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Consistent Copy Number Changes and Recurrent *PRKAR1A* Mutations Distinguish Melanotic Schwannomas from Melanomas: SNP-Array and Next Generation Sequencing Analysis

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Abstract

Melanotic Schwannomas (MS) are rare tumors that share histological features with melanocytic tumors and schwannomas. However, their genetics are poorly understood. To elucidate the genetic characteristics of MS, we performed genome-wide studies in a series of cases. Twelve MS cases were available for the study. Genomic DNAs extracted from formalin-fixed paraffin embedded tumor tissues were subjected to copy number (CN) and allelic imbalance (AI) analysis by Single Nucleotide Polymorphism (SNP)-array and screened for mutations in coding exons of 341 key cancer-associated genes using a hybrid capture-based next-generation sequencing (NGS) assay. Sanger sequencing was used to further verify recurrent mutations detected by NGS study. SNP-array analysis revealed remarkably stereotypic chromosomal abnormalities in MS. Hypodiploidy was common, typically involving monosomies of chromosomes 1, 2, and 17. All 12 samples showed mutations in *PRKAR1A* gene, including 2 cases with 2 mutations each. The 14 mutations were scattered across *PRKAR1A*, and most were inactivating mutations. AI on 17q, presenting as loss of heterozygosity with or without CN losses, combined with a *PRKAR1A* mutation was observed in 9/12 MS cases. The remaining 3 cases included the two samples harboring two mutations in *PRKAR1A*. MS exhibits a stereotypic pattern of chromosomal losses. In contrast, melanomas are typically characterized by the presence of multiple CN aberrations, without demonstrable differences in the frequency of losses and gains. Inactivation of both alleles of *PRKAR1A* by “two hits” observed in almost all cases underscores the central role of *PRKAR1A* in the pathogenesis of this neoplasm.

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Additional Supporting Information may be found in the online version of this article.

INTRODUCTION

Melanotic schwannoma is a rare tumor with less than 200 cases reported since its first description in 1932 by Millar (Millar, 1932; Rowlands et al., 1987; Thornton et al. 1992; Ranjan et al., 1995; Akiyoshi et al., 2004; Er et al., 2007; Torres-Mora, et al., 2014). These neuroectodermally derived tumors share histopathological and immunohistochemical features with melanomas (Scheithauer et al., 2007; Wadasadawala et al., 2010). Additionally, the biological behavior of these tumors is unpredictable with no established clinical and/or pathological criteria for recognition of malignant counterparts. Several theories have been proposed regarding their etiology including melanocytic differentiation of Schwann cells, phagocytosis of melanin by Schwann cells and simultaneous presence of two neoplastic populations of proliferating Schwann cells and melanocytes (Smith et al., 2009). Melanotic schwannomas (MS) can be sporadic or occur in Carney complex (CNC). CNC is an autosomal dominant disorder which was originally described in 1985 as “the complex of myxomas, spotty pigmentation and endocrine overactivity” (Carney et al., 1985). Psammomatous melanotic schwannoma is seen in up to 8% of the individuals with CNC (Espiard and Bertherat, 2013).

MS occurs in adulthood, and tumors arising in association with CNC tend to occur at earlier age than sporadic tumors (a peak incidence in the 3rd vs. 4th decade of life) (Hilton and Hanemann, 2014). The tumors are usually deep seated and commonly occur in the spinal canal, paraspinal regions and rarely in organ systems such as the gastrointestinal tract. Their potential for aggressive behavior is underscored by reports of metastases ranging from 13 to 26% (Vallat-Decouvelaere et al., 1999). Their morphological overlap with melanomas poses additional diagnostic challenges. The genetic characteristics of MS are largely unknown except in the context of CNC. In about two-third of CNC cases, germline mutations in *PRKARIA* gene on 17q24 encoding protein kinase A regulatory subunit 1- α (RI α) have been reported (Salpea and Stratakis, 2014). To delineate the genetics of apparently sporadic MS, we utilized a genome-wide high-resolution Single Nucleotide Polymorphism (SNP)-array and next-generation sequencing (NGS)-based molecular profiling platform to analyze CN changes, allelic imbalances (AIs) as well as somatic gene mutations. Furthermore, to better distinguish MS from melanomas, we compared the genome-wide DNA CN changes of MS to previously obtained data in melanomas.

MATERIALS AND METHODS

Tumor Collection

This study was conducted with the approval of the Memorial Sloan Kettering Cancer Center (MSKCC) Institutional Review Board (IRB Waiver Number: WA0256-13). We collected formalin-fixed paraffin embedded (FFPE) tumor blocks and slides of 12 well-characterized MS cases (SCH1–12) and one tumor of conventional schwannoma with melanin pigment from the case archives of the Department of Pathology at MSKCC. The histopathologic and immunohistochemical findings of all cases were further reviewed by experienced bone and soft tissue pathologists and neuropathologists (MH, MR, and NA). Tumor-rich areas were circled to enrich tumor pure population to enhance sensitivity of genome-wide SNP-array

and next generation sequencing analysis. Clinical follow-up was obtained from medical records.

SNP-Array Analysis of the Tumor Genome

Genomic DNA was extracted from FFPE tumor tissues using Qiagen DNeasy Tissue & Blood kit. Genome-wide DNA CN alterations and AIs were analyzed by SNP-array using Affymetrix Onco-Scan FFPE Assay (Affymetrix, CA). We used 80 ng of genomic DNA for each sample. Processing of samples was performed according to the manufacturer's guidelines (Affymetrix). OncoScan SNP-array data were analyzed by the software couple of OncoScan Console (Affymetrix) and Nexus Express (BioDiscovery, CA) using Affymetrix TuScan algorithm. All array data were also manually reviewed for subtle alterations not automatically called by the software.

NGS-Targeted Sequencing

The tumor DNA samples used for SNP-array analysis were also screened for gene mutations in 341 key cancer-associated genes (gene list is available in Supporting Information Table 1) using solution-phase exon capture and next generation sequencing MSK-Integrated Mutation Profiling of Actionable Cancer Targets (MSK-IMPACT). MSK-IMPACT was designed to focus on somatic mutation detection by filtering out alterations also present in matched normal samples. Matching normal with enough material for MSK-IMPACT study was available for 3 out of 12 MS cases (SCH2, SCH5, and SCH12), and the normal DNAs were sequenced in parallel with the corresponding tumor DNAs. For the remaining 9 MS tumor samples, a mixture of pooled FFPE normal DNA was used as unmatched normal control for mutation calling. Briefly, barcoded sequences are prepared and captured by hybridization with custom biotinylated DNA probes for all exons and selected introns of 341 oncogenes and tumor suppressor genes using 100–250 ng of input DNA. Captured libraries are sequenced on an Illumina HiSeq (2 × 100 bp paired-end reads). Bioinformatics analysis included alignment of reads to the human genome (hg19) using BWA-MEM; duplicate read removal, base recalibration and indel realignment using GATK (v 2.6–5) following best practices; variant calling using MuTect (v 1.1.4) for single nucleotide variants and Somatic Indel Detector (GATK 2.3–9) for indels. Annovar was used to annotate the variants for cDNA and amino acid changes as well as presence in dbSNP database (v137) and COSMIC database (v68) and 1,000 Genomes minor allele frequencies.

PCR-Based Confirmatory Tests for *PRKAR1A* Gene Mutations

Somatic mutations in *PRKAR1A* detected by MSK-IMPACT were further verified by Sanger sequencing following PCR (exonic mutations) or RT-PCR (intronic mutations which interrupted splicing). RNA was extracted from SCH1, SCH7, and SCH8 for RT-PCR verification of splicing mutations. Sequences of primers are available in Supporting Information Table 2.

RESULTS

Clinical and Pathologic Features of Melanotic Schwannoma Cases

Tumor sites of the 12 MS cases included sacrum (1), cervical spine (1), paraspinal (3), chest wall (2), thoracic spine (1), spine not specified (1), orbital metastases (1), metacarpal bone (1), and lung (1). The additional case of schwannoma with melanin pigment arose in the presacral space. Histological diagnoses for MS cases were epithelioid melanotic schwannoma (6), psammomatous melanotic schwannoma (2), and the rest 4 samples were spindle or spindle and epithelioid melanotic schwannoma. Four patients (SCH3, 6, 7, and 12) developed metastatic disease (33%), of which two ultimately died of the disease. In all cases mitoses, necrosis, results of immunohistochemical stains included Ki-67 when available were recorded and summarized in Table 1. For genomic analysis, 10 samples were from the primary tumor, SCH6 was from a recurrent lesion, and SCH12 was from an orbital metastases.

All patients were adults with a median age of 40. Medical record was not available for patient SCH10. As for the other patients, there was no clinical documentation of CNC for anyone, and MS was their presenting tumor except case SCH9. SCH9 was a 19-years old male with a history of atrial myxoma (presenting sign) which had been removed elsewhere and presented to our institution with a back mass that was diagnosed as melanotic schwannoma. Although this patient had a history of atrial myxoma, there was no clinical documentation of CNC in the patient's medical record.

Copy Number and Allelic Imbalance Analysis by SNP-Array

SNP-array analysis showed predominantly CN losses across the genome in MS tumor samples. In contrast, unbalanced genomic aberrations were not detected in the individual case diagnosed with conventional Schwannoma with melanin pigment. CN changes and loss of heterozygosity (LOH) without CN change are displayed for each individual MS case in Figure 1a. The aggregated DNA CN analyses (Fig. 1b) revealed several recurrent unbalanced genomic aberrations. The most frequent changes included losses of 1p (50%), 1q (42%), 2 (50%), and 17 (42%). When integrating LOH with CN alterations, we noticed that unbalanced genomic alterations (AI with or without CN changes) involving chromosomes 1, 2, and 17 were even more dominant in MS (Fig. 1c), and the most frequent recurrent aberration was AI of chromosome arm 17q observed in nine samples (75%), five of which presented as monosomy of chromosome 17, and four had LOH of 17q without DNA CN loss. In the latter group, three cases (SCH2, SCH4, and SCH12) had copy neutral LOH (CN-LOH) of 17q and one case (SCH3) had a trisomy 17q with LOH (uniparental trisomy [UPT]). It is worth noting that cases SCH3 and SCH12 showed multiple chromosomal gains rather than stereotypic pattern of chromosomal losses across the genome, however, LOH without CN loss was also identified in multiple chromosomes typically affected in MS, including chromosome 17, in both samples. Therefore, the combined CN and AI analyses of SCH3 and SCH12 indicated that the distinct tumor genome of each sample may result from doubling of a hypodiploid clone, respectively.

Mutation Screening by NGS-Targeted Sequencing

Analysis of sequence variant data showed an average of 4.3 mutations per sample for cases without a matched normal and 1.67 for the three cases with matched normal. All the mutations detected in each MS tumor sample are listed in Supporting Information Table 2 and the distribution of mutated genes in 12 MS samples is illustrated in Figure 2. Interestingly, the targeted sequencing revealed *PRKARIA* gene mutations in all MS tumor samples. In contrast, the case of conventional schwannoma with melanin pigment did not show *PRKARIA* mutation. SCH7 and SCH8 harbored two mutations in *PRKARIA* gene, and the others had one mutation for each sample. The two mutations in SCH8 were occurring *in-trans* (Supporting Information Figure 1). A schematic presentation of the type and the approximate location of the 14 mutations in *PRKARIA* identified in this study are illustrated in Figure 3a. The 14 mutations were scattered across the gene, and most were overtly inactivating mutations which included 5 nonsense mutations and 4 indels that resulted in frame shifts and premature stop codons. Other *PRKARIA* mutations included 3 intronic mutations and one missense mutation. Two intronic mutations altered the acceptor splicing site (gt/ag) and the remaining one was a possible splicing mutation (c.441-3C>A in SCH1). The only missense mutation identified in this study, p.C347R (c.1039T>C) in SCH6, is within the cyclic nucleotide binding domain, being adjacent to the cAMP binding sites and affects a residue that is well conserved across different species (Fig. 3b). Therefore, the substitution C347R may also impair PRKAR1A function. In addition, *PRKARIA* mutations were verified to be somatic in three cases in which matched normal sample was available (SCH2, SCH5, and SCH12).

A comprehensive summary of all the *PRKARIA* gene mutations identified in this study as well as a comparison to the *PRKARIA* mutation database (<http://prkar1a.nichd.nih.gov>) is presented in Table 2.

Confirmatory PCR

All exonic mutations except c.535C>T in SCH2 and c.851dupT in SCH9 were confirmed by PCR-Sanger sequencing (see Table 2 for a summary). Among the three intronic splicing mutations, one (c.708+1G>C in SCH7) was confirmed by RT-PCR demonstrating a variant transcript with exon 7 skipped (Supporting Information Figure 2); another one (c.441-1G>C in SCH8) was confirmed at the DNA level. RT-PCR failed in SCH1 and SCH8 due to the very poor quality of FFPE-RNA. PCR-Sanger sequencing data are available in Supporting Information Figure 3. Mutations identified by MSK-IMPACT that could not be evaluated by Sanger sequencing due to unavailability of tumor materials are presented in Supporting Information Figure 4.

DISCUSSION

MS are uncommon pigmented Schwann cell tumors. These tumors show ultrastructural evidence of melanosome formation and are immunoreactive for melanocytic proteins. Therefore, MS often cannot be distinguished from primary melanocytic tumors (Mooi and Krausz, 2007). The genomic aberrations of benign melanocytic tumors and melanomas have been well investigated in the past several years and the CN signature of melanomas are well-

established and are used in the diagnostic setting (Curtin et al., 2005; Wang et al., 2013). The genomic characteristics of MS have not been studied so far.

Using the Affymetrix genome-wide SNP-array, we found remarkably stereotypic chromosomal abnormalities in MS. Hypodiploidy was common, and typically involves monosomies of chromosomes 1, 2, and 17. In contrast, melanomas were characterized by the presence of multiple genomic CN aberrations, without differences in the frequency of losses and gains. Recurrent unbalanced genomic aberrations known to occur in association with melanomas are CN increases of 1q, 6p, 7, 8q, 17q, and 20q and frequent losses of 6q, 8p, 9p, 10, and 21q (Curtin et al., 2005; Wang et al., 2013). Therefore, a genome-wide CN analysis can aid in distinguishing melanotic schwannoma and melanoma in histologically ambiguous cases. Additionally, melanocytomas which arise from leptomeninges can pose diagnostic challenges due to morphological and immunohistochemical similarities with melanotic schwannoma. However it has been shown that over 50% of melanocytomas harbor *GNAQ* and/or *GNA11* mutations which can serve as a marker to distinguish these neoplasms (Küsters-Vandeveldt et al., 2010a; Koelsche et al., 2014). As reported previously (Küsters-Vandeveldt et al., 2010b), none of the MS in our series showed *GNAQ* or *GNA11* mutation.

In addition to the stereotypic CN changes, AI on 17q, inactivation of both alleles of *PRKARIA* by “two hits,” that is, one mutation coupled with LOH of 17q or by two mutations, was observed in almost all cases. A correlation of *PRKARIA* gene mutation status and AI of 17q in all 12 MS cases is summarized in Table 2. In brief, 5 cases showed monosomy of chromosome 17 with one mutation in *PRKARIA*; 4 cases showed CN/CG (copy neutral/copy gain)-LOH of 17q with one mutation in *PRKARIA*. In the remaining three cases with allelic-balance 17q, two different mutations in *PRKARIA* gene were identified in two (SCH7 and SCH8), and MSK-IMPACT data demonstrated that the two mutations in SCH8 presented *in-trans* which indicated that they occurred in different alleles of *PRKARIA* gene, respectively. We were unable to verify whether or not the two mutations identified in SCH7 occurred in two different alleles due to the distance between them. With the combination of SNP-array and mutation screening analyses, we conclude that inactivation of both alleles of *PRKARIA* plays a central role in the pathogenesis of this neoplasm.

Inherited mutations in *PRKARIA* have been reported to be present in up to 70% of the patients diagnosed with CNC (Kirschner et al., 2000; Bertherat et al., 2009). *PRKARIA* (Protein Kinase, cAMP-Dependent, Regulatory, type I, Alpha) gene encodes one of the regulatory subunits (R1- α) of type I protein kinase A (PKA). The inactive holoenzyme PKA is a tetramer composed of two regulatory and two catalytic subunits. Activation occurs when 2 cAMP molecules bind to each regulatory subunit, eliciting a reversible conformational change that releases active catalytic subunits (Horvath et al., 2010). The gene is located in 17q24 and consists of 11 exons. More than 120 pathogenic mutations of *PRKARIA* gene have been reported in CNC patients. A review of the *PRKARIA* mutation database (<http://prkar1a.nichd.nih.gov>) shows that the mutations in *PRKARIA* are spread along the entire coding sequence, without significant preference for an exon or a domain. Almost all of the mutations reported result in premature stop codons caused by either nonsense mutations or frameshift mutations, and the mutant mRNAs are predicted to be degraded via nonsense-

mediated decay (NMD). As such, *PRKAR1A* haploinsufficiency was hypothesized to be pathogenic. However, studies examining the consequences of *Prkar1a* haploinsufficiency in mice have demonstrated that *Prkar1a* haploinsufficiency acts as a relatively weak tumorigenic signal, which can cooperate with other tumor suppressors or tissue-specific factors or even chemicals to induce tumor (Almeida et al., 2010). In our study, only one case (SCH11) harbored only one heterozygous *PRKAR1A* mutation, and no evidence of inactivation of the other allele. We speculate that other genetic or epigenetic aberrations may synergize with *PRKAR1A* haploinsufficiency to induce melanotic schwannoma in this case. Mutations in other genes detected in SCH11 by MSK-IMPACT are listed in Supporting Information Table 2.

Another chromosome locus that has been reported to be associated with CNC is 2p16 (Stratakis et al., 1996; Matyakhina et al., 2003); however, the specific gene(s) remains unknown. In our study, 6 out of the 12 MS tumor samples had CN changes involving chromosome 2, including loss of the entire chromosome 2 in 5 cases and CN-LOH of the entire chromosome 2 in the remaining one case (Fig. 1A). Focal CN change on 2p16 was not found. In addition to the CN and AI analysis, somatic mutations in coding exons of genes *EPCAM*, *MSH2*, *MSH6*, and *REL* in 2p16 were studied by targeted next generation sequencing (MSK-IMPACT). Somatic mutations were not detected in any of these genes. Please note that other genes in 2p16 were not captured in our targeted-sequencing, therefore, were not studied.

Based on the genome-wide CN and AI findings, the MS cases in this study can be classified as two groups. Group I (SCH1, 2, 3, 5, 6, 9, 10, 12) is characterized by the stereotypic multiple losses of chromosomes and or chromosome arms, and Group II (SCH4, 7, 8, 11) with fairly stable genome (Fig. 1A). It is worth noting that, in addition to inactivation of both alleles of *PRKAR1A* by “two hits,” *TP53* haploinsufficiency/inactivation or complete inactivation of a common tumor suppressor gene (most of which are associated with *TP53* pathway) was seen in Group I samples (detailed below). SCH1 and 2 had *TP53* haploinsufficiency due to deletion of 17p. SCH5 and SCH10 had monosomy of chromosome 17 and *TP53* mutations. Others included a missense mutation of *BRIP1* associated with a monosomy of 17 (SCH3); a nonsense mutation of *MEN1* gene associated with a monosomy of chromosome 11 (SCH6); a missense mutation of *BRCA1* gene associated with a monosomy of chromosome 17 (SCH9); and a missense mutation of *LATS2* gene associated with CN-LOH of chromosome 13 (SCH12).

Several mouse and human cancer models have shown that an increase of aneuploid tumor cells was specifically correlated with loss of function of *TP53* and/or tumor suppressors such as *LATS2* and *BRCA1* that synergize with *TP53* in ploidy guarding (Aylon and Oren, 2011). Therefore, inactivation of these tumor suppressor genes may lead to a tolerance of aneuploidy, and the aneuploid cell may eventually thrive by accumulating more growth-promoting and transforming genomic alterations. Perhaps reflecting this, clinical follow-up showed that metastasis was seen in three Group I cases and two died of the disease (Table 1). In the remaining cases of Group I, one was disease free at last follow-up (8 years) and no follow-up was available for 4 cases. In contrast, in Group II, three cases were disease free at last follow-up (6 months–3 years) and one is alive with metastasis. Further studies are

necessary to verify the correlation between clinical outcome of MS and alterations in the TP53 pathway and other tumor suppressor pathways.

In conclusion, we find that *PRKARIA* gene mutation is a recurrent genetic event in MS, which also exhibits a stereotypic pattern of chromosomal losses. In contrast, melanomas are typically characterized by the presence of multiple CN aberrations, without demonstrable differences in the frequency of CN losses and gains. Regarding *PRKARIA* mutations, the TCGA data for cutaneous melanoma study at *The cBioPortal for Cancer Genomics* (Cerami et al., 2012; Gao et al., 2013), showed 3 out of 375 (0.8%) samples bearing *PRKARIA* gene mutation. It is worth noting that the *PRKARIA* mutations were seen concurrently with *BRAF* or *NRAS* mutations in the three samples which had the typical high mutation loads seen in melanoma. This implies that the *PRKARIA* mutations likely represent rare secondary genetic events in these cutaneous melanomas. In addition, according to our experience of mutation screening of 34 melanoma samples using MSK-IMPACT, *PRKARIA* gene mutation was not identified in any cutaneous melanoma samples. Therefore, we conclude that stereotypic CN changes and recurrent *PRKARIA* mutations identified by genome-wide molecular profiling in this study can help distinguish MS from melanomas. Furthermore, inactivation of both alleles of *PRKARIA* by “two hits,” that is, either by one mutation coupled with LOH of 17q or by two mutations, was observed in almost all cases, underscoring the central role of *PRKARIA* in the pathogenesis of this neoplasm.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.

CN changes and AIs detected in 12 MS tumor samples by OnsoScan SNP-array analysis. A. Heat map of SNP-array results. CN gains (blue) and losses (red) as well as LOH without copy loss (yellow) are displayed for each individual MS case (rows) with chromosomes organized in columns and indicated by labels on the top. B: Genome-wide frequency plot of DNA CN gains (blue) and losses (red) for all 12 melanotic schwannoma samples. C. Frequency plot of unbalanced genomic alterations (AI with or without CN changes) of 1p/1q, 2 and 17 for all 12 MS samples. The most frequent recurrent aberration was AI on chromosome arm 17q observed in nine samples (75%). [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

Figure 2. Mutation screening of 12 MS tumor samples by MSK-IMPACT NGS-based analysis. Count of different mutation types in the genes sequenced by MSK-IMPACT. Counts are color coded for the different mutation types [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

Figure 3. *PRKAR1A* gene mutations detected. A. Protein domain structure of *PRKAR1A* along with mutations observed in the study. Different mutation types are color coded along the protein axis. B. Multiple alignment of *PRKAR1A* gene sequence from different organisms showing the highly conserved nature of the site of missense mutation C347R. The alignment also shows the close proximity of the missense mutation to the cAMP binding sites. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

TABLE 1

Clinical and Histological Data of All 12 Melanotic Schwannoma (MS) Cases

Case #	Age/Sex	Presenting Tumor	Site	MS Histology	Mitoses/HPF	Necrosis	KI-67	S100 & HMB45	Follow-up
SCH1	48/F	Melanotic Schwannoma	Paraspinal	Epithelioid	<1/10	Present	5%	pos	NA
SCH2	36/F	Melanotic Schwannoma	Paraspinal	Epithelioid	None	Present	ND	ND	NA
SCH3	44/M	Melanotic Schwannoma	Chest wall	Epithelioid	3/10	present	ND	ND	Mets/DOD
SCH4	25/F	Melanotic Schwannoma	metacarpal	Spindle	None	None	ND	pos	NED-3 years-lost to follow-up
SCH5	44/F	Melanotic Schwannoma	Chest wall	Epithelioid	<1/10	None	1%	pos	NED-8 years
SCH6	53/M	Melanotic Schwannoma	Cervical Spine/recurrent	Psammomatous	None	None	5–10%	ND	Mets/DOD
SCH7	72/M	Melanotic Schwannoma	Thoracic spine	Spindle and epithelioid	None	None	5–10%	Pos	Alive with mets
SCH8	51/M	Melanotic Schwannoma	Sacrum	Epithelioid	None	None	1%	pos	NED-2 years
SCH9	19/M	Atrial Myxoma followed by Melanotic Schwannoma	Paraspinal	Epithelioid	<1/10	None	<5%	ND	NA
SCH10	33/M	Melanotic Schwannoma	Spine	Psammomatous	<1/10	None	ND	Pos	NA
SCH11	31/M	Melanotic Schwannoma	Lung	Spindle	none	None	2%	Pos	NED-6 months
SCH12	51/F	Melanotic Schwannoma	Orbital met	Spindle and epithelioid	10/10	Present	ND	ND	Alive with mets

DOD, died of disease; NED, no evidence of disease; ND, not done; NA, not available, Met: metastasis.

TABLE 2

Summary of All *PRKARIA* Mutations Identified in This Study, Correlation with 17q CN Status and Comparison to *PRKARIA* Mutation Database (<http://prkar1a.nichd.nih.gov>)

SCH #	<i>PRKARIA</i> Exon	<i>PRKARIA</i> cDNA	Effect of the mutation	Sanger confirmation	Reported in <i>PRKARIA</i> mutation database	17q Status by SNP-array	Both Alleles of <i>PRKARIA</i> Inactivated
SCH1	5	c.441-3C>A	Likely alters the splice acceptor site	NA	No	monosomy 17	Yes
SCH2	6	c.535C>T	p.Q179X	NA	No	CN-LOH 17q	Yes
SCH3	3	c.286C>T	p.R96X	Confirmed	Yes	trisomy of 17q21.3-25.3-LOH (UPT)	Yes
SCH4	8	c.761_762delCT	p.S254Yfs×15	Confirmed	No	CN-LOH 17q	Yes
SCH5	2	c.124C>T	p.R42X	Confirmed	Yes	Monosomy 17	Yes
SCH6	11	c.1039T>C	p.C347R	Confirmed	No	Monosomy 17	Yes
SCH7	2	c.124C>T	p.R42X	Confirmed	Yes	Normal	Yes
	7	c.708+1G>C	exon 7 skipped (shorter <i>PRKARIA</i> protein)	Confirmed	Yes		
SCH8	5	c.441-1G>C	Alters the splice acceptor site	Confirmed	No	Normal	Yes
	5	c.496C>T	p.Q166X	Confirmed	Yes		
SCH9	9	c.851dupT	p.Q285Afs×7	NA	No	Monosomy 17	Yes
SCH10	4	c.380_402del23	p.A127Efs×8	Confirmed	No	Monosomy 17	Yes
SCH11	10	c.944delT	p.V315Gfs×16	Confirmed	No	Normal	No
SCH12	8	c.750_761delinsG	p.S251Yfs×15	Confirmed	No	CN-LOH of 17	Yes

NA: not available, confirmatory Sanger sequencing could not be performed due to sample insufficiency CN-LOH, copy neutral-loss of heterozygosity; UPT, uniparental trisomy.