

## Frequent *hSNF5/INI1* Germline Mutations in Patients with Rhabdoid Tumor

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### Abstract

**Purpose:** Germline *hSNF5/INI1* mutations are responsible for hereditary cases of rhabdoid tumors (RT) that constitute the rhabdoid predisposition syndrome (RPS). Our study provides the first precise overview of the prevalence of RPS within a large cohort of RT.

**Experimental Design:** *hSNF5/INI1* coding exons were investigated by sequencing and by multiplex ligation-dependent probe amplification.

**Results:** Seventy-four constitutional DNAs from 115 apparently sporadic RT were analyzed from 1999 to 2009. Germline mutations were found in 26 patients (35%). Data from 9 individuals from 5 RPS families (siblings) were also studied. The median age at diagnosis was much lower (6 months) in patients with germline mutation ( $P < 0.01$ ) than in patients without (18 months). Nevertheless, 7 of 35 patients with germline mutation (20%) developed the disease after 2 years of age. The mutation could be detected in only 1 parent whereas germline blood DNA was wild type in the 20 other parent pairs, therefore indicating the very high proportion of germ-cell mosaicism or of *de novo* mutations in RPS. The former hypothesis could be clearly documented in 1 case in which prenatal diagnosis was positive in a new pregnancy. Finally, the 2 years' overall survival was 7% in mutated and 29% in wild-type patients, mainly due to the worse outcome of RT in younger patients.

**Conclusions:** Our results show a high proportion of germline mutations in patients with RT that can be found at any age and up to 60% in the youngest patients. Genetic counseling is recommended given the low but actual risk of familial recurrence. *Clin Cancer Res*; 17(1); 31–8. ©2011 AACR.

### Introduction

Rhabdoid tumors (RT) are rare and aggressive tumors of children, initially characterized by the presence of undifferentiated cells with eosinophilic cytoplasmic inclusions, uncondensed chromatin, and large nucleolus, defining the rhabdoid phenotype (1). Miscellaneous anatomic locations can be involved. Beckwith et al. first described rhabdoid cells in aggressive renal tumors, therefore individualizing rhabdoid tumors of the kidney

(RTK) from Wilms' tumors (1). Tsuneyoshi et al. extended the observation of a rhabdoid phenotype in soft-part undifferentiated tumors, which were subsequently named extrarenal malignant rhabdoid tumors (ER-MRT; ref. 2). Finally, Rorke et al. described central nervous system (CNS) tumors of early onset, with high aggressiveness and also harboring rhabdoid cells. Because of their pleomorphic aspect, these CNS tumors were designated as atypical/teratoid rhabdoid tumors (ATRT; ref. 3). Although affecting different parts of the

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**Note:** Supplementary data for this article are available at Clinical Cancer Research Online (<http://clincancerres.aacrjournals.org/>).

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### Translational Relevance

Our study provides more accurate advices for the genetic counseling of patients with rhabdoid tumor. As germline mutations are frequent, a predisposition should now be searched for systematically. In particular, our study shows possible late occurrence of tumors in carriers of a germline mutation; hence, the age at tumor diagnosis should not be a criteria for genetic counseling anymore. We also illustrate that prenatal diagnosis is feasible and should be considered.

body, RTK, ER-MRT, and ATRT were shown to share the same genetic driver event, that is, the biallelic inactivation of the tumor-suppressor gene *hSNF5/INI1* that can be documented in more than 80% of cases (4–6). The genetic full inactivation of *hSNF5/INI1* according to the two-hits Knudson model is somatically acquired in the tumors. However, early-onset, multifocal disease and familial cases strongly supported the possibility of a rhabdoid predisposition syndrome (RPS). This was confirmed by the presence of constitutional mutations of *hSNF5/INI1* in the rare familial cases (7–11) and, more broadly, in a subset of patients with apparently sporadic rhabdoid tumors (4, 11–13). Analysis of the presently reported familial cases indicates that RPS is highly penetrant and, due to the high mortality rate of RT, very rarely affects more than 1 generation (9, 11, 13, 14). However, the precise genetic epidemiology of RPS is sparsely known, as only small cohorts and brief reports are available. In particular, genetic counseling for families remains cautious; hence, better knowledge on the frequency of RPS, of its penetrance and expression, and of the familial recurrence risk is warranted. To assess these issues, we have conducted a retrospective study on a large cohort of rhabdoid tumors with available constitutional DNA. Our study brings some more accurate genetic epidemiologic data and offers new lights for the genetic counseling.

### Materials and Methods

#### Tumors

From 1999, frozen tumors samples from 30 institutions in France and 5 institutions in Europe with a presumptive diagnosis of RT were referred to our institution for DNA extraction and *hSNF5/INI1* gene assessment. Since 2006, we also use immunohistochemistry (IHC) with anti-BAF47 antibody to assess the expression of *hSNF5/INI1* in tumor cells. In a previous report, we described some RTs with a maintained *hSNF5/INI1* expression and without any mutation (15). In the present study, we have included all tumors with a compatible histologic phenotype and (i) a biallelic inactivation as documented by molecular biology and/or (ii) loss of *hSNF5/INI1* expression by IHC referred to our unit between 1999 and 2009. Thus, 115 tumors were

included in this study. Constitutional DNA and informed consent were prospectively collected in 74 of these 115 index cases but were unavailable in 41 other cases.

#### Patients and families

Clinical data of the patients were collected from the referent physicians, including age at diagnosis, anatomic location of the tumor, pathologic report, uni- or multifocal status, follow-up, and outcome.

Patients' and relatives' peripheral blood samples were prospectively obtained by the referent physicians, with informed consent for genetic screening.

In case of a germline mutation in the index case and of a new pregnancy, genetic counseling was proposed to the parents. For prenatal diagnosis, DNA was extracted from trophoblasts biopsy or from cells obtained by amniocentesis.

#### *hSNF5/INI1* and 22q11.2 locus analyses

DNA was extracted from frozen tumors, blood lymphocytes, and chorionic villi according to classical procedures. All coding exons and splice sites regions were sequenced using the Sanger method and ABI automated fluorescent sequencer (primers available in Supplementary Table 1). Large size deletions were searched for using the multiplex ligation-dependent probe amplification assay (Salsa MLPA KIT P258-B1 SMARCB1). For patients with whole *hSNF5/INI1* deletion in the germline, the deletion borders were studied using Affymetrix SNP6-arrays (following manufacturer's recommendation and as published in ref. 16).

#### Statistical analyses

Statistics were performed on the patients with a constitutional mutation screening only, excluding the 41 of 115 patients for whom no mutation screening was done. Median age at diagnosis between patients groups was compared with the Kruskal–Wallis test. The association of constitutional mutations with anatomic locations was assessed by the Fisher exact test. Overall survivals (OS) of patients were estimated using the log-rank test and plotted according to the Kaplan–Meier method; survival was calculated from the date of diagnosis to the date of last follow-up. Log-rank tests and Cox regression models were used to investigate the impact of mutation status and age at diagnosis on OS.

### Results

#### Sporadic presentations

Among the whole cohort of 115 RT, patients with a germline genetic analysis were mostly the youngest patients, median age at diagnosis being 11 months in this group as compared with 32 months in the unstudied group. A germline mutation of the *hSNF5/INI1* gene was detected in 26 of 74 analyzed patients (35%); a second inactivating event was evidenced in all the analyzed tumors (Table 1).

**Table 1.** Summary of genetic and clinical characteristics of patients with germline mutations

| Patient           | Germline         | Consequence            | Tumor                 | Location        | Age <sup>a</sup> | Outcome |
|-------------------|------------------|------------------------|-----------------------|-----------------|------------------|---------|
| 1                 | c.93G > C        | Affect the splice site | Del Ex1-9             | ATRT            | 28               | DOD     |
| 2                 | c.105C > G       | p.Tyr35X               | Del Ex1-9             | ATRT            | 29               | NED     |
| 3                 | c.118C > T       | p.Arg40X               | c.118C > T            | Orbita          | 0                | DOD     |
| 4                 | c.122dup         | p.Ser42PhefsX29        | Del Ex1-9             | Thorax          | 2                | NA      |
| 5                 | c.157C > T       | p.Arg53X               | Del Ex1-9             | ATRT            | 1                | DOD     |
| 6                 | c.442_449del     | p.Ser148HisfsX19       | Del Ex1-9             | ATRT            | 14               | DOD     |
| 7                 | c.472C > T       | p.Arg158X              | c.472C > T            | Multifocal MRT  | 0                | DOD     |
| 8                 | c.564del         | p.Ile189SerfsX20       | c.564del <sup>b</sup> | RTK             | 3                | DOD     |
| 9                 | c.601C > T       | p.Arg201X              | c.601C > T            | ATRT            | 0                | DOD     |
| 10                | c.747dup         | p.Thr250HisfsX3        | Del Ex1-9             | Pelvis          | 7                | DOD     |
| 11                | c.760dup         | p.Leu254ProfsX27       | Del Ex1-9             | RTK             | 6                | NA      |
| 12                | c.757_796-659del | p.Ile253_Lys265del     | ND                    | ATRT            | 5                | NA      |
| 13                | c.778del         | p.Gln260SerfsX7        | Del Ex1-9             | ATRT            | 6                | DOD     |
| 14                | c.902del         | p.Leu301ArgfsX19       | c.902del              | ATRT            | 7                | NED     |
| 15                | Dup Ex6-7        | Frameshift             | Del Ex1-9             | ATRT + RTK      | 7                | DOD     |
| 16                | Del Ex2          | Frameshift             | Del Ex2               | Leg             | 0                | DOD     |
| 17                | Del Ex4-5        | Frameshift             | ND                    | ATRT            | 24               | DOD     |
| 18                | Del Ex6          | Frameshift             | Del Ex6               | ATRT            | 2                | DOD     |
| 19                | Del Ex7-9        | No transcript          | Del Ex7-9             | ATRT + RTK      | 5                | DOD     |
| 20                | Del Ex1-9        | No transcript          | Del Ex1-9             | Buttock + ATRT  | 3                | DOD     |
| 21                | Del Ex1-9        | No transcript          | ND                    | ATRT + RTK      | 3                | DOD     |
| 22                | Del Ex1-9        | No transcript          | Del Ex1-9             | ATRT            | 8                | DOD     |
| 23                | Del Ex1-9        | No transcript          | c.1148del             | ATRT            | 25               | DOD     |
| 24                | Del Ex1-9        | No transcript          | Del Ex1-3             | Thorax          | 101              | CR      |
| 25                | Del Ex1-9        | No transcript          | c.927del              | Retroperitoneum | 297              | DOD     |
| F1.1 <sup>c</sup> | c.978C > A       | p.Tyr326X              | c.978C > A            | Paraspinal      | 4                | DOD     |
| F1.2 <sup>c</sup> | c.978C > A       | p.Tyr326X              | Del Ex7               | ATRT            | 0                | DOD     |
| F2.1 <sup>d</sup> | c.472C > T       | p.Arg158X              | Del Ex1-9             | Multifocal MRT  | <24              | NA      |
| F2.2 <sup>d</sup> | c.472C > T       | p.Arg158X              | Del Ex1-9             | RTK             | 7                | NA      |
| F3.1              | c.952dup         | p.Gly318ProfsX43       | c.952dup              | ATRT            | 4                | DOD     |
| F3.2              | c.952dup         | p.Gly318ProfsX43       | c.952dup              | Thorax          | 73               | CR      |
| F5.1              | c.472C > T       | p.Arg158X              | ND                    | ATRT            | 4                | DOD     |
| F5.2              | c.472C > T       | p.Arg158X              | ND                    | ATRT            | 8                | DOD     |

Abbreviations: Del, deletion; DOD, dead of disease; NED, no evidence of disease; dup, duplication; NA, not available; ND, not done; CR, complete remission.

<sup>a</sup>Age in months.

<sup>b</sup>Loss of heterozygosity with no copy number variation assessed by MLPA, suggesting a duplication of the mutated allele in the tumor.

<sup>c</sup>Patients in reference 8.

<sup>d</sup>Patients in reference 10.

Among the parents of the 26 apparently sporadic index cases with germline mutation, constitutional DNA could be studied in 20 fathers and 20 mothers; no mutation was evidenced. The median ages of mothers and fathers at birth time of the germline mutation carriers were 30.9 and 35.4 years, respectively.

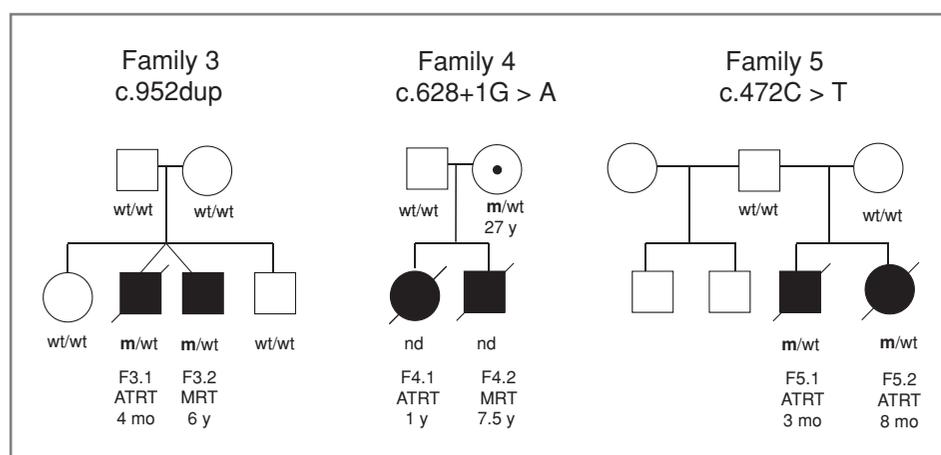
#### Prenatal studies

Following the identification of a germline mutation in the index case, a new pregnancy occurred in 13 cases. After genetic counseling, a prenatal diagnosis was carried out in all cases. Twelve fetuses were negative and 1 was positive for

the mutation previously detected in the older brother (family 5, Fig. 1). The parents decided not to undergo medical abortion. The child was normally born at term and followed by monthly abdominal and transfontanelar ultrasonography; but she ultimately developed a spinal ATRT, a location imperfectly explored by abdominal ultrasonography, at 8 months of age and died 3 months later.

#### Familial cases

Constitutional DNAs from 3 pairs of siblings (2 pairs of twins) with RT were also studied. Two families were previously reported (10). All the affected children in this



**Figure 1.** Pedigrees of the 3 unpublished new families. Numbers refer to Table 1. Sequencing results are reported under each individual: m: mutated; wt: wild type; nd: not done.

familial cohort carried a germline *hSNF5/INI1* mutation (Table 1).

For 1 additional pedigree with 2 children affected by RT, constitutional DNA was available for the parents only. A nucleotide substitution affecting the donor splice site of exon 5 (c.628 + 1G > A) was identified in the 27 years old unaffected mother (family 4, Fig. 1).

Including the family with the positive prenatal diagnosis, only 2 familial cases were observed in the French cohort within the 10 years period of our study (families 1 and 5). The 3 newly described families are indicated in Figure 1.

#### Clinical characteristics of patients with germline mutations

Altogether, a germline mutation was clearly documented in 34 individuals (26 apparently sporadic index cases, 1 prenatal diagnosis, and 7 with a known familial history of RT) and was highly likely in 2 additional individuals (F4.1 and F4.2); only 1 individual remained asymptomatic (mother of patients F4.1 and F4.2). A total of 17 of 40 ATRT, 3 of 9 renal tumors, 9 of 26 extrarenal and extracerebral tumors, and 6 of 8 multifocal RT carried a germline mutation. We tested whether the presence of a germline mutation preferentially predispose to a particular tumor location, including CNS, kidney, or extrarenal extracerebral locations; we found no such significant association (Fisher's exact test,  $P = 0.159$ ), suggesting that germline mutations equally predispose to all kind of tumor locations.

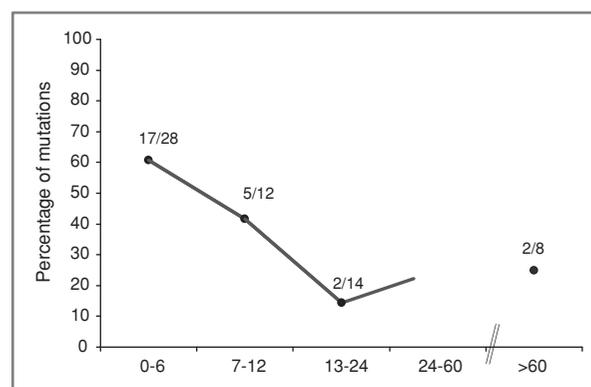
The median age at the diagnosis of RT in patients with germline mutations was 6 months, compared with a median age of 18 months for RT occurring in the patients with wild-type alleles (Kruskal-Wallis test,  $P < 0.0055$ ).

The frequency of germline mutation according to age at tumor diagnosis is shown in Figure 2. Of note, among the 7 of 25 patients affected after 2 years of age that carried a germline mutation, 3 had a whole gene deletion and 1 a splice site mutation (Table 1).

#### Impact of germline mutation on survival

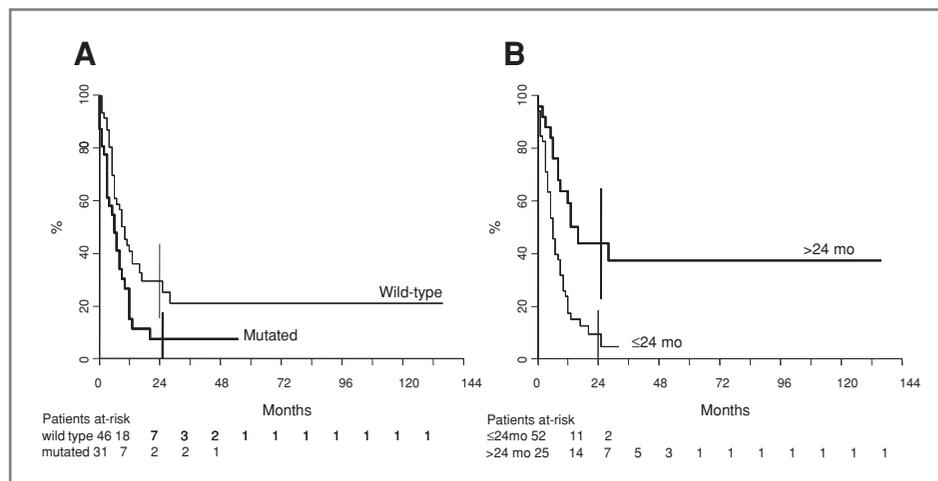
Clinical variables thought to impact the prognosis of RT include metastases, total gross resection, irradiation,

germline mutations, and age at diagnosis. Among these, we considered that the only unambiguous variables in this wide inhomogeneous and retrospective series were age and germline mutation, precluding an accurate multivariate analysis but still allowing addressing the impact of these 2 variables. On univariate analysis, overall survival at 2 years was 7.6% (0%–17%) in patients with germline mutation whereas it was 29.4% (15%–43%) in patients with wild-type germline alleles (Kaplan–Meier method, log-rank,  $P \leq 0.02$ ; Fig. 3A). Furthermore, and as previously observed (13), the comparison of survival between patients older or younger than 2 years at diagnosis showed a highly significant difference in a univariate analysis (Fig. 3B; log-rank,  $P \leq 0.0004$ ). Given the strong association of germline mutation with earlier tumor onset, we assessed whether germline mutation may influence the prognosis independently of age. A Cox regression model including age and presence of germline mutation as variables finally shows that age was the most strongly significant



**Figure 2.** Observed frequency of germline mutation according to the age at tumor onset. The black line shows the risk of carrying a germline mutation at each age range; the risk is estimated by the ratio of the number of patients with a germline *hSNF5/INI1* mutation on the total number of patients (mutated and wild-type) for whom constitutional DNA could be studied.

**Figure 3.** Overall survival of patients according to mutation status (A) and age at tumor onset (B). The estimated survival is calculated using the log-rank test and reported with the Kaplan–Meier method. Wild type, patients with normal constitutional *hSNF5/INI1* gene; mutated, patients with germline mutations.



factor ( $P \leq 0.0004$ ) and attenuated the impact of the germline mutation ( $P \leq 0.035$ ).

**Multifocal disease**

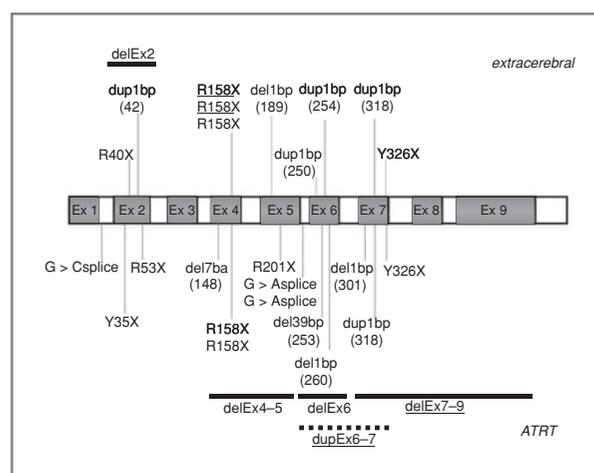
A total of 8 multifocal cases were investigated, with 6 of 8 carrying a germline mutation. Hence, in 2 patients, a multifocal neonatal disease was not associated with an obvious germline mutation. The first case presented at birth with 3 different tumors in soft parts (leg, axillary, and flank). The second case was a neonatal presentation of multiple tumors: 1 compressive cervicothoracic tumor arising from the cervical spine and leading to active hydrocephaly, and 2 tumors in the adrenal glands and multiple lesions suggestive of metastases in lymph nodes, lungs, bone marrow, and subcutaneous tissues. Although somatic mutation of *hSNF5/INI1* was documented in both cases, no mutation was found in blood lymphocytes. We also searched for mutation in the DNA extracted from skin fibroblasts, but the absence of mutation could not document the hypothesis of a somatic mosaicism in these patients. We could not compare tumor samples from the different sites because only one biopsy was done for both children. Hence, either a somatic mosaicism or a single tumor with unusual metastatic sites can be discussed in these 2 cases.

**Molecular characteristics of the mutations**

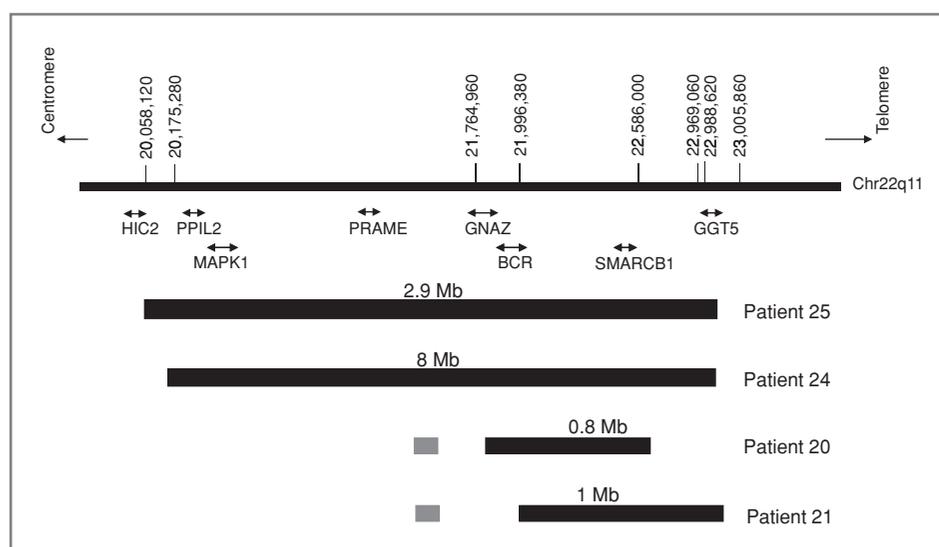
Germline mutations are summarized in Figure 4. Point mutations affected exons 2, 4, 5, 6, and 7. We found 2 mutations affecting donor splice sites (exons 1 and 5), 10 intragenic insertions/deletions leading to frameshifts (including 5 single base insertions, 3 single base deletion, 1 deletion of 7 bases, and 1 complex rearrangement), and 11 nonsense mutations. No missense mutation was observed. The p.Arg158X(c.472C > T)Ex4 mutation is the only recurrent one, found in 5 cases. In addition, 6 patients harbored a complete germline deletion of *hSNF5/INI1* whereas 4 cases showed intragenic deletions. Of note, 3 of 6 of the patients with complete deletion were older than 2 years at tumor onset (25 months, 8

years, and 22 years). The oldest patient (patient 25) also presented a complex phenotype with bladder exstrophy, mild mental retardation, and agenesis of one rib. The other patients with *hSNF5/INI1* deletion did not harbor any major malformation.

The boundaries of germline 22q11 deletions could be studied for 4 of 6 patients with complete *hSNF5/INI1* deletion (Fig. 5). The size of the deletion varied from 819 kb to 2.9 Mb. No other chromosomal rearrangement was observed; in particular, no duplication of 22q11.2 was seen in the patient with bladder exstrophy. The precise breakpoints are reported in Supplementary Figure 1; 6 of 8 of the breakpoints were located within the low copy repeat regions (LCR) D, F, and G of chromosome 22q11.2 (17). Duplications of approximately 100 kb that flank the proximal breakpoints of patients 20 and 21 (between positions 22:19,806,574 and 19,900,956) correspond to known segmental duplications.



**Figure 4.** Summary of germline mutations. On the bottom are indicated mutations found in patients with CNS tumors. On the top, mutations observed in all other locations are indicated. Underlined mutations refer to patients with multifocal disease. Del, deletion; dup, duplication; bp, base pair.



**Figure 5.** Boundaries of the germline whole gene deletions. These proximal breakpoint is considered to be between the last nondeleted single nucleotide polymorphism (SNP) and the first deleted SNP; the distal breakpoint is considered to be between the last deleted SNP and the first nondeleted SNP. Gray bars, duplications; black bars, deletions. The names of genes are reported under the arrows corresponding to their chromosomal location. Precise locations of the breakpoints are reported on the upper line figuring the chromosome, centromere being in the left, and telomere in the right.

The somatic event acquired in the tumor was assessed in 28 cases (Table 1); it consisted in a point mutation in 2 cases, an intragenic deletion in 2 cases, a loss of the wild-type allele in 13 cases, and a loss of the wild-type allele combined with a reduplication of the mutated allele in 11 cases.

## Discussion

The first important conclusion is that germline mutations are frequent and therefore certainly should be searched for in any RT cases. As the presence of a germline mutation was more frequently searched for among young patients, the overall predisposing rate of 35% (26/74) may possibly be overestimated. However, when taking into account all investigated RTs, whether germline DNA was available or not, our study reveals that the presence of germline mutation concerns not less than 23% (26/115) of apparently sporadic tumors. Moreover, in the youngest patients for whom constitutional DNA was almost systematically analyzed (54 patients  $\leq 2$  years of age analyzed from 70 in the whole 10 years period), we assume that our study provides a relevant estimation of the risk for predisposition at least in the youngest children (Fig. 2). Thus, our data indicate that almost 60% of RTs occurring before 6 months of age are linked to the presence of a germline mutation. However, our study also points out that, though less frequent and poorly assessed in our series, the oldest cases can also be associated with a germline mutation in a significant proportion that warrants further systematic analysis.

The poor outcome that is observed in this cohort may be linked to an overrepresentation of children younger than 2 years. Previous articles suggested that patients with germline mutations have a poorer prognosis (13, 18). However, the real impact of the germline mutation on outcome, regarding the small size of the studied population and the low number of survivors whatever the germline status, was hard to investigate. Nevertheless, our data and others' suggest that the impact of a germline mutation on prognosis is mainly

due to the low age at diagnosis (18, 19), which, in turn, might essentially reflect the less intensive treatment that physicians feel allowed to administer to infants. However, it is interesting to mention that the 2 mid-term survivors we report were both affected at much later ages (6 and 8 years).

As 3 of 5 families were referred to our laboratory from abroad in a retrospective manner, only 2 familial cases (families 1 and 5, Table 1 and Fig. 1) were observed in the French cohort of RTs in a 10-year experience. Hence, although rare, the possible recurrence of the disease in siblings has to be clearly exposed to the parents (7, 8, 10, 11, 14, 20). This justifies both the parental DNA screening and the proposal for genetic counseling with possible prenatal diagnosis in case of a new pregnancy. Indeed, the single mutation observed in 2 generations (donor splice site of exon 5) among the 21 studied couples is consistent with the previously mentioned rarity of heritable asymptomatic mutation, due to the high penetrance and mortality of RPS (7, 9, 14). Familial recurrence may more usually be due to a gonadal mosaicism, which is again proven by our observation of a positive prenatal diagnosis; hence, even though the parents do not carry the mutation in the blood DNA, the low but concrete risk of recurrence has to be anticipated. However, presently, no ascertained estimation of the risk of familial recurrence can be given to families.

A main issue of genetic counseling is to predict the phenotype associated with a genotype, which is of particular importance in case of prenatal diagnosis. Interestingly enough, we report at least 4 very instructive observations regarding the delay for RT development in the context of constitutional mutation. First, patients F3.2 and F4.2 were diagnosed an RT at 6 and 7.5 years of age, respectively, whereas their siblings (F3.1 and F4.1) were affected at much earlier ages (4 and 12 months, respectively), indicating that the same germline point mutation may lead to the occurrence of RT at different ages (Fig. 1). Secondly, as germline *hSNF5/IN11* mutation is known to predispose not only to early aggressive RTs with rapid fatal outcome but

also to late-onset indolent multiple schwannomas (21–27) and meningiomas (21, 28), predicting the outcome of a mutation carrier seems somewhat hazardous. Indeed, different tumor types may selectively affect the members of a family carrying the same mutation in a so far unpredictable manner (26). Thirdly, our data combined with these of the literature suggest some trends in genotype–phenotype correlations. Indeed, splice sites mutations are predominantly observed in schwannomatosis (12/20 reported; refs. 21–24, 27), whereas they are rare in RTs and particularly associated with asymptomatic carriers (3/5 heritable mutations of *hSNF5/INI1*; refs. 7, 14). Missense mutations and mutations affecting exon 1 seem exclusively linked to schwannomatosis (22, 23, 25). In contrast, frameshift and nonsense mutations are mostly associated with RTs, with early development. Finally, germline 22q11.2 deletion, so far only observed in RTs, may be linked to a later onset of tumor (17, 29–31). In this respect, patients 24 and 25, both with germline 22q11.2 deletions, harbored the latest RTs ever reported in a context of *hSNF5/INI1* constitutional mutations (8 and 22 years, respectively). Miscellaneous polymalformative conditions, such as velocardiofacial and Goldenhar syndromes, have been reported in patients with large 22q11.2 deletions (17, 30), but bladder exstrophy, occasionally related to 22q11.2 duplication (32, 33), has not been reported in these cases. Hence, the association between RPS and bladder exstrophy in our case remains unexplained.

In conclusion, our study provides some guidelines to help physicians and geneticists in their counseling. Indeed, we propose that a germline analysis should be provided to all individuals with RTs, whatever the age. In case of mutation, parental screening may be proposed, with a low probability of positive result except, presumably, in case of splice site alterations. Gonadal mosaicism

should systematically be evoked and justifies that genetic counseling with possible prenatal diagnosis should be considered in case of pregnancy. Finally, our failure to detect the tumor by ultrasonography only in the prenatally diagnosed infant outlines that while monthly sedation for whole-body MRI is unacceptable in the first weeks of life, the most appropriate procedure still remains to be consensually defined for such a screening in mutation carriers.

### Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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