastic; NA, not applicable; SHH, sonic hedgehog; WNT, wingless.

Subgroup Analysis

Molecular subgrouping was performed on the HSC screening and the MAGIC validation cohort using a custom nanoString codeset designed to assess the expression of 22 medulloblastoma subgroup-specific signature genes as previously described.[20] Samples were processed at the University Health Network (UHN) Microarray Facility using an input of 100 ng of total RNA. Subgrouping of the DKFZ samples were determined by gene expression profiling or by immunohistochemistry and nanoString codeset as previously described.[8,21,22] Subgrouping of the Newcastle samples was determined by a limited gene expression signature as previously described.[13]

TP53 Mutation Analysis

TP53 sequencing for the HSC and validation cohorts was performed on the entire coding sequence (exons 2 through 11) with primers and methodology as previously described.[23,24] *TP53* sequencing for the Heidelberg and the Newcastle cohort was performed as previously described.[8,9] For patients with *TP53* mutant tumors when blood DNA was available, germline mutation status was assessed to determine the diagnosis of Li-Fraumeni syndrome (LFS).[25]

Statistical Analysis

Statistical analysis was performed in the R statistical environment (v2.15). Univariate survival analysis was performed using the log-rank test as implemented in the survival R package (v2.36). Multivariate Cox proportional hazards regression was used to adjust for additional covariates using the survival R package (v.2.36). In all cases, $P < .05$ was considered significant. Stabilities of prognostic markers were assessed by bootstrap resampling as previously described,[26] using the Akaike information criterion for variable selection by backward elimination in 1,000 bootstrap replicates. For correlative studies, the Fisher's exact test was used.

RESULTS

Characteristics of TP53 Mutated Medulloblastomas

In the discovery cohort, we identified *TP53* mutations in 41 (10%) of 397 medulloblastomas. Median age at diagnosis for patients with *TP53* mutant tumors was 11.7 years (range, 1.1 to 45 years). The male to female ratio was 1:1.2. Only 10.5% of *TP53* mutant tumors were metastatic at diagnosis (Table 1). Diffuse anaplasia was observed in 42.5% of mutant tumors. Only one tumor had a concomitant *MYCC* amplification, whereas eight tumors demonstrated simultaneous *MYCN* amplification. Interestingly, of these nine patients, six are alive with a mean follow-up of 4.5 years. At a median follow-up of 49 months (range, 3 to 226 months), 5-year OS for all patients in the discovery cohort was 77% ± 6% and 55% ± 8% for *TP53* wild-type and mutant medulloblastomas, respectively ($P < .001$; Fig 1A).

TP53 Mutational Pattern by Subgroup

TP53 mutations were found predominantly in the SHH and WNT groups. Specifically, *TP53* mutations were observed in 11 (16%) of 66 WNT and 28 (21%) of 133 SHH tumors. In contrast, *TP53* mutations were observed in only one of 122 group 4 and in 0 of 72 group 3 tumors (Fig 2A; $P < .001$). The age distribution observed in patients with SHH/*TP53* mutant medulloblastoma revealed a Gaussian curve peaking at approximately age 15 years, whereas a bimodal age distribution was observed in SHH/*TP53* wild-type tumors (Fig 2B). Most of the patients with SHH/*TP53* mutant tumors (25 of 28) were between the ages of 5 and 18 years, which differs dramatically from SHH/*TP53* wild-type ones (31 of 105; $P < .001$). None of the WNT/*TP53* mutant tumors demonstrated anaplastic features, whereas 68% of SHH/*TP53* mutant tumors had severe anaplasia ($P < .001$). Similarly, none of the WNT/*TP53* mutant tumors harbored *MYC* or *MYCN* amplifications, whereas 33% of SHH/*TP53* mutant tumors exhibited these genetic alterations ($P = .05$). *TP53* mutations were observed in the DNA-binding domain (exons 4 through 8; Fig 2C). The most common mutations were in codons 248 and 175, which are the most commonly mutated residues in both somatic and germline *TP53* mutation databases (International Agency for Research on Cancer).[26a] Of the patients for whom *TP53* germline status was available (n = 20), nine patients (45%) harbored germline mutations consistent with LFS. All these individuals had SHH tumors. Although 56% of SHH/*TP53* mutant tumors had a concomitant germline mutation, germline mutations were not seen in WNT/*TP53* mutant tumors.

TP53 Mutations and Survival by Subgroup

A striking association between biologic subgroups and survival for patients with *TP53* mutant tumors was observed. Specifically, patients with SHH/*TP53* mutant tumors had 5-year OS of 41% ± 19%, whereas patients with WNT/*TP53* mutant tumors had 5-year OS of 90% ± 9% ($P = .018$; Fig 1B). Five-year OS was 41% ± 9% and 81% ± 5% for patients with SHH tumors with and without *TP53* mutations ($P < .001$; Fig 1C). However, for children between the ages of 5 and 18 years with SHH tumors, *TP53* mutations accounted for 72% of deaths. Within the limitations of a small cohort, children with confirmed LFS had survival similar to that of the remaining patients with *TP53* mutant SHH medulloblastomas (Appendix Fig A1, online only). In contrast, 5-year OS was 90% ± 9% and 97% ± 3% for patients with WNT tumors with and without *TP53* mutations ($P = .21$; Fig 1D). A multivariate Cox proportional hazards regression model of 5-year survival for SHH tumors accounting for age, sex, histology, presence of metastases, and *TP53* status demonstrated that *TP53* mutation status is the single most important independent risk factor for this group (Table 2).

Validation of Survival Analysis for SHH and WNT Tumors

To validate the survival difference between WNT and SHH *TP53* mutant medulloblastomas observed in our new assembled cohort, we performed additional Sanger sequencing of *TP53* in a separate cohort of 156 patients with medulloblastoma from the SHH and WNT groups with adequate clinical data, who were available through MAGIC. The rate of *TP53* mutations in this cohort was similar to the rate seen by our group. Most patients (81%) had nonmetastatic tumors, and the male to female and age distributions were similar to those of our initial cohort. Diffuse anaplasia was reported in 54% of SHH/*TP53* mutant tumors and 12% of SHH/*TP53* wild-type ones ($P = .002$). None of the WNT/*TP53* mutant tumors were defined as anaplastic. The OS of this cohort mimicked the observed findings in our initial cohort (Figs 2E and 2F). At a median follow-up of 42 months (range, 2 to 300 months), 5-year OS for patients with SHH tumors with and without *TP53* mutations was 41% ± 17% and 76% ± 4%, respectively ($P = .012$); in contrast, 5-year OS for patients with WNT tumors with and without *TP53* mutations was 86% ± 13% and 94% ± 5%, respectively ($P = .41$).

Additional Data From All Patients in the Study

Summarizing all data from all patients from whom all clinical and molecular data were available enabled us to further clarify several observations. Combining all patients with SHH medulloblastomas

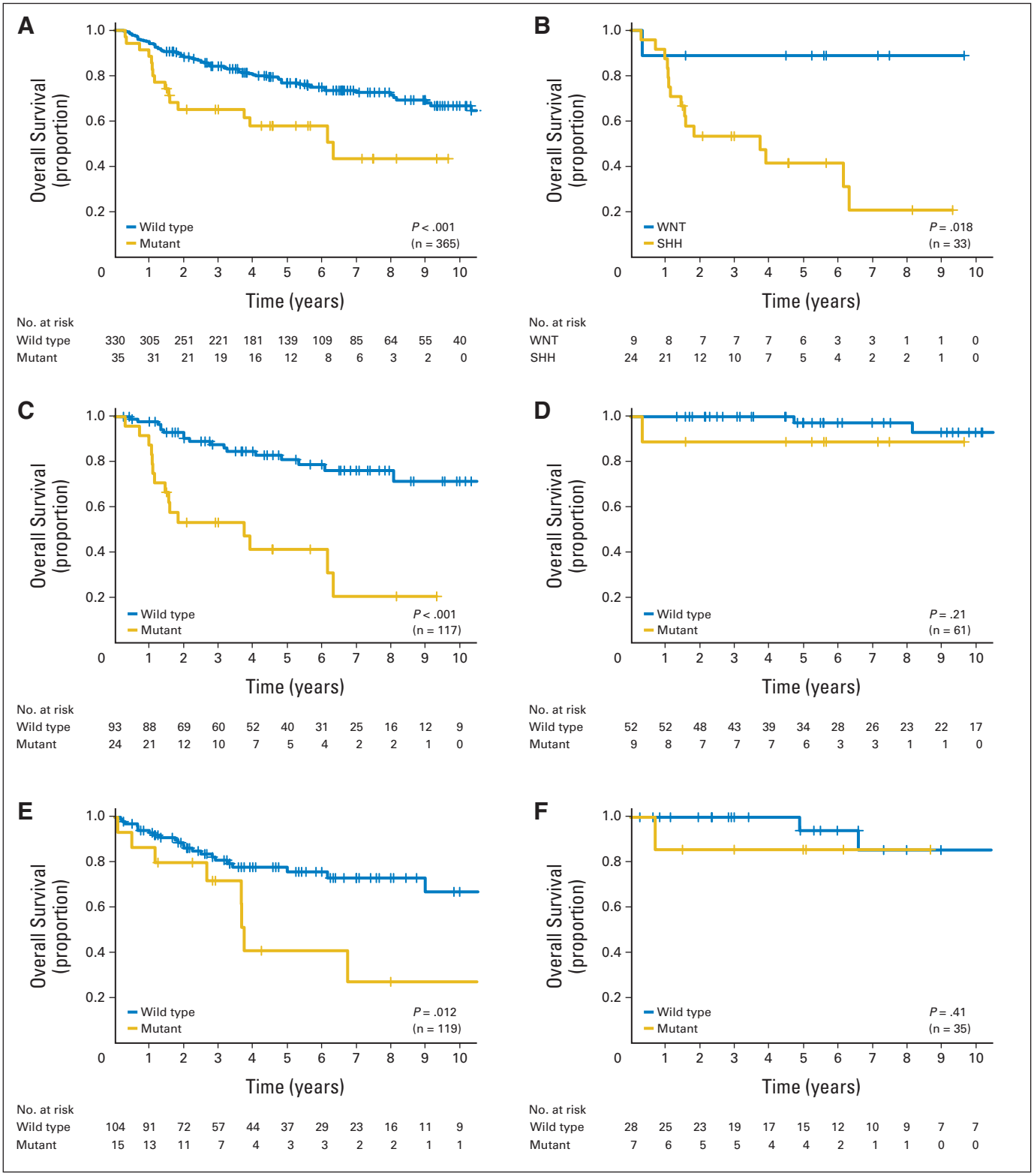

Fig 1. Kaplan-Meier estimates of overall survival for study patients by group. (A) All discovery cohort patients by *TP53* mutations. Blue line, *TP53* wild type, wingless (WNT) group; gold line *TP53* mutant, sonic hedgehog (SHH) group. (B) *TP53*-mutant tumors stratified by subgroup analysis. Blue line, WNT group; gold line, SHH group. (C) Blue line, *TP53* wild type, WNT group; gold line, *TP53* mutant, SHH group. (D) WNT tumors from the discovery cohort. Blue line, *TP53* wild type, WNT group; gold line, *TP53* mutant, SHH group. (E) SHH tumors from the validation cohort. Blue line, *TP53* wild type, WNT group; gold line, *TP53* mutant, SHH group. (F) WNT tumors from the validation cohort. Blue line, *TP53* wild type, WNT group; gold line, *TP53* mutant, SHH group.

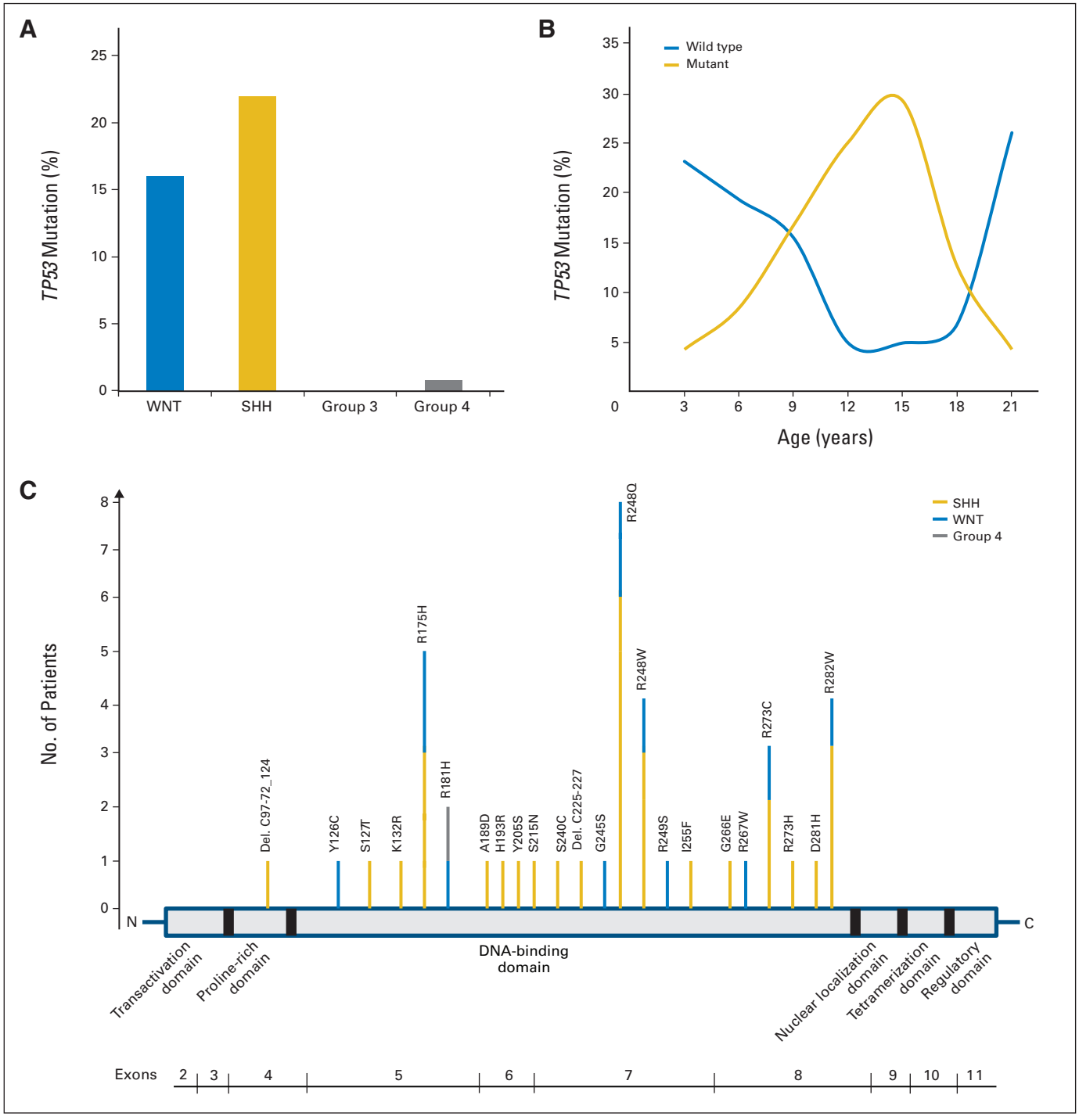

Fig 2. Characteristics of *TP53* mutation in medulloblastoma. (A) Percentage of *TP53* mutations in medulloblastoma subgroups; (B) Age distribution of sonic hedgehog (SHH) medullablastoma according to *TP53* status. (C) Distribution of *TP53* mutations according to functional domains. WNT, wingless.

between the ages of 5 and 18 years (where radiation is usually given to all children, n = 84) revealed that tumors carrying *TP53* mutations were associated with 21 (72%) of 29 deaths ($P < .001$; Appendix Fig A2, online only). The combined cohorts included 42 adults, of whom six (14%) had *TP53* mutant tumors. Five of these patients died of their disease. Interestingly, two patients had WNT tumors, and none survived.

DISCUSSION

The genetic and genomic understanding of medulloblastoma has evolved dramatically in the past few years. International collaborations have resulted in novel classification of this brain tumor and the potential for targeted therapies for patients within specific subgroups.

Table 2. Multivariate Cox Proportional Hazards Regression Models of Overall Survival in SHH Subgroup Medulloblastoma

Variable	Hazard Ratio	95% CI	BIF	*P*
Discovery cohort				
TP53 mutation	4.39	1.70 to 11.29	95.7	.002
LCA *v* classic	1.07	0.39 to 2.90	20.9	.90
Desmoplastic *v* classic	0.45	0.17 to 1.22	54.0	.11
Sex (male *v* female)	1.21	0.56 to 2.58	24.3	.63
M+	0.38	0.11 to 1.37	58.6	.14
Age < 3 years	1.41	0.51 to 3.87	25.1	.51
Validation cohort				
TP53 mutation	14.7	3.44 to 62.9	97.6	< .001
LCA *v* classic	2.90	0.79 to 10.6	47.1	.11
Desmoplastic *v* classic	0.41	0.08 to 2.14	43.9	.29
Sex (male *v* female)	0.71	0.24 to 2.11	24.4	.54
M+	26.4	5.96 to 117.5	99.4	< .001
Age < 3 years	4.15	0.93 to 18.6	60.7	.062

Abbreviations: BIF, bootstrap inclusion frequency; LCA, large cell/anaplastic; SHH, sonic hedgehog.

Nevertheless, except for patients with WNT tumors, for whom outcome is superior to all other subgroups, survival of children with SHH, group 3, and group 4 tumors is still unsatisfactory.[17]

In this study, we demonstrate that characterization of *TP53* mutation status can segregate individuals with SHH medulloblastoma into favorable and extremely poor survival groups. Specifically, patients with SHH/*TP53* mutant medulloblastomas have profoundly worse outcome than those with SHH/*TP53* wild-type tumors. The importance of this observation is further highlighted by the fact that most patients with *TP53* mutant medulloblastomas have average-risk tumors, as measured by conventional nonmolecular methods, for which survival is expected to be excellent with current protocols.[1,2] To validate our observations from the discovery cohort, we examined a separately ascertained cohort that included a large number of children and adults with SHH and WNT tumors. The impressive similarity in outcomes between the two cohorts argues against an unobserved variable confounding the results of our discovery cohort. Moreover, large whole-genome and exome sequencing efforts recently published by separate groups revealed an additional, albeit small number, of *TP53* mutations in medulloblastoma.[27-29] Interestingly, these independent groups found *TP53* mutations enriched in the SHH group and associated with poor survival. In fact, of 14 SHH tumors studied by Robinson et al,[29] deaths were only reported in the three *TP53* mutant tumors. The observation that a significant number of deaths in the SHH cohort were associated with *TP53* mutations in both our and other cohorts suggests that somatic *TP53* mutations analysis should be performed on patients with SHH medulloblastomas (Fig 3).

Our study also highlights how genomic and molecular stratification could explain differences in clinical and biologic behavior of cancers with a specific genetic alteration. Indeed, although patients with SHH *TP53* mutated tumors fared miserably, this aggressive genotype was completely modified in WNT tumors. Our group has recently shown that WNT activation through constitutive β-catenin activation can abrogate the radioresistance conferred by *TP53*-mutated medulloblastomas as a potential explanation for this difference in outcome.[30] The separation of SHH subgroup patients by *TP53* status would significantly improve risk stratification of this subgroup, analogous to the role of *FSTL5* in stratifying group 3 and 4 patients.[22] Indeed, our multivariate modeling would suggest that a simple model

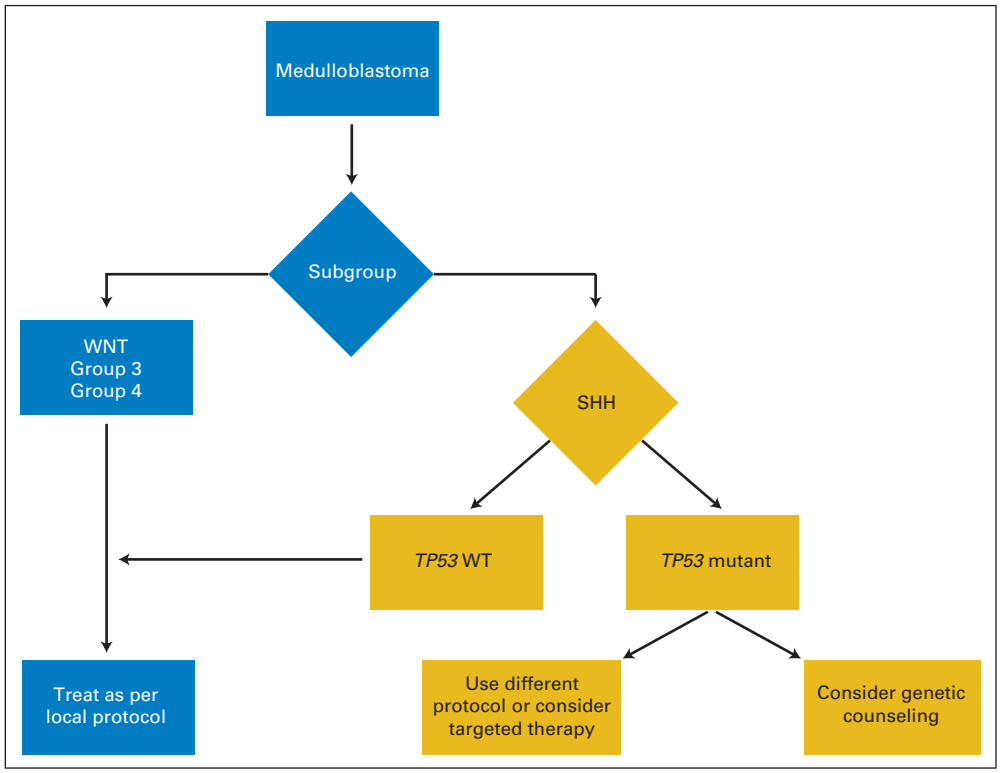

Fig 3. Risk stratification for patients with medulloblastoma based on molecular subgroups and *TP53* status. SHH, sonic hedgehog; WNT, wingless; WT, wild type.

of subgroup along with *TP53* mutation status is highly predictive of outcome.[31]

SHH/*TP53* mutant medulloblastomas have several biologic characteristics that can explain their clinical behavior. These tumors reveal a high rate of *MYCN* amplification. Furthermore, our group was recently able to demonstrate that these cancers have a high rate of single chromosomal shattering, or chromothripsis.[18] This catastrophic event is associated with poor survival and a high degree of genomic instability in other cancers[31-33] and therefore offers a plausible explanation for the poor survival of these patients.

The primary aim of this study was to assess somatic *TP53* alterations in medulloblastoma. However, our germline data from a subgroup of these patients demonstrate a high rate of germline *TP53* mutations. These findings have important implications to both the patient and other family members in terms of their subsequent cancer risk. Individuals with LFS, which is a devastating cancer predisposition syndrome, were recently shown to derive benefit from a clinical surveillance protocol.[34] Therefore, genetic counseling should be offered to all patients and families with SHH *TP53* mutant medulloblastomas.

Although our WNT *TP53* mutant tumors had a poor outcome among adults, the data are based on a small number of tumors, albeit suggesting that adults might deserve a different approach then children regarding genetic and molecular stratification.[35]

Although the effects of *TP53* mutation were different in SHH and WNT subgroups, the interactions between *TP53* mutation and subgroup (SHH *v* WNT) was not statistically significant in the discovery ($P = .62$) and validation cohorts ($P = .70$). Our data are consistent with the proposition that patients with mutant *TP53* WNT tumors do not have poorer survival compared with their wild-type counterpart, although the alternative possibility cannot be ruled out because of the rarity of *TP53* mutation in patients with WNT tumors. In this study and others,[36] *TP53* mutations were highly associated with anaplasia. In our study, anaplasia was seen in 19 (66%) of 29 SHH *TP53* mutant tumors and only in 14 (10%) of 136 SHH *TP53* wild-type ones ($P < .001$). This suggests that high index of suspicion for *TP53* mutations should exist for SHH tumors with diffuse anaplasia.

Finally, we suggest a clinicopathologic approach to medulloblastoma based on molecular subgroups and *TP53* status (Fig 3). In institutions where molecular group stratification is available, determination of molecular subgroup should be performed. Although some centers use RNA-based assays that may be difficult to reproduce by all institutions, efforts are being made to optimize antibody-based assays,[14,22] which aim to define SHH tumors in a robust and simple way for most clinical laboratories.[37] SHH subgroup tumors should be sent for *TP53* sequencing by clinically approved laboratories. For most institutions where molecular subgroups are not available, determination of *TP53* status should be performed in conjunction with nuclear β-catenin status. *TP53* mutational analysis is preferably done by direct sequencing. However, p53 immunostaining is a very sensitive (albeit not specific) surrogate method for *TP53* mutation detection in brain (and other) tumors and could be performed as an initial screening tool.[7,38,39] In our previous cohort, all *TP53* mutant tumors were immunopositive.[7] In this cohort and in our larger pediatric brain tumor cohort, immunostaining is more than 90% sensitive for detection of *TP53* mutations, and false negatives are generally restricted to deletions and splice site mutations in which no or truncated protein is expressed.[7,39,40] Tumors harboring *TP53* mutations should be then sent for subgroup analysis for determination of SHH status. Because current treatment protocols for patients with average-risk medulloblastoma result in dismal survival for those with *TP53* mutant SHH medulloblastomas, these patients should be considered for either enrollment in clinical trials of targeted therapy and/or other modified therapies. Furthermore, careful history of prior cancers in the family and genetic counseling should be offered to these individuals because of the high rate of LFS in this population. This practical approach can be also used in future clinical trials that are trying to stratify patients for reduced therapy.

In summary, this study serves as a proof of principle that collaborative efforts can resolve discrepancies between reports of an uncommon event in cancer. Specifically, this approach validated the prognostic role of *TP53* mutations in medulloblastoma once molecular subgroup is taken into account. This study also adds another dimension to the evolving role of genomic and genetic approaches to cancer by demonstrating that SHH-driven medulloblastomas, which account for a third of medulloblastomas seen in children, should be stratified according to their *TP53* status, whereas *TP53* mutation status has little prognostic effect in the setting of a WNT medulloblastoma. Ongoing collaborations between centers will allow for elucidation of the role of *TP53* alterations in adult medulloblastomas as well as the design of rational therapies for children with medulloblastoma in an era of precision medicine.

AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

The author(s) indicated no potential conflicts of interest.

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Appendix

Table A1. Patient Distribution According to Subgroups and *TP53* Status (discovery cohort)

	Newcastle		DKFZ		Sick Kids		Boston		Total	
	No.	%	No.	%	No.	%	No.	%	No.	%
WNT										
Wild type	13	65	35	11	7	11.7	0	0	55	13.9
Mutant	2	10	6	1.9	1	1.7	2	66.7	11	2.8
SHH										
Wild type	3	15	90	28.7	12	20	0	0	105	26.4
Mutant	1	5	18	13.3	8	13.3	1	33.3	28	7.1
Group 3										
Wild type	0	0	59	18.8	13	21.7	0	0	72	18.1
Mutant	0	0	0	0	0	0	0	0	0	0
Group 4										
Wild type	0	0	105	33.4	16	26.7	0	0	121	30.5
Mutant	0	0	1	0.3	0	0	0	0	1	0.3
Missing										
Wild type	0	0	0	0	3	5	0	0	3	0.8
Mutant	1	5	0	0	0	0	0	0	1	0.3
Total	20	100	314	100	60	100	3	100	397	100

Abbreviations: DKFZ, Heidelberg database; SHH, sonic hedgehog; WNT, wingless.

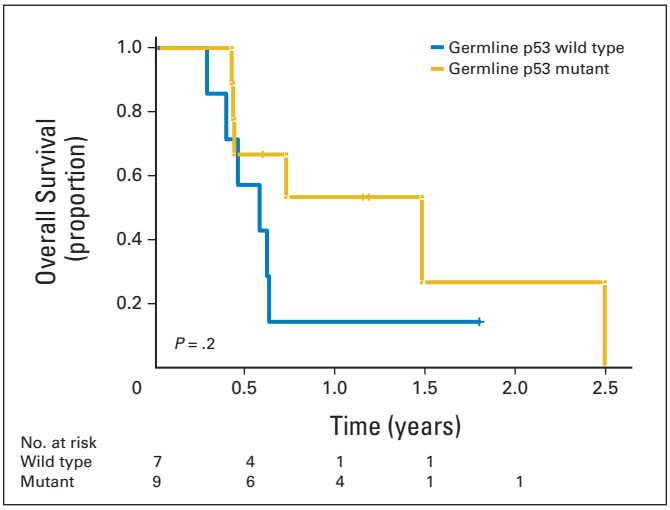

Fig A1. Survival estimates for children with sonic hedgehog (SHH)/*TP53*-mutant tumors when germline *TP53* status was available (n = 16).

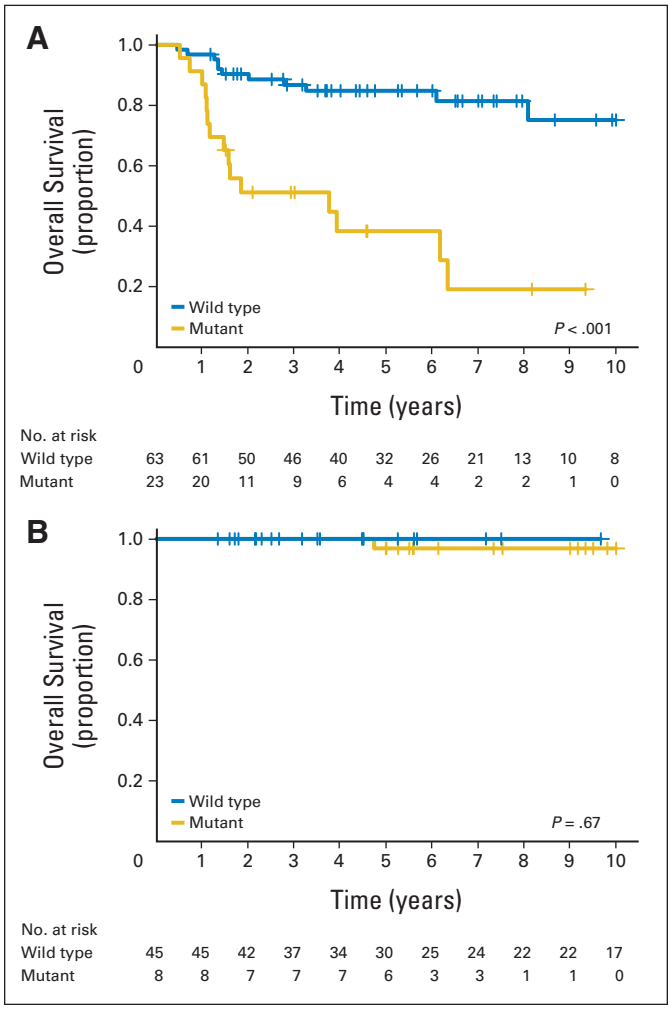

Fig A2. Kaplan-Meier estimates of overall survival for children and infants by group. (A) Sonic hedgehog (SHH) tumors from the discovery cohort. (B) Wingless (WNT) tumors from the discovery cohort.