Sacroccocygeal Chordomas in Patients with Tuberous Sclerosis Complex Show Somatic Loss of TSC1 or TSC2

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Chordomas are rare sacrococcygeal/sacral, sphenoccipital/clivus, and spinal tumors whose molecular etiology remains relatively understudied. As several anecdotal reports had described chordomas in individuals with tuberous sclerosis complex (TSC), a multisystem hamartoma syndrome, we hypothesized that the genes that cause TSC may have an etiological role in chordomas. In two cases of sacrococcygeal chordomas in individuals with TSC, one with a germ-line TSC2 mutation and the other with a germ-line TSC1 mutation, we confirmed somatic inactivation of the corresponding wild-type allele by loss of heterozygosity analysis and immunohistochemistry. These data provide the first evidence of a pathogenic role by TSC genes in sacrococcygeal chordomas.

Chordomas [MIM 215400] are rare tumors that originate from notochordal remnants along the axial skeleton in the sacrococcygeal/sacral (45%), sphenoccipital/clivus (40%), and spinal (15%) regions (Stepanek et al., 1998; Börgel et al., 2001). They are characterized by slow growth, local destruction of bone, extension into adjacent soft tissue, and, rarely, distant metastatic spread (Stepanek et al., 1998). The incidence of sacrococcygeal chordomas is only 1 in 40,000 births (Harbon and Pheline, 1989). However, they are the most prevalent type of chordoma in children (Sasson and Chutorian, 1967). Chordomas are usually sporadic. However, 8 families have been reported to have multiple family members affected by chordomas (Foote et al., 1958; Enin, 1963; Kerr et al., 1975; Chetty et al., 1991; Korczak et al., 1997; Stepanek et al., 1998; Dalprà et al., 1999), suggesting that inherited variants may predispose to these tumors. Three of these families demonstrated autosomal dominant transmission (Korczak et al., 1997; Stepanek et al., 1998; Dalprà et al., 1999). To date, knowledge of the molecular pathology of chordomas is rudimentary. Of the limited cytogenetic data available on approximately 40 cases of chordoma worldwide (Dalprà et al., 1999; Miozzo et al., 2000; Sawyer et al., 2001; Scheil et al., 2001), no recurrent aberrations have been found (Scheil et al., 2001). A previous molecular cytogenetic study using comparative genomic hybridization identified chromosomal imbalances in 16 of 16 chordomas analyzed (5 clivus, 10 sacrococcygeal, and 1 spinal). However, none showed losses of 9q34 or 16p13, to which TSC1 and TSC2 map (Scheil et al., 2001). The only previous loss of heterozygosity (LOH) study of chordomas was of RB1 (13q14), at which LOH was identified in 2 of 7 sphenoccipital/clivus chordomas (Eisenberg et al., 1997). The tumors demonstrating 13q14 LOH were noted to be particularly aggressive, and consequently the LOH may have been a late genetic event in these tumors. Two further loci have been implicated in the pathogenesis of chordomas. A susceptibility allele at 7q33 was suggested by linkage analysis of one family with apparent autosomal dominant transmission (Kelley et al., 2001). A putative tumor-suppressor gene that may play a role in both familial and sporadic chordomas also was reported (Miozzo et al., 2000) after linkage studies and cytogenetic studies that identified somatic losses of 1p in chor-
domas (Mertens et al., 1994; Butler et al., 1995; Dalprà et al., 1999).

Because of the limited molecular data available on chordomas, we searched the published literature for unusual associations of chordomas with tumor predisposition syndromes, as genes involved in such syndromes often play a role in the pathogenesis of sporadic tumors. We identified three reports of chordomas found in patients with tuberous sclerosis complex (TSC; Dutton and Singleton, 1975; Schroeder et al., 1987; Borgel et al., 2001). TSC [MIM 191100] is an autosomal dominant syndrome characterized by hamartomas in multiple organs, epilepsy, mental retardation, and behavioral problems, with a prevalence of 1 in 6,800 (Osborne et al., 1991). TSC is caused by germ-line mutations in either of two genes, TSC1 [MIM 605284] and TSC2 [MIM 191092]. These genes behave like tumor-suppressor genes, as inactivation of the wild-type allele has been demonstrated in hamartomas, consistent with Knudson’s two-hit hypothesis. The involvement of TSC1 and TSC2 in other unusual tumors in individuals with TSC was reported previously, including identification of biallelic inactivation of TSC2 in a malignant islet cell tumor in a TSC patient (Verhoef et al., 1999). Furthermore, somatic biallelic inactivation of TSC genes also has been established in lymphangioleiomyomatosis (Sato et al., 2002) and some primary bladder cancers (Hornigold et al., 1999). We hypothesized that TSC1 and TSC2 are candidate genes in the genesis of chordomas.

We identified two previously unreported cases of TSC with coexisting sacrococcygeal chordoma and have undertaken molecular genetic analysis to investigate somatic involvement of the TSC genes in these tumors. Appropriate informed consent was obtained from both families. The first case was a 33-week female fetus from a family with no history of TSC that was terminated because of prenatal ultrasound detection of multiple cardiac rhabdomyomas, suggesting a diagnosis of TSC. Histological examination confirmed the cardiac tumors to be rhabdomyomas, but the brain was not examined because of cephalocelesthesia and severe disruption of the cranium. A sacrococcygeal chordoma was present. The tumor comprised lobules separated by fibrous septae. Within the lobules, small round cells were arranged in a cordlike fashion in a myxoid stroma. The majority of cells were physaliphorous, with marked vacuolization of the cytoplasm (Fig. 1A).

In view of the clinical findings, mutation analysis of all coding exons of TSC1 and TSC2 was undertaken by single-strand conformational polymorphism (SSCP) analysis, as described in Beauchamp et al. (1998), using constitutional DNA (obtained from paraffin-embedded pancreas and left lung by standard proteinase K digestion and phenol chloroform extraction following deparaffinization in xyylene). SSCP shifts were characterized by direct sequencing and revealed the TSC2 nonsense mutation, CAG (glutamine, Q)→TAG (stop, X) at amino acid residue 1010, Q1010X (arrowed), in exon 26 (Fig. 1B), and a 4-bp deletion polymorphism in intron 39 of TSC2, 5179-30_5179-27delAGTG (data not shown). Both parents were clinically normal and were not tested for the mutation. This mutation was used for LOH studies by a seminested PCR of TSC2 exon 26, using the primers LLJ1 (reverse 5’-GCCGTGAAGTGGAGAAGACG-3’) and LLJ2 (reverse 5’-GAGCATCATGTCAGAAGAGG-3’) in the first and second rounds of amplification, respectively, in conjunction with the TSC2 exon 26 forward primer detailed in Jones et al. (1999, 2000), generating a product of 206 bp in the second step. A seminested PCR was used, as the DNA was extracted from paraffin sections and insufficient product was detectable by denaturing high-performance liquid chromatography (DHPLC) following 32 cycles of amplification. DHPLC analysis of the PCR product from the second round of amplification demonstrated a marked reduction of one of the peaks, indicating LOH (data not shown). Sequencing confirmed that this profile change was attributable to loss of the wild-type allele (Fig. 1B). Assay of the 4-bp deletion polymorphism using the same approach confirmed LOH at the TSC2 locus (data not shown). Immunohistochemistry was performed using polyclonal antibodies, HF6 for hamartin and TSDF for tuberin, as described in Murthy et al. (2001), and showed consistent immunopositivity for hamartin (Fig. 1C) and focal, very weak staining for tuberin (Fig. 1D). Antihamartin antibody, HF6, was generated in rabbits against the GST fusion protein derived from a GST fusion construct of exon 15 of human hamartin (amino acids 480–666; Murthy et al., 2000). Antituberin antibody, TSDF, was generated in rabbits against amino acids 1165–1393, as detailed in Han et al. (2004).

The second case was born at term following an uneventful pregnancy and normal delivery. Antenatal ultrasound scans were normal. At birth, she was noted to have a 3.5-cm sacrococcygeal lesion. The tumor was excised at 5 days of age. Histology revealed this to be a sacrococcygeal chordoma. Microscopic analysis demonstrated a skin-covered
Figure 1.
nodule composed of compact cords of clear cells arranged in a lobular pattern. Thick strands of fibrous tissue intersected the nodule. There were typical physaliphorous cells present, with a clear, bubblelike cytoplasm and vesicular nucleolus (Fig. 1E). Periodic acid Schiff was weakly positive, whereas Alcian-blue stained the mucinous material strongly. Immunohistochemistry was positive with cytokeratin, S100, and epithelial membrane antigen. The cytological features were benign, and no mitotic figures were seen. The findings were consistent with chordoma and were confirmed by electron microscopy. The patient did not receive any subsequent treatment for the chordoma.

Ultrasonography of renal and pelvic regions were normal on follow-up. From 11 months, hypomelanotic macules were noted on her skin, which increased in number with age. A formal diagnosis of TSC was made when the patient was 6 years of age, following detection of subependymal nodules on a brain MRI scan. Two lipomas were found on her back, and an early shagreen patch was found on her left hip. No spinal abnormalities were found by MRI, and no further cutaneous signs of TSC were present on reassessment at 8 years of age. Renal ultrasond and fundoscopy were normal. The patient has no history of seizures and is intellectually normal. Both parents were clinically normal.

Analysis of all coding exons of TSC1 and TSC2 in constitutional DNA extracted from EDTA-treated blood was performed using the conditions detailed in Jones et al. (1999, 2000). DHPLC analysis with a WAVE DNA Fragment Analysis System (3500HT; Transgenomic, Crewe, UK), using the methods described on http://www.uwcm.ac.uk/uwcm/mg/tsc_db/dhplc2.html, demonstrated a profile variant in exon 4 of TSC1 (data not shown) that on sequencing revealed a 9-bp in-frame deletion, 402_410delCTGACCACC (Fig. 1F). Parental DNA samples were analyzed, which confirmed that this mutation had occurred de novo. Biological pat-
GTTGTACTC-3') also revealed apparently reduced signal from the wild-type allele, suggestive of LOH. Tumor DNA quality precluded systematic assays for an intragenic somatic mutation, but we performed immunohistochemistry in order to address expression levels of hamartin and tuberin. Immunostaining with the HP6 antibody showed absence of hamartin in the chordoma (Fig. 1G), consistent with somatic inactivation of TSC1, whereas the tumor cells were strongly immunopositive for tuberin (Fig. 1H).

Our data are consistent with biallelic inactivation of each gene that causes TSC in the cases studied. Case 1, with a germ-line TSC2 mutation, demonstrated LOH of TSC2, which was supported by the focal loss of tuberin staining in the chordoma on immunohistochemistry. The tumor from Case 2, with a germ-line TSC1 mutation, did not unequivocally demonstrate LOH of TSC1 but showed absence of hamartin, suggesting that a more subtle intragenic mutation or hypermethylation caused loss of function of the second allele in this tumor. Analysis of all the coding exons of TSC1 would have enabled us to address this but was precluded by the poor-quality DNA obtained from the paraffin-embedded tissue.

This report provides the first evidence for the role of somatic mutations of TSC1 and TSC2 in the development of chordomas. There have been three previous reports of chordomas in TSC patients. One was a sacrococcygeal chordoma (Dutton and Singleton, 1975), and two were clivus chordomas (Schroeder et al., 1987; Börgel et al., 2001), but no examination of somatic changes of the TSC genes was undertaken in those studies. Chordomas are clearly associated with TSC only rarely. Although MRI of the spine is not part of the routine workup of patients with TSC, it is unlikely that spinal or sacrococcygeal chordomas would escape clinical detection. Perhaps the rarity of this tumor type in individuals with TSC is more likely to reflect the small number of target cells (i.e., notochordal remnants) susceptible to somatic second-hit mutations or a requirement for further somatic events. All chordomas associated with TSC have been single lesions, a situation also seen with subependymal giant-cell astrocytoma but not with most other TSC-associated lesions, which are usually multiple.

Case 2 reported here, with a TSC1 mutation, had minimal clinical manifestations of TSC. The case reported by Börgel et al. (2001), presented at less than 4 years of age with chordoma and had no recognized clinical signs of TSC at the time of his death, at age 6. However, he did have a nonsense mutation, E609X; 2046G>T, in exon 15 of the TSC1 gene that was also present in his father, who had a definitive clinical diagnosis of TSC. The manifestations of TSC can be so subtle that unless specific investigations are done, the diagnosis can be missed. It is possible that the association between chordoma and TSC has therefore been overlooked in some cases. However, it would be premature to suggest additional investigations in patients with apparently sporadic chordoma. Careful clinical and family history assessment should suffice.

The average age of onset of chordomas not associated with TSC is 38 years for sphenoidocipital (Heffelfinger et al., 1973) and 56 years for sacrococcygeal chordomas (Sundaresan et al., 1979), although sacrococcygeal chordomas are the most frequent of these tumors in children (Sassin and Chutorian, 1967) and may be congenital. The TSC-associated cases of sphenoidocipital chordomas reported by Schroeder et al. (1987) and Börgel et al. (2001) were in children less than 5 years of age, and the TSC-associated sacrococcygeal chordoma reported by Dutton and Singleton (1975) was discovered during the first few days of life. The early onset of these tumors is also consistent with genetic predisposition to chordomas mediated via germ-line mutation in a TSC gene.

Although our data support a role for the TSC genes in the etiology of chordomas, this may be true only for a subset of these tumors. Future studies of this rare tumor should investigate involvement of TSC1 or TSC2 in cases arising in patients who have been carefully assessed to exclude any manifestations of TSC.

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