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Rapid Publication

HRAS Mutation Analysis in Costello Syndrome: Genotype and Phenotype Correlation

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Costello syndrome is a rare condition comprising mental retardation, distinctive facial appearance, cardiovascular abnormalities (typically pulmonic stenosis, hypertrophic cardiomyopathy, and/or atrial tachycardia), tumor predisposition, and skin and musculoskeletal abnormalities. Recently mutations in *HRAS* were identified in 12 Japanese and Italian patients with clinical information available on 7 of the Japanese patients. To expand the molecular delineation of Costello syndrome, we performed mutation analysis in 34 North American and 6 European (total 40) patients with Costello syndrome, and detected missense mutations in *HRAS* in 33 (82.5%) patients. All mutations affected either codon 12 or 13 of the protein product, with G12S occurring in 30 (90.9%) patients of the mutation-positive cases. In two patients, we found a mutation resulting in an alanine substitution in position 12 (G12A), and in one patient, we detected a novel mutation (G13C). Five different *HRAS* mutations have now been reported in Costello syndrome, however genotype–phenotype correlation remains incomplete. © 2005 Wiley-Liss, Inc.

Key words: bladder cancer; gain-of-function; *HRAS*; overgrowth syndrome; rhabdomyosarcoma

INTRODUCTION

Costello syndrome (OMIM #218040) is a rare disorder with a distinctive prenatal phenotype (polyhydramnios, overgrowth, edema), postnatal feeding difficulties and failure to thrive, characteristic facial appearance, abnormalities of the heart, skin and musculoskeletal system, and tumor predisposition [reviewed by Hennekam, 2003; Gripp, 2005; Lin et al., 2005]. The risk of neoplasia (approximately 10–15%) [Gripp et al., 2002] influences clinical care, morbidity, and mortality. While the papillomata, which develop throughout childhood in the peri-oral and/or perianal region are the most common benign tumors, the most common malignancy is rhabdomyosarcoma (RMS), typically with embryonal histologic findings [reviewed by Gripp, 2005]. Less common are neuroblastoma, ganglioneuroblastoma, and transitional cell carcinoma of the bladder [Gripp, 2005].

Costello syndrome shares many phenotypic traits with cardio-facio-cutaneous (CFC) syndrome (OMIM #115150), and in some children it may be difficult if not impossible to be certain about the diagnosis. Although Costello, CFC, and Noonan syndrome (OMIM #163950) all share the familiar cardiac phenotype of pulmonic stenosis and/or hypertrophic cardiomyopathy [summarized in Table VII, Lin et al., 2002], the facial appearance and overall phenotype of Noonan syndrome is much less similar

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to Costello syndrome except in the fetal and neonatal period. Noonan syndrome is caused by missense mutations in *PTPN11*, encoding the tyrosine phosphatase SHP2, in about 50% of patients [Tartaglia et al., 2001]. These *PTPN11* mutations lead to a gain-of-function of SHP2 with enhanced phosphatase activity, resulting in increased activation of the mitogen activated protein kinase (MAPK) pathway. Aoki et al. [2005] hypothesized that the gene mutated in Costello syndrome encodes a molecule that functions upstream or downstream of SHP2 in the signal pathway. They identified the *RAS* genes as potential candidates and subsequently showed that germline mutations in *HRAS* are the underlying cause of Costello syndrome. The mutations identified by Aoki et al. [2005] affect one of two amino acids (position 12 and 13 of the protein) previously found to be mutated in malignant tumors.

To increase our understanding of the molecular definition of Costello syndrome and to provide clinical correlation, we report the results of mutation analysis and phenotypic review in 40 North American and European patients.

MATERIAL AND METHODS

Patients

Patients with Costello syndrome were identified at the 2003 and 2005 International Costello Syndrome Meetings, through the Costello Syndrome Family Network and through physician referral. Patients 1–27 and 29–36 (Table I) were enrolled in a research study approved by the Institutional Review Board of the A. I. duPont Hospital for Children (#2003–006). Clinical information was obtained by self-report by the families who completed a standardized data collection form which was updated every 2 years, when possible, and supplemented by review of medical records and interview of the families. Additional patients (Table I, Patients 28, 37–40) were clinically identified by P.L. and A. G.-M. and studied under an IRB approved protocol (CEIC-HULP-2003-PI-362) at the Hospital Universitario La Paz, Madrid, Spain.

Although a patient may have been diagnosed by a local geneticist or other professional, the diagnosis of Costello syndrome was confirmed independently by K.W.G. and A.E. L. based on diagnostic guidelines [Table 14.1, Lin et al., 2005; Proud et al., 2005]. Emphasis was placed on the characteristic growth pattern (especially severe feeding problems and failure to thrive), developmental delay or mental retardation, skin abnormalities, and distinctive hands, especially ulnar deviation, and ligamentous laxity of the fingers. The characteristic craniofacial appearance (macrocephaly, high forehead, unusually curly hair, hypertelorism, fleshy nasal tip, full lips, wide mouth, full cheeks, and fleshy ear lobes) was the most discriminatory and created the most discussion and doubt in diagnosis when atypical. In this series of well-scrutinized patients, all patients had many of these facial findings. Cardiac hypertrophy included hypertrophic cardiomyopathy (also known as asymmetric septal hypertrophy and idiopathic hypertrophic subaortic stenosis), but excluded mild septal thickening [Lin et al., 2002]. Cardiovascular malformations referred to structural congenital heart defects, and excluded valve prolapse, regurgitation, dysplasia, or thickening.

Laboratory Techniques

DNA was extracted from blood, saliva, or cell lines using standard methods. All DNA represents constitutional samples, no tumor samples were analyzed. In the patients enrolled in the North American protocol, genomic DNA was extracted from buccal cells, blood, or from previously established fibroblast cultures using the PureGene DNA Isolation Kit (Gentra Systems, Minneapolis, MN). Genomic DNA was isolated from saliva samples using the Oragen purification kit. A discrete 575 bp region of the *HRAS* gene containing the first translated exon (Exon 2) and flanking intronic regions was amplified by polymerase chain reaction using these primers: forward-ATTTGGGTGCGTGGTTGA, reverse-CCTCTAGAGGAAGCAGGAGACA. PCR was performed with 150 ng genomic DNA in a 25 μl reaction containing 1× Qiagen Taq Buffer plus Q solution, 3 mM $MgCl_2$, 500 μM each dNTP, 1 μM of each forward and reverse primers and 0.75 U Taq polymerase (Qiagen, Valencia, CA). Reactions were run on a Stratagene robocycler for 30 cycles (30 sec at 94°C, 30 sec at 60°C, and 1 min at 72°C). Genomic fragments containing the remaining translated exons were amplified in the presence of Q-solution and an annealing temperature of 63°C, using primers previously described [Aoki et al., 2005]. Sequencing was performed in both directions using the ABI BigDye Terminator Cycle Sequencing Ready Reaction kit v 3.1, using a 1/4 dilution of the terminator mix, and analyzed on an ABI3130XL Genetic Analyzer.

The protocol used for the Spanish patients varied regarding the primer sequences and reaction conditions used. Polymerase chain reaction and sequencing were performed following standard protocols [Cheng et al., 1994; Williams and Soper, 1995].

We sequenced the entire coding region in all patients in whom no disease causing mutation was identified. Parental samples were sequenced as available, for the amplicon of interest only.

RESULTS

Table I presents the clinical and molecular characteristics of 40 patients (34 North American,

TABLE I. Genotype and Phenotype Analysis in 40 Patients With Costello Syndrome

	Mutation				Growth			Clinical features								Cardiac abnormalities		
Pt#	Nucleotide substitution	Amino acid change	Sex	Age	Ht centile	OFC centile	GH deficiency	Polyhydramnios	FTT	Tone	CNS abnormality	Nystagmus	Ulnar deviation	Papillomata	Tumor	Cardiac hypertrophy	Arrhythmia	CVM
1[a]	34G → A	G12S	F	4	−5 SD	90th	−	+	+	↑	−	+	+	−	−	−	SVT	PS
2	34G → A	G12S	F	10	−5 SD	50–75th	−	+	+	↓	Chiari I syrinx	+	+	−	−	−	−	−
3[b,c]	34G → A	G12S	M	22	−5 SD	90th	−	+	+	↓	−	−	+	+	−	HCM severe concentric	SVT, PACs, PVCs	BAV
4[c]	34G → A	G12S	F	28	−4 SD	>98th	+	+	+	−	−	+	−	+	−	−	−	−
5[b]	34G → A	G12S	F	8	−5 SD	3–10th	−	+	+	↓	−	−	+	+	−	HCM	SVT	ASD
6	34G → A	G12S	M	4	−3 SD	90–97th	−	+	+	↓	−	+	−	−	−	−	SVT	−
7[c]	34G → A	G12S	F	20	10th	75th	+Rec'd GH	+	+	↓	−	−	+	+	−	−	+	−
8[a]	34G → A	G12S	F	2	−4SD	75–90th	−	+	+	↓	−	+	+	−	−	HCM	Tachycardia NOS	−
9	34G → A	G12S	M	5	−4 SD	50–75th	+Rec'd GH	+	+	↓	−	−	+	−	−	−	−	−
10[a,e]	34G → A	G12S	F	6	−4 SD	>98th	+Rec'd GH	+	+	↓	−	+	+	+	−	LVH concentric	−	PSV, VSD
11[b,f,g]	34G → A	G12S	F	9	−5 SD	>98th	+Rec'd GH	+	+	−	VM	+	+	+	RMS	HCM Severe	SVT, EAT	PSV, MS
12[c]	34G → A	G12S	M	35	−4 SD	>98th	−	−	+	↓	−	−	+	+	−	−	−	PSV, ASD
13	34G → A	G12S	F	3	−5 SD	75th	+	+	+	↓	Chiari I	−	−	−	−	HCM	Severe sinus tachycardia	−
14	34G → A	G12S	M	7	−5 SD	50–75th	+	+	+	−	+	−	−	−	−	−	−	
15[b]	34G → A	G12S	F	9	−5 SD	50th	−	+	+	↓	HC, VPS, Chiari I	+	+	+	−	−	Tachycardia, Bradycardia	PSV
16	34G → A	G12S	F	17	−3 SD	>98th	+ Rec'd GH	+	+	↓	−	+	+	+	Benign bladder tumor	−	−	−
17	34G → A	G12S	F	10	1–3rd	90th	+ Rec'd GH	−	+	↓	−	−	+	+	−	−	−	−
18[d]	34G → A	G12S	M	16	−5 SD	90th	+ Rec'd GH	+	+	↓	VM	+	+	−	−	LVH	−	−
19[a,b]	34G → A	G12S	F	10	1–3rd	50–75th	−	+	+	↓	−	−	−	−	−	−	−	−
20	34G → A	G12S	M	3	−5 SD	50–75th	−	+	+	↓	−	−	+	+	−	HCM	SVT	−
21	34G → A	G12S	F	6	−5 SD	50–75th	−	+	+	−	−	−	−	−	−	−	−	−
22	34G → A	G12S	F	13	−4 SD	75–90th	+ Rec'd GH	+	+	−	−	−	+	+	−	−	LQTS	−
23	34G → A	G12S	M	19	−4 SD	>98th	−	+	+	↓	−	−	+	+	−	HCM	−	−
24	34G → A	G12S	F	6	−5 SD	>98th	−	+	+	−	−	+	+	−	−	HCM Severe	+ NOS	−
25	34G → A	G12S	M	10	−4 SD	75th	+	+	+	↓	−	−	+	−	−	−	−	−
26[b,h]	34G → A	G12S	M	10	−5 SD	25th	+ Rec'd GH	+	+	−	Chiari I	−	+	−	−	−	−	PSV
27	34G → A	G12S	F	9	−3 SD	>98th	−	+	+	−	HC, VPS	+	−	−		−	−	−
28[k]	34G → A	G12S	M	8	−4 SD	25–50th	−	+	+	↓	HC	+	+	−	−	IHSS	SVT	PS, ASD
29	34G → A	G12S	M	2	−4 SD	50th	+	+	+	−	−	−	+	−	RMS	+	EAT	−
30[c,h,i]	34G → A	G12S	F	16	5th	>98th	+ Rec'd GH	−	+	↓	−	−	+	+	−	+	−	−
31[a]	35G → C	G12A	M	6	−4 SD	75th	−	+	+	↓	HC, VPS	+	+	+	−	−	Sinus tachycardia, persistent	−
32[c,j]	35G → C	G12A	F	21	−5 SD	75–90th	+ Rec'd GH	−	+	−	−	−	−	+	Bladder CA	−	−	−
33[b]	37G → T	G13C	M	12	5th	>98th	−	+	+	↓	VM	−	−	−	−	LVH	−	−
Patients with No *HRAS* Mutation:																		
34*[a]	None		M	6	−3 SD	50–75th	−	−	+	↓	−	−	+	−	−	−	−	−
35[a]	None		F	2	−5 SD	10–25th	−	+	+	↓	Increased extra axial fluid	+	+	−	−	HCM	−	PSV
36*	None		M	6	−5 SD	>98th	−	+	+	↓	HC	+	+	−	−	HCM	EAT, PVC, PAC	−
37*[,k]	None		F	1	−3 SD	10th	−	−	+	↓	−	−	−	−	−	−	−	SVPS
38*[,k]	None		M	3	−3 SD	10–25th	−	+	+	↓	VM	+	+	−	−	−	−	−
39*[,k]	None		M	4	−3 SD	NA	−	−	+	↓	Hypoplastic CC, Mega cisterna magna	−	−	−	−	−	−	PS
40*[,k]	None		F	4	−4 SD	50–75th	−	+	−	↓	−	−	−	−	−	+ NOS	SVT	PS

ASD, atrial septal defect; BAV, bicuspid aortic valve; CA, carcinoma; CAR, chaotic atrial rhythm; CC, corpus callosum; CNS, central nervous system; CVM, cardiovascular malformation; EAT, ectopic atrial rhythm; F, female; FTT, failure to thrive; GH, growth hormone; HC, hydrocephalus; HCM, hypertrophic cardiomyopathy; Ht, height; IHSS, idiopathic subaortic stenosis; LQTS, prolongation of QT interval; M, male; MS, mitral stenosis; NA, not available; NOS, not otherwise specified; PSV/SV, pulmonic stenosis (valvar, supravalvar); PAC, premature atrial contraction; Pt, patient; RMS, rhabdomyosarcoma; SVT, supraventricular tachycardia; Tone increased (↑), tone decreased (↓); VM, ventriculomegaly; VPS, ventriculo-peritoneal shunt; VSD, ventricular septal defect. Age in years, at time measurements for height and OFC were obtained. Height as percentile, or standard deviation when below 1st centile.

Reported previously.

[a]Gripp et al. [2004].

[b]Lin et al. [2002].

[c]White et al. [2005].

[d]Johnson et al. [1998].

[e]Lin et al. [2004].

[f]Dearlove and Harper [1997].

[g]Kerr et al. [1998].

[h]Stein et al. [2004].

[i]Legault and Gagnon [2001].

[j]Gripp et al. [2000].

[k]Spanish patients.

*Indicates overall appearance which was not classic or atypical for Costello syndrome.

6 European) with Costello syndrome. There were 22 females (55%). Ages ranged from 2 to 35 years.

We identified heterozygous *HRAS* mutations in 33 of 40 (82.5%) patients. All mutations occurred de novo, since none of the 19 sets of analyzed parents carried the sequence change. The *HRAS* mutations were identified in different cell types in two patients (Table I, Patient 14: buccal cells and lymphocytes; Patient 18: fibroblasts and lymphocytes) for which different tissues were available, thus indicating that these mutations occurred in the parental germline. Most (30 of 33, 90.9%) of the mutations are 34G > A nucleotide transitions resulting in the substitution of a serine for the glycine in position 12 (G12S). We identified two additional patients with a 35G > C transversion resulting in an alanine substitution in position 12 (G12A). A 37G > T mutation seen in one patient causing a cysteine substitution of amino acid 13 (G13C) has not previously been reported in Costello syndrome (Table II). Table I lists the presumed disease causing nucleotide changes. Several novel single nucleotide polymorphisms (SNPs) were identified in mutation-positive and -negative patients (data not shown). These SNPs were also present in parents and control DNAs isolated from unrelated volunteers and do not appear related to Costello syndrome.

Table III presents a comparison of the clinical characteristics between patients with and without mutations, and between the different mutations.

DISCUSSION

Our results confirm that germline *HRAS* mutations cause Costello syndrome in most patients [Aoki et al., 2005]. All mutations occurred de novo among those triads tested (slightly over half). The patients' missense mutations result in amino acid substitutions of a glycine residue in position 12 or 13 of the protein product. These particular amino acids are located at the GTP binding site and mutations at these sites have previously been shown to cause constitutive activation of HRAS, in turn causing increased activation of downstream effectors in signaling pathways controlling cell proliferation and differentiation [Oliva et al., 2004].

Based on a total of 45 (12 Aoki et al., 2005; 33 in this study) patients with mutations, mutations affecting HRAS amino acids 12 and 13 seem to define a mutational hotspot for Costello syndrome. The phosphate (PO_4) box of the HRAS GTP binding domain encoded by amino acid 10–15 in Exon 2 includes several 5′-CG-3′ (CpG) sites, which could account for this mutational hotspot. When these CpGs are methylated, they become vulnerable to mutations affecting not only the cytosines of either DNA strand, but also the neighboring guanines [Pfeifer, 2000]. Spontaneous mutations can occur at these sites, especially C → T or G → A transitions, with the G → A mutation resulting in the G12S change seen in 30 Costello patients reported here, and 7 previously reported (Table II).

In contrast, nearly 80% of codon 12 mutations seen in tumors [Sanger Institute Catalogue of Somatic Mutations in Cancer, 2005], involve a G → T transversion resulting in amino acid changes G12V or G12C (Table II). The frequency of these mutations is increased in response to mutagens acting on methylated CpG and are very common in many tumor tissues, indicating a high oncogenic potential resulting from the constitutive activation of the protein product. As pointed out by Aoki et al. [2005], the *HRAS* mutation spectrum seen in Costello syndrome differs both qualitatively and quantitatively from the mutation spectrum seen in tumors. The lack of mutations affecting codons other than those in malignancies suggests that there are a limited number of codons in which missense mutations can lead to constitutive activation of the protein product.

Heterozygous missense mutations causing constitutive activation of the protein product often occur in the paternal germline, as suggested by Penrose [1955] who proposed that mitotic replications errors accumulate in male germ cells. Supporting this hypothesis are the findings in Apert syndrome, achondroplasia and Muenke syndrome, due to missense mutations in *FGFR2* and *FGFR3*, respectively, with exclusive paternal origin of new mutations resulting in constitutive activation or increased ligand binding of the protein product [Moloney et al., 1996; Rannan-Eliya et al., 2004]. The paternal age effect observed in Costello syndrome [Lurie, 1994], in combination with

TABLE II. *HRAS* Mutations in Patients With Costello Syndrome and in Tumor Samples

Amino acid change	Nucleotide substitution	Aoki et al. [2005]	This report	Total (%)	Frequency in tumors[a]
G12S	34G → A	7	30	37 (82.2 %)	6.5%
G12A	35G → C	2	2	4 (8.8 %)	0.4%
G13D	38G → A	2	—	2 (4.4 %)	4.4%
G12V[b]	35GC → TT; 35G → T	1	—	1 (2.2%)	44.2%
G13C	37G → T	—	1	1 (2.2%)	0.6%

[a]Frequency in tumors was calculated based on 477 *HRAS* missense mutation positive tumor samples on the Sanger Institute Catalogue of Somatic Mutations in Cancer [2005]. Percentages in the tumors do not add up to 100 because only the amino acid changes seen in Costello syndrome are listed.
[b]The G12V mutations is typically due to a G to T transversion at position 35 in tumors; however, in the Costello patient, a double mutation occurred resulting in the same predicted amino acid change.

TABLE III. Clinical Characteristics and *HRAS* Mutation Status in Patients With Costello Syndrome: Combined Series

		HRAS mutation present					
Clinical characteristic	No mutation 7 (7,0) pts	Total 40[a] (33,7) pts	G12S 33 (30,3) pts	G12A 3 (2,1) pts	G13D 2 (0,2) pts	G12V 1 (0,1) pt	G13C 1 (1,0) pt
Failure to thrive	6/7 (86%)	40/40 (100%)	33/33 (100%)	G13D 2 (0,2) pts	G12V 1 (0,1) pt	G13C 1 (1,0) pt	1/1 (100%)
Polyhydramnios	4/7 (57%)	29/33 (87%)	27/30 (90%)	1/2 (50%)	N/A	N/A	1/1 (100%)
Hypotonia	7/7 (100%)	24/33 (72%)	22/30 (73%)	1/2 (50%)	N/A	N/A	1/1 (100%)
Ulnar deviation	4/7 (57%)	25/33 (75%)	24/30 (80%)	1/2 (50%)	N/A	N/A	0/1 (0%)
Any cardiac abnormality	5/7 (71%)	30/40 (75%)	22/33 (66%)	2/3 (66%)	2/2 (100%)	1/1 (100%)	1/1 (100%)
Cardiac hypertrophy	4/7 (43%)	19/40 (47%)	15/33 (45%)	1/3 (33%)	1/2 (50%)	1/1 (100%)	1/1 (100%)
Arrhythmia	2/7 (28%)	17/40 (42%)	15/33 (45%)	1/3 (33%)	1/2 (50%)	0/1 (0%)	0/1 (0%)
CVM	4/7 (57%)	10/40 (25%)	9/33 (27%)	0/3 (0%)	1/2 (50%)	0/1 (0%)	0/1 (0%)
Papillomata	0/7 (0%)	19/40 (47%)	16/33 (48%)	2/3 (66%)	1/2 (50%)	0/1 (0%)	0/1 (0%)
GH deficiency	0/7 (0%)	15/33 (45%)	14/30 (46%)	1/2 (50%)	N/A	N/A	0/1 (0%)
Nystagmus	3/7 (43%)	14/33 (42%)	13/30 (43%)	1/2 (50%)	N/A	N/A	0/1 (0%)
Tumor	0/7 (0%)	6/40 (15%)	4/33 (12%)	2/3 (66%)	0/2 (0%)	0/1 (0%)	0/1 (0%)
CNS abnormality	4/7 (57%)	9/33 (27%)	8/30 (27%)	1/2 (50%)	N/A	N/A	1/1 (100%)

Figures are rounded.

CNS, central nervous system abnormality; CVM, cardiovascular malformation; GH, growth hormone.

[a]Patient total includes the 33 new patients listed on Table I in this report, and the 7 Japanese patients listed on the supplementary Table I (online version) of Aoki et al. [2005]; no information was available on the five Italian patients from that series. Patients are listed as the total, followed in parentheses by the number in the present series and Japanese patients. There was no information on polyhydramnios, growth hormone deficiency, hypotonia, nystagmus, and ulnar deviation was provided by Aoki et al. [2005], and thus, denominators reflect the number of informative patients.

the nature of the missense mutations, suggests a paternal origin of the mutations. In this context, the loss of heterozygosity (LOH) of 11p15.5 in tumor tissue from Costello syndrome cases is of particular interest. Kerr et al. [2003] analyzed five embryonal RMS from Costello syndrome patients and showed loss of heterozygosity for 11p15.5 in all samples, with retention of the paternal allele confirmed in two cases. This finding may be consistent with the monoallelic expression of the mutated allele in the ganglioneuroblastoma described by Aoki et al. [2005]. It remains to be seen if LOH for *HRAS* is a consistent finding in all tumors in Costello syndrome, or if it is typical only for embryonal tumors as reported by Kerr et al. [2003] and Aoki et al. [2005]. While the constitutional *HRAS* mutation in Costello patients represents the first step in tumorigenesis, the second step may vary with LOH in embryonal tumors and mutations in additional genes in bladder cancer and other malignancies of adulthood. Jebar et al. [2005] reviewed *FGFR3* and *RAS* mutations in urothelial cell carcinoma and did not identify LOH, rather they reported mutually exclusive sequence changes in the genes whose protein products share the MAPK pathway as common effector.

The lack of mutations in seven patients led us to review their respective clinical presentation in detail. All patients except Patients 34 and 36 enrolled under the North American study were thought to have the typical facial changes of Costello syndrome. Upon review of facial photographs of the patients enrolled under the Spanish protocol, only Patient 28 had the completely characteristic facial appearance of Costello syndrome, and Patients 37–40 had facial findings consistent with either Costello or CFC syndrome. At this time, we cannot be certain that the lack of an identifiable *HRAS* mutation excludes the diagnosis of Costello syndrome. The possibility that these patients do not have Costello, but possibly CFC syndrome needs to be considered. If this was confirmed, the phenotype of CFC syndrome would include elevated catecholamine metabolite levels and cardiac arrhythmia.

It is noteworthy that we identified *HRAS* mutations in Patients 11, 29, and 30, who each had a malignancy, and Patient 16, who reportedly had a benign bladder tumor. Patient 30 developed a transitional cell carcinoma of the bladder [Gripp et al., 2000], she carries a mutation predicted to result in a G12A amino acid substitution. This mutation is found in less than 1% of malignancies with an *HRAS* mutation (Table II), specifically in one chondrosarcoma and one papillary thyroid carcinoma [Sanger Institute Catalogue of Somatic Mutations in Cancer, 2005]. In contrast, the G12S change present in Patients 11 and 29 with RMS represents the most common mutation in Costello syndrome and occurs in a variety of malignancies including soft tissue and synovial sarcoma and carcinoma of the gastro-intestinal and urinary tract [Sanger Institute Catalogue of Somatic Mutations in Cancer, 2005]. This mutation was seen in a Japanese patient with rhabdomyosarcoma [Aoki et al., 2005]. We identified one novel CS mutation, resulting in a cysteine substitution of amino acid 13 (Table I, Patient 33). This particular mutation is relatively rare in malignancies, but has been identified in three bladder cancer samples [Visvanathan et al., 1988; Levesque et al., 1993]. While it may be tempting to speculate on the oncogenic potential of the different mutations, we need more data to evaluate if the cancer risk varies by mutation.

Most of our patients and those reported by Aoki et al. [2005] share a common mutation (Table II). Rare phenotypic findings in these patients, for example

the long QT syndrome in Patient 22, may be coincidental, or caused by the mutation with a low incidence or in combination with modifying factors. A correlation between the cardiac abnormalities and the specific mutations is also hampered by the fact that we have few patients with mutations other than G12S. Of the three patients with mutations other than G12S, one each had left ventricular hypertrophy and tachycardia. While none had pulmonic stenosis or other structural anomalies, these numbers are too small to draw conclusions. Of note are the cardiac anomalies seen in some of the *HRAS* mutation-negative patients: Three had hypertrophic cardiomyopathy, two showed tachyarrhythmia, and four had pulmonic stenosis. Concerning the short stature seen in almost all Costello patients, Patient 33, the only person reported to date with the G13C mutation, is noteworthy. He is the tallest mutation-positive patient who never received growth hormone, and at age 12 years, he has not developed papillomata. This may suggest that G13C causes a slightly less severe phenotype.

The identification of *HRAS* mutations as the underlying cause for Costello syndrome is very helpful in respect to the ability to confirm a clinical diagnosis of Costello syndrome. Based on the data available today, a *HRAS* missense mutation leading to constitutive activation of the protein, in combination with consistent clinical findings, is likely diagnostic of Costello syndrome. In contrast, we cannot be certain that the lack of such a mutation precludes a diagnosis of Costello syndrome. It is too early to revise recommendations for clinical care based on the mutation status, but we hope to collect additional data in order to achieve this goal. Lastly, one may speculate that the identification of these mutations in Costello syndrome in combination with the knowledge from cancer research on *HRAS* and the MAPK pathway will allow for the use of medications directed at this pathway.

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