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Male to Male Transmission of Costello Syndrome: G12S *HRAS* Germline Mutation Inherited from a Father with Somatic Mosaicism

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Abstract

Costello syndrome is a rare congenital anomaly syndrome associated with mental retardation and predisposition to benign and malignant tumors, caused by heterozygous missense mutations in the *HRAS* oncogene. Previously, all molecularly analyzed mutations appeared de novo, and most arose in the paternal germline. A single patient with somatic mosaicism for a Costello syndrome causing *HRAS* mutation has been reported. Here we describe the first documented transmission of an *HRAS* mutation from a parent with somatic mosaicism to a child with typical Costello syndrome. Prior to the identification of the underlying gene mutation in Costello syndrome, this family had been identified clinically. The proband was subsequently found to carry a G12S *HRAS* germline mutation. Testing of the parents for parental origin identified his father as mosaic for the same *HRAS* mutation. The mother was found not to carry an *HRAS* mutation. The causative familial mutation is identified as a c. 34G>A, which is the most common mutation in the *HRAS* gene in patients with Costello syndrome. The father carries the mutation in 7–8% of his alleles. This is the second case of mosaicism observed in Costello syndrome and the first direct molecular evidence of father to son transmission of the disease-causing mutation. Our observation underlines the importance of parental evaluation, and may have implications for genetic counseling and clinical practice.

Keywords

Ras oncogenic mutation; allelic specific amplification; MAPK dysregulation

INTRODUCTION

Costello syndrome [OMIM 218040] typically encompasses severe failure-to-thrive, cardiac abnormalities including tachyarrhythmia and hypertrophic cardiomyopathy, a predisposition

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Web Resources

The URL for data presented herein is as follows: Online Mendelian Inheritance in Man (OMIM), <http://www.ncbi.nlm.nih.gov/Omim/>

to papillomata and malignant tumors, and neurologic abnormalities including nystagmus, hypotonia, developmental delay and mental retardation [Gripp and Lin, 2006]. A clinical diagnosis can now be confirmed through the identification of an *HRAS* mutation [Aoki et al., 2005] and the diagnosis should be reconsidered if no *HRAS* mutation is seen [Gripp et al., 2007; Kerr et al., 2008]. More than 80% of patients with Costello syndrome share the same underlying *HRAS* mutation, resulting in a G12S amino acid change [Aoki et al., 2005; Estep et al., 2006; Gripp et al., 2006a; Kerr et al., 2006; Sol-Church et al., 2006, van Steensel et al., 2006; Zampino et al., 2007]. The mutations were de novo in all analyzed cases, and in the vast majority occurred in the paternal germline [Sol-Church et al., 2006; Zampino et al., 2007]. Only one case of somatic mosaicism for an *HRAS* mutation in Costello syndrome has been confirmed [Gripp et al., 2006b]. We report here on a second individual with a mosaic *HRAS* mutation, resulting in transmission to a child with Costello syndrome.

SUBJECTS AND METHODS

Informed consent was obtained based on the protocol approved by the institutional review board at the A. I. duPont Hospital for Children (#2005-051).

Patient 1

The patient is a 21-year-old male in whom a clinical diagnosis of Costello syndrome was made in early childhood based on the typical medical history of severe feeding difficulties and failure to thrive requiring the use of a feeding tube until age 4 ½ years. He had developmental delay. On his most recent exam his height was 150 cm (50th centile for 12.5 years). He had deep palmar creases, joint laxity and nasal papillomata. His facial findings and his hyperkeratotic skin were typical for Costello syndrome.

Cardiac hypertrophy was detected at age 17 years. He has had hypertrophic cardiomyopathy for nearly 9 years, which has steadily increased and remains stable at moderate severity. He has no syncope or palpitations.

The most recent echocardiogram performed at age 20 years showed mild concentric left ventricular hypertrophy with additional focal thickening in the mid-septal region. The peak Doppler gradient averaged 100 mm hg (range 90–120 mm Hg, mean 48 mm). Treatment has included atenolol, and he is currently treated with a calcium channel blocker, verapamil (180 mg orally daily, 4.3 mg/kg/day). Echocardiograms have not shown congenital heart defects or valve dysplasia, nor did he have serious arrhythmia in infancy. Electrocardiograms have been obtained on a regular basis (at least annually) and have shown increased ventricular voltages, without significant repolarization abnormalities, and increased atrial complexes. Holter monitoring performed at ages 18 and 19 years showed rare ventricular couplets and triplets. The most recent one performed at age 21 years showed similar rare ventricular and atrial ectopy with mild nonsustained ventricular tachycardia. He had a treadmill exercise stress test at age 20 years which was successful up to stage 3, and limited due to his lack of cooperation.

Patient 2

The father of Patient 1 came to medical attention years ago when physical findings suggestive of mosaic Costello syndrome were noted by the physician (L.A.D) treating Patient 1 [Bodkin et al., 1999]. Patient 2 had a history of feeding problems and severe failure to thrive in early childhood. He has hyperkeratosis on both hands and his left foot, but not on the right foot. Papillomata were present on the right, but not the left perianal region. Nasal papillomata had been removed. Hyperpigmentation, thick ear lobes, and patches of curly hair in his otherwise straight hair were noted. His medical history includes kidney stones in

his 20's; and bilateral inguinal hernia repair in his 50's. He also had coronary artery disease, which was treated with stenting, ballooning and pharmacotherapy. A cardiac echo performed in his 50s did not show structural anomalies, in particular no hypertrophy.

The patient had developmental delay, and was academically challenged in school, succeeding with family support. Prior to confirmation by DNA analysis, he expressed awareness that this son and he had similar physical and developmental Costello syndrome traits.

To respect the family's preference for privacy, no patient's pictures are included in this report.

Molecular Techniques

DNA from the patients, Patient 1's mother and an unrelated normal control individual was extracted from buccal cells using the PureGene DNA Isolation Kit (Gentra Systems, Minneapolis, MN, USA). A second sampling of the buccal swab was collected on Patient 2 to validate the original result. Parent-child biologic relationships were confirmed using the AmpFISTR® Profiler Plus™ kit from Applied Biosystems (Foster City, CA), as previously described [Sol-Church et al., 2006].

All primers are listed in Table 1. For the G12S mutation screen, a discrete 575bp region of *HRAS* containing exon 2 was amplified using primers F1 and R1. Two additional overlapping PCR products were obtained for Patient 2 using primers F2 and R2 (490bp), and F3 and R1 (649bp). The latter product was subjected to a second round of PCR amplification using primers F3 and R1, in order to rule out preferential allelic amplification. Additional regions of the *HRAS* gene were screened using primers that amplified exons 3, exons 4–5, and exon 6 (supplemental Table 1). All PCR products were purified and sequenced in both directions using the ABI BigDye Terminator Cycle Sequencing Ready Reaction kit v 3.1 and analyzed on an ABI3130XL Genetic Analyzer. All sequences were analyzed by direct observation of the electropherogram and using Sequencher 4.8 (Gene Codes Corporation, Ann Harbor MI).

Allele specific primers RW and RM were designed to selectively amplify either the wild type G12 or the mutated S12 alleles. Patient 1's alleles were amplified using F4/RW and F4/RM and sequenced using F4. Longer allele specific PCR products were generated for Patient 2 using F3/RW and F3/RM, and the sequencing performed using primer F3. Standard PCR conditions were used and supplemental Table 1 lists the products size and annealing temperature of each primer set.

SgrA1 digestion of the 575 bp and 649 bp products derived from Patient 1 and Patient 2 was performed as follows. Unpurified products were incubated with the restriction endonuclease for 3 hours at 37°C, separated by gel electrophoresis on a 2% agarose gel and photographed under UV light. For the sample derived from Patient 2, the *SgrA1*-resistant 649 bp band containing the mutated allele was gel purified and subjected to a second round of PCR amplification, followed by purification and sequencing.

Allelic quantification was performed using pyrosequencing technology. Briefly, a 177 bp biotinylated PCR product was generated using primers F5 and R5. Following immobilization of the biotin tagged single stranded PCR product onto Streptavidine Sepharose HP (Amersham Pharmacia Biotech, Uppsala, Sweden) sequencing primer R6 was annealed, and pyrosequencing was initiated using the PSQ96 Gold Reagent Kit following the manufacturer's recommendation (Pyrosequencing AB, Uppsala, Sweden). Data analysis was performed using the PSQ96 allelic quantitation (AQ) option.

RESULTS

Patient 1 and 2's DNAs were sequenced across a region of the *HRAS* gene containing the first translated exon (exon 2) and flanking intronic regions. Patient 1 carries a known SNP (–10C>T) and a germline c.34G>A mutation, resulting in a heterozygous G12S change in the ras protein (Figure 1A). Both DNA samples isolated from his father, Patient 2, showed the heterozygous SNP as well as evidence of the G12S mutation, albeit at a lower level suggesting somatic mosaicism (Figure 1B and 1C). Results for Patient 2 were confirmed using independently amplified overlapping PCR products (data not shown). The mother of Patient 1 is homozygous C at the –10 SNP and carries no mutation in exon 2 (Figure 1D). The SNP is an informative marker for this family as Patient 1's –10C allele can be traced back to his mother, while the –10T allele is inherited from the father (Patient 2). Preferential amplification of either allele in Patient 2 was ruled out by performing a second round of amplification using the 649 bp PCR product as a template and primers F3 and R1. No other mutation was detected in exons 3, 4, 5 and 6 in either patient. As previously reported in Sol-Church et al. [2006] the G12S mutation leads to loss of a cognate site for endonuclease *SgrA1*. Restriction endonuclease analysis of PCR products using *SgrA1* was performed on the patients and a control sample (Figure 2). Compared to the normal control, Patient 1 and Patient 2 carry alleles that are resistant to hydrolysis, thus confirming the presence of the mutation in both individuals (Figure 2, panels A and B). Consistent with Patient 2's low level mosaicism observed by direct sequencing, the father's undigested product is less compared to Patient 1. Patient 2's 649bp *SgrA1*-resistant product seen in Figure 2B (lane 2) was gel purified and sequenced. The sequencing profiles depicted in Figure 2C indicate that this restriction endonuclease resistant product is enriched in mutant allele and shows no preferential amplification when subjected to second round amplification. Interestingly, the –10T allele is also enriched suggesting that this allele may be present in *cis* with the CS-causing mutation.

Pyrosequencing was used to quantify the fraction of mutated alleles in the DNA from both affected individuals (supplemental Fig. 4). Patient 1's observed allelic ratio (48.2%) is close to that expected of a germline mutation (50%), while only 7.5% of Patient 2's alleles are mutated, indicating somatic mosaicism. Patient 2's buccal sample consists of a mixed population of cells: most cells are wild type and carry a normal *HRAS* gene and a small fraction of mutant "CS" cells carry the heterozygous G12S mutation. Allele specific amplification (ASA) was performed to verify the paternal origin of Patient 1's germline mutation, and also to clarify whether or not the father's mutation had occurred randomly on his chromosome homologs. During ASA specific primers were used to selectively amplify either the wild type (ASA-G12) or the mutant (ASA-S12) alleles (Figure 3). Sequencing results show that Patient 1's wild type amplicons carry in *cis* the –10C allele, which he has inherited from his mother. Conversely, his mutated allele carries the –10T paternal allele thus demonstrating that the germline mutation was inherited from the father.

As expected from a mosaic population of cells, Patient 2's wild type allele revealed the presence of the heterozygous C>T SNP, contributed from both normal and mutant cells. In contrast, Patient 2's mutant allele generated via ASA-S12, clearly identifies in *cis* the presence of the homozygous –10T SNP allele. These results unequivocally demonstrate that the clonal nature of the mutated cells in the father arose from a single hit to the SNP T-allele.

DISCUSSION

Before the gene defect for Costello syndrome was identified, Bodkin et al [1999] postulated somatic mosaicism in the father of a male with typical Costello syndrome. The father had a

history of feeding problems, a patchy distribution of skin and hair abnormalities, and nasal papillomata. The authors suggested that this apparent male-to-male transmission was consistent with autosomal dominant inheritance, in contrast to the previously considered recessive inheritance pattern. Since then, a discrete number of heterozygous germline mutations in the oncogene *HRAS* have been reported to cause Costello syndrome. Currently, 13 different DNA variants of the *HRAS* gene, affecting amino acid 12, 13, 22, 58, 63, 117 or 146, have been reported in Costello syndrome patients [reviewed in Sol-Church and Gripp, 2008 in press]. Substitutions of glycine 12 or 13 in the ras protein account for 95% of these mutations. The most common changes (G12S, G12A) result in the typical phenotype, while milder cases can be due to mutations resulting in presumably less functional impairment of the abnormal protein product [Gripp et al., 2008]. Another mechanism resulting in an apparently mild Costello syndrome phenotype despite a typical G12S *HRAS* mutation is somatic mosaicism [Gripp et al., 2006b]. The first molecular evidence of mosaicism in Costello syndrome was seen in a female with developmental delay, short stature, sparse hair, coarse facial features, and nasal papillomata. She had irregular skin hyper- and hypopigmentation, a finding not typical for Costello syndrome, but often associated with mosaicism for chromosome abnormalities. Standard assays performed on this patient's white blood cell derived DNA did not show an *HRAS* mutation. However, buccal cell derived DNA revealed a sequence change qualitatively consistent with the G12S mutation. Allelic quantitation demonstrated the presence of this mutation in ~25–30% of the cells' alleles. It was noted that this patient with somatic mosaicism had regular periods, whereas most females with Costello syndrome have delayed or incomplete puberty or amenorrhea due to central hypogonadism [White et al 2005]. Thus, this patient was considered at risk for offspring with Costello syndrome [Gripp et al., 2006b].

Through molecular analysis of the family originally reported by Bodkin et al 1999, we have now shown for the first time that parental somatic mosaicism can result in an offspring with typical Costello syndrome. Similar to the case reported here, parental somatic mosaicism could explain the findings in the family with mild mental retardation, short stature, macrostomia, thick lips, hyperlax and hyperpigmented skin and facial papillomata in the mother of siblings with presumed typical Costello syndrome [Ioans and Fryns, 2002]. However, while this mother's findings could be consistent with somatic mosaicism for a typical Costello syndrome mutation, they may also be consistent with the "attenuated phenotype" apparently associated with rare Costello syndrome causing mutations [Gripp et al., 2008]. In contrast to the families reported here and by Ioans and Fryns, no physical findings suggestive of parental somatic mosaicism were noted in the few other reported siblings with Costello syndrome [Zampino et al., 1993; Johnson et al., 1998]. Thus, parental germline mosaicism appears to be a more likely explanation than parental somatic mosaicism for the recurrence in these families. Detailed molecular studies will need to be performed in these families in order to confirm the underlying mechanism.

Costello syndrome in a mosaic individual may be difficult to recognize on clinical grounds, but it should always be considered when evaluating parents of affected individuals. The incidence of somatic mosaicism for Costello syndrome is unknown, but the identification of two mosaic individuals has important consequences for clinical diagnosis, molecular testing and genetic counseling. In addition to the obvious concern for the risk to offspring, somatic mosaicism may have important consequences regarding medical problems related to the *HRAS* mutation. For example, patients with Costello syndrome are at increased risk for transitional cell carcinoma of the bladder, presumably due to the *HRAS* mutation representing the first "hit" in carcinogenesis. An individual with somatic mosaicism may or may not carry the *HRAS* mutation in the transitional cell epithelium of the bladder, and the presence of cells with two wild type alleles may mitigate the tumor risk. We do not know if the risk for bladder cancer in our Patient 2 is increased compared to the general population.

In order to better understand the consequences of a somatic *HRAS* mutation, long term follow up of these rare patients will be required, and it is important to keep in mind the variability inherent in mosaic states.

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Figure 1. Genotyping status of *HRAS* in family members as detected by direct sequencing Buccal cell genomic DNA was collected from Patient 1 (A), Patient 2 (B and C, two independently collected samples) and Patient 1's mother (D). Patient 1 shows a heterozygous c.34G>A change, causing a G12S mutation, while Patient 2 carries low levels of the mutated allele. Father (B) and child (A) are heterozygous C/T at the -10 SNP, while the mother (D) is homozygous C and carries a wild type c.34G base.

Figure 2. Confirmation of the presence of the G12S allele in Patient 2 by restriction endonuclease analysis

Panel A and B: Restriction digest analysis of genomic DNAs isolated from Patient 1 (lane 1), Patient 2 (lanes 2 and 3), and a wild type control (lane 4). Genomic DNAs were amplified with primers F1R1 (panel A) or F3R1 (panel B) and subjected to *SgrA1* digestion. The arrows indicate the location of the *SgrA1*^R resistant DNA fragments (*SgrA1*^R) that were gel purified and sequenced. Panel C: Sequencing results of Patient 2's F3R1 PCR before (uncut) and after digestion with *SgrA1*.

Figure 3. Parental origin of the germline G12S mutation in Patient 1 and evidence of mosaicism in Patient 2

Allelic specific amplification (ASA) was used to amplify specific alleles in Patients 1 and 2. The location of the informative SNP at bp-10 is indicated. Each chromatogram depicts the sequence of either the wild type (G12) or mutated S12 alleles for both patients. The ASA base permitting allelic discrimination is underlined.

Table 1

HRAS Primers

Forward	Sequence	Reverse	Sequence	Size (bp)	Ta (°C)
F1	ATTGGGTGCGTGGTTGA	R1	CCTCTAGAGGAAGCAGGAGACA	575	58
F2	GCAGGAGACCCCTGTAGGAGGAC	R2	CGGTATCCAGGATGTCCAACAG	490	60
F3	ACCTGTCTCTGGAGGACGGTAA	R1	CCTCTAGAGGAAGCAGGAGACA	649	59
F3	ACCTGTTCTGGAGGACGGTAA	RW	TATATACTCTTGGCCACACCGCC	462	60
F3	ACCTGTTCTGGAGGACGGTAA	RM	ACTCTTGGCCACACCGCT	457	60
F4	TCTAATTTGGGTGCGTGTGA	RW	TATATACTCTTGGCCACACCGCC	392	60
F4	TCTAATTTGGGTGCGTGTGA	RM	ACTCTTGGCCACACCGCT	387	60
F5	Biotin-AGGAGCGATGACGGAATATAAGC	R5	TCTATAGTGGGGTCTGTATTCTGTCC	117	61
		R6	TCTTGGCCACACCGC		
3F	GAGAGGCTGGCTGTGTGAATC	3R	TCTGCTCCCTGAGAGGTGGAA	410	59
4F	GCTTCCTGTGTGTGTTGCCA	4R	CCAGTAGCCCCCACTAAGACTCAGA	768	59
6F	GGCACCTGTGGTTCTGAGTCTT	6R	TTCTTCTCTCTCTCTCTCTCTCT	639	59