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Transmission of the Rare *HRAS* Mutation (c. 173C > T; p.T58I) Further Illustrates Its Attenuated Phenotype

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Abstract

Costello syndrome was delineated based on its distinctive phenotype including severe failure-to-thrive with macrocephaly, characteristic facial features, hypertrophic cardiomyopathy, papillomata, malignant tumors, and cognitive impairment. Heterozygous germline mutations in the proto-oncogene *HRAS* cause Costello syndrome, and its inheritance pattern would thus be autosomal dominant. With exception of two instances of parental mosaicism, one presumed gonadal and the other proven somatic mosaicism for the p.G12S change, all published cases resulted from de novo mutations, typically arising in the paternal germline. More than 90% of these mutations affect the glycine residues in position 12 or 13, and result in a gain-of-function of the altered protein. A rare heterozygous *HRAS* alteration (c.173C > T; p.T58I) associated with an attenuated phenotype was previously reported in one patient. We identified two additional individuals with this mutation, father and son. Further studies supported origin of the alteration in the grand-paternal germline. Transmission of the mutation underscores its attenuated phenotype compatible with reproduction. We reviewed the phenotype in the newly identified individuals (Patient 1, 2) and include updated information on the first previously reported individual with *HRAS* p.T58I (Patient 3). Macrocephaly was present in all three. Cardiac findings included hypertrophic cardiomyopathy with double-chambered right ventricle; or mitral valve prolapse in one patient each. While subtle neurologic abnormalities or developmental delay were present in all, only one showed significant cognitive and functional impairment. None developed papillomata or a malignant tumor. Genetic counseling for Costello syndrome needs to take into consideration the particular *HRAS* mutation.

Keywords

costello syndrome; *HRAS*; p.T58I; vertical transmission; recurrence risk

INTRODUCTION

Costello syndrome encompasses severe failure-to-thrive, cardiac abnormalities including tachyarrhythmia and hypertrophic cardiomyopathy, a predisposition to papillomata and malignant tumors, and neurologic abnormalities including intellectual disability, nystagmus, and hypotonia [Gripp and Lin, 2009, 2011]. Costello syndrome is caused by heterozygous germline point mutations in the proto-oncogene *HRAS* located on chromosome 11p15.5 [Aoki et al., 2005]. The majority of patients share an *HRAS* mutation predicting a p.G12S amino acid change, and the glycine residue in position 13 is the second most commonly altered amino acid [Gripp et al., 2011a]. Mutations affecting other amino acids are very rare, and may be associated with an attenuated phenotype for p.T58 and p.A146 [Zampino et al., 2007; Gripp et al., 2008], or a slightly variant phenotype with a duplication of the p.E37 [Gremer et al., 2010].

With few exceptions, Costello syndrome is the result of a de novo mutation arising in the paternal germline [Sol-Church et al., 2006; Zampino et al., 2007]. Here, we report transmission of the rare *HRAS* p.T58I change from an apparently heterozygous father to his son. These are the second and third individuals reported with this rare change.

MATERIALS AND METHODS

In our ongoing clinical and molecular study on Costello syndrome and related disorders, we gathered information on 96 probands with Costello syndrome and their presumably pathogenic germline *HRAS* mutations. Identification of an *HRAS* mutation in a proband is followed by testing of both biological parents for the respective change and for associated polymorphic markers whenever feasible. The study is approved by our institutional review board (A. I. duPont Hospital for Children #2005-051).

Clinical Reports

Patient 1—This male (CS# 261) was born to his 39-year-old mother and 38-year-old father (Fig. 1II-2 and II-1, respectively), he had an older maternal half-sister. The pregnancy was complicated by reported “borderline polyhydramnios” noted on ultrasound, there was no gestational diabetes or intrauterine exposure. He was born by vaginal delivery at full term with a length of 53.3 cm (90th centile); weight 3.94 kg (90–95th centile) and OFC 36.5 cm (75–90th centile). The patient did not have medical or feeding difficulties as a neonate. Per review of the pediatrician’s records, his OFC increased rapidly, measuring 41 cm at age 9 days (>95th centile); 48 cm at age 6 months (>95th centile); 50 cm at 1 year (>95th centile); and 53 cm at 18 months (>95th centile, 50th centile for age 9 years). His height and weight increased within the normal range for age.

A cardiac murmur noted at age 5 weeks led to an echocardiography performed at age 2 months, which showed a double-chambered right heart with an anomalous muscle bundle at the level of the right ventricular outflow tract, causing mild subpulmonic stenosis. A re-evaluation at age 3 years revealed moderate left ventricular septal hypertrophy with narrowing of the left ventricular outflow tract (peak gradient 25–65 mm Hg), and mild right ventricular outflow tract narrowing (peak gradient 11 mm Hg). The hypertrophic

cardiomyopathy remained stable until his most recent evaluation at age 5 10/12 years, when an increase in septal thickness resulted in treatment with atenolol.

Bilateral strabismus was noted in early childhood, and surgical repair at age 3 years and 4 years was successful. Patient 1 had normal motor development, he walked independently at age 14 months. He was toilet trained at age 3 years. His speech development is age appropriate, and he spoke in complex sentences at age 4 years. Patient 1 was very active, he enjoyed being the center of attention and his behavior was at times disruptive in a peer setting. While no formal cognitive testing has been performed, he appeared age appropriate as reported by his preschool teacher.

At age 4 8/12 years, his height was 102 cm (10–25th centile), weight 16.6 kg (10th centile), and OFC 54.7 cm (>97th centile for age; 50th centile for age 15 years). He was dolichocephalic and had a high forehead. His hair was fine and not curly, it grew slowly but was never “easily pluckable”. His facial features were not coarse and essentially non dysmorphic (Fig. 2). Both ear lobes were slightly prominent and anteverted. Very mild pectus excavatum and narrow shoulders with full range of motion were noted. Slight redundancy of the soft tissue in both hands with ligamentous laxity of fingers and thumbs were present, and plantar creases were slightly deeper than typical. He did not have tight Achilles tendons or foot positional abnormality. His genitalia were normal with descended testicles. A single café-au-lait lesion was seen. His muscle tone and strength appeared normal, and he did not have clonus.

The identification of the hypertrophic cardiomyopathy at age 3 years led to a number of diagnostic tests, and normal results were reported for a skeletal survey and bone age study; karyotype and oligonucleotide microarray; oligosaccharides and organic acids in urine; creatine kinase, lactate, pyruvate, and acylcarnitine profile. Diagnostic testing for mutations associated with rasopathies revealed an *HRAS* mutation (c.C173T; p.T58I), considered pathogenic. No clinically significant DNA sequence variants were detected in the other genes studied: *PTPN11*, *SOS1*, *RAF1*, *KRAS*, *BRAF*, *MEK1*, and *MEK2*.

Patient 2—This male (CS#262) is Patient 1’s father (Fig. III-1), he was found to carry the familial *HRAS* mutation upon parental testing. He was reportedly born 3 weeks prior to the due date. As a young child he was shorter than his peers and he reportedly had very little body fat. His hair grew normally, but became very light blonde in the summer. A suspected diagnosis of very mild cerebral palsy was mentioned to his mother once in early childhood, but Patient 2 was never formally diagnosed with cerebral palsy or spasticity. He required speech therapy until first grade. Patient 2 now reports hand tremors. The tremors occur at rest and affect the left hand more than the right, rarely the head is also affected. Despite the tremors, he reports good strength and endurance and is able to work as a flight attendant. He reports that he may have attention deficit disorder and he has a long history of difficulties focusing, but never used medication for this issue. He has psoriasis. He has worn glasses since age 3 years, and in adulthood has severe myopia. An echocardiography performed at age 41 years showed “top normal sized left atrium” and “top normal left ventricular wall thickness” with preserved left ventricular systolic function. No central nervous system imaging studies were available, and the patient declined additional medical evaluation.

At age 41 years his height was 180 cm (75–90th centile for adult males), weight 86.3 kg and OFC 59.5 cm (>98th centile for adult males). His facial features were essentially non dysmorphic, bilateral limited elbow extension was noted on exam (Fig. 2).

Patient 3: Updated information on the previously reported individual with

HRAS p.T58I—This male (CS#184) was the first individual reported with a paternal germline-derived HRAS p.T58I change [Gripp et al., 2008] and is unrelated to Patients 1 and 2. He came to medical attention for failure-to-thrive with macrocephaly, hypotonia, nystagmus, pyloric stenosis, and mitral valve prolapse (Table I). Developmental delay and behavior problems were noted. He continued to show developmental progress, but his school performance was significantly below grade level. His behavior problems and aggression worsened and were treated with olanzapine from age 9 years. Anxiety was noted. He had intermittent joint pain affecting hands and feet with erythema, this was not further evaluated. Night-time enuresis continued.

At his most recent medical evaluation at age 9 9/12 years, his height was 132.2 cm (10–25th centile), weight 27.7 kg (10–25th centile), and OFC 55 cm (>98th centile; 50th centile for age 15 years). Pertinent positive findings on examination included full lips, fine hair, long eyelashes, and prominent ear lobes (Fig. 3). Deep palmar and plantar creases with prominent digital pads and mildly increased hyperextensibility of the fingers were noted. Cubitus valgus and limited supination were newly identified findings. Papillomata were absent.

Molecular Analyses

Buccal cell genomic DNA was extracted from Patient 1, Patient 2, Patient 1's mother, and Patient 2's parents using the Qiagen Puregene kit (Gentra Systems, Minneapolis, MN). Gender and familial relationship was verified using the AmpFISTR Profiler Plus kit or AmpFISTR Identifiler kit from Applied Biosystems (Foster City, CA), as previously described [Sol-Church et al., 2006]. Primers were designed to amplify discrete regions of the *HRAS* locus containing coding exons 2–6 [Gripp et al., 2011b; Supplemental Table I: 2F/2R, 3F/3R, 4F/4R, 5F/5R]. Samples from Patient 1 and Patient 2 were sequenced for exons 2–6. Only exons 2 and 3 were sequenced in samples from Patient 1's mother and Patient 2's parents.

Allele specific amplification (ASA) using the p.T58I mutation site (c.173C > T) was performed on both, Patient 1 and Patient 2, using 5'~GCAGGAGACCCTGTAGGAGGAC~3' as the forward primer and reverse primers 5'~ACATATCCTCCTGGCCAGCGG~3' (WTc.173C) and 5'~ACATATCCTCCTGGCCTGCGA~3' (MTc.173T). Both primer sets amplify a 508 bp product. ASA primers were optimized by adding 5' tails and a mismatch 5 bp from the 3' end (underlined sequence).

Mutation analysis of *SHOC2* was performed by PCR amplification of a discrete portion of *SHOC2* exon 2 containing amino acid 2. Purified PCR products were sequenced in both directions on an ABI 3130xl Genetic Analyzer and results compared to genomic *SHOC2* (NG_028922) using the Sequencher v5.0 software.

RESULTS

In our ongoing study on Costello syndrome we completed parental mutation testing on either buccal cell or PWBC-derived DNA in 59 families. We identified somatic mosaicism in the father of one proband [Sol-Church et al., 2009], and have indirect evidence for maternal gonadal mosaicism in another family [Gripp et al., 2011b].

Biologic relationships and gender assignment of Patient 1's family members were confirmed using an ABI profiling kit (results not shown). Molecular testing for *HRAS* mutations was performed clinically in Patient 1 and revealed the heterozygous *HRAS* c.173C > T mutation, predicted to result in a p.T58I amino acid change (Fig. 4). Subsequent parental studies showed a normal *HRAS* sequence for Patient 1's mother. The familial sequence change was seen in PWBC-derived DNA in his father, Patient 2. Buccal cell genomic DNA from Patient 2 also showed the sequence change in a heterozygous state (Fig. 4).

In order to further analyze the origin of this mutation, studies were performed on Patient 2's parents (Fig. 1I-1,I-2). Clinical testing performed on PWBC-derived DNA did not reveal a mutation. Similarly, buccal cell derived DNA was wildtype in both individuals (Fig. 4). Sequencing also revealed a heterozygous SNP rs41258054 G > A, located in intron 2 of *HRAS*, and shown in Figure 4. ASA using the T58I (c.173C > T) mutation site showed that SNP allele A segregated with the *HRAS* mutation in Patients 1 and 2. Presence of this SNP in the paternal grandfather (I-1; Fig. 4) in the absence of the pathogenic *HRAS* mutation documents the paternal origin of this de novo mutation in Patient 2, subsequently transmitted to Patient 1.

The *SHOC2* mutation predicting p.G2S was absent in Patient 1, 2, and 3.

DISCUSSION

Patients 1 and 2 are, to our knowledge, the second and third individuals with apparently germline-derived *HRAS* p.T58I mutations, thus furthering our understanding of the associated phenotype previously described as attenuated Costello syndrome [Gripp et al., 2008].

Attenuated Costello Syndrome Phenotype Associated With *HRAS* p.T58I

Macrocephaly is present in all three patients (Table I), this is in keeping with the relative or absolute macrocephaly seen in all 28 Costello syndrome individuals whose brain imaging studies were available for review [Gripp et al., 2010]. In Patient 1, the combination of macrocephaly and hypertrophic cardiomyopathy led to molecular diagnostic testing. Hypertrophic cardiomyopathy is the most common cardiac manifestation of Costello syndrome, occurring in 61–68% [Lin et al., 2011]. Failure-to-thrive and developmental delay resulted in testing in the previously reported Patient 3. In contrast, Patient 2 was identified through parental testing, and only in hindsight was his history of “no body fat in childhood” interpreted as possibly Costello syndrome related failure-to-thrive. Similarly, the concern for “very mild cerebral palsy” and his need for speech therapy may have been manifestations of the attenuated Costello syndrome phenotype. Patient 2's “top normal left

ventricular wall thickness” could reflect mild Costello syndrome associated hypertrophic cardiomyopathy. While we do not have formal cognitive test results it is noteworthy that Patient 1 and 2 appear to have normal cognitive function, based on the teacher’s report in Patient 1 and the successful employment history in Patient 2. In contrast, Patient 3’s cognitive function and adaptive behavior are more similar to those seen in the majority of Costello syndrome individuals with significant intellectual disability [Axelrad et al., 2009]. No patient with HRAS p.T58I shows the coarse facial features typically seen in Costello syndrome, and neither has developed papillomata or a malignant tumor. Thus, the impression of an attenuated phenotype associated with HRAS p.T58I is supported by the presentation of the two newly identified patients. Interestingly, both younger individuals have very blonde hair, described as “slow growing” in Patient 1 and as “fine” in Patient 3. Their hair resembles that seen in individuals with HRAS p.G13C [Gripp et al., 2011a], more than the tight curls typical for Costello syndrome with HRAS p.G12S. Several patients with HRAS p.G13C have been diagnosed with loose anagen hair syndrome, a condition more often associated with a specific missense mutation in SHOC2 [Cordeddu et al., 2009]. This SHOC2 mutation was absent in Patient 1, 2, and 3. Modifiers governing the expression of certain findings such as hypertrophic cardiomyopathy, intellectual disability, or loose anagen hair syndrome in the presence of HRAS p.T58I may be identified in the future.

Pathogenicity of HRAS p.T58I

It is impossible to prove that the HRAS p.T58I alteration is responsible for any particular manifestation in our patients. The general pathogenicity of this mutation is supported by the effect of the p.T58I change in the homologous KRAS, and through functional studies showing an associated gain-of-function of the abnormal protein product [Schubbert et al., 2006]. Since HRAS and KRAS share 97% amino acid sequence identity in their G-domain, and are 100% identical in regions responsible for interactions with other proteins, their structural and biochemical properties are very similar. Thus, data obtained by studying KRAS variants and their interaction with effectors and regulators likely parallels functional changes in similar HRAS variants. KRAS variants were analyzed and grouped into five classes of altered signal transduction [Gremer et al., 2011], placing KRAS p.T58I in class A, defined by the lack of major biochemical alterations compared to wild-type in vivo. However, because p.T58I results in higher downstream signaling, the authors concluded that this mutation does impact RAS function, but cannot be monitored by the current tools of RAS biochemistry [Gremer et al., 2011]. These complex functional study results further illustrate the intricate regulation of RAS signaling, disturbed by apparently subtle effects of missense mutations, which in turn affect the developing embryo and young infant in similarly complex and likely modifier dependent ways.

Reproduction in an Individual With a Germline *HRAS* Mutation

While transmission of a pathogenic *HRAS* mutation from a father with somatic mosaicism to his son with typical Costello syndrome has been reported [Sol-Church et al., 2009], vertical transmission of a pathogenic mutation from a parent with a heterozygous germline *HRAS* mutation has not been reported before. Using allelic specific amplification, we confirmed that the de novo change in Patient 2 arose from his father’s germline, consistent with the predominantly paternal origin of de novo germline *HRAS* changes [Sol-Church et al., 2006;

Zampino et al., 2007; Goriely et al., 2009]. It is likely that the attenuated phenotype associated with this particular *HRAS* change was critical for Patient 2's reproductive fitness. This transmission of a pathogenic *HRAS* mutation illustrates the need for parental testing, not only to identify carriers of low level mosaicism, but also to identify heterozygote parents and the inherent recurrence risk. Interestingly, Stark et al. [2011] recently reported the first case of vertical transmission of a *KRAS* p.Y71H change. Genetic counseling needs to take into consideration the variable phenotype associated with the different pathogenic *HRAS* mutations. Evidence for distinctive features associated with certain Costello syndrome causing *HRAS* changes, such as the dolichocilia associated with *HRAS* p.G13C [Gripp et al., 2011a], and statistically significant differences as measured by a Costello syndrome specific severity score [McCormick et al., 2012] underscore the importance of the mutation identification. This reflects the value of an "axis diagnosis" as proposed by Robin and Biesecker [2001] specifying the clinical diagnosis on axis 1, molecular change on axis 2, and environmental factors on axis 3. This approach equally values the clinical diagnosis and the specific molecular cause, rather than stressing one over the other. For the individuals reported here, the diagnosis would be specified as Axis 1: Costello syndrome, attenuated phenotype; Axis 2: *HRAS* p.T58I; and Axis 3: N/A. We anticipate that recommendations for medical care for Costello syndrome individuals will be differentiated based on the specific disease causing mutation in the future. Further genotype-phenotype correlation studies are required to clearly delineate the critical differences between the associated phenotypes, which may in turn guide recommendations for tumor screening and other preventive studies.

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FIG. 1.

Simplified pedigree, showing family members on whom molecular studies were completed. + denotes presence of the heterozygous HRAS change (c. 173C > T; p.T58I); - denotes absence of this familial HRAS change. Filled symbols represent individuals with attenuated Costello syndrome phenotype.

FIG. 2.

Images of Patient 1: A: lateral view at age 3 10/12 years, with dolichocephaly and slightly prominent and anteverted ear lobe; (B) facial findings showing a high forehead, straight hair and absence of coarse facial features at age 4 10/12 years; (C) Patient 1's palms showing slight redundancy of the soft tissue, and (D) demonstrating ligamentous laxity in his thumb at age 4 10/12 years. (E,F) Patient 2's facial features at age 41 years are essentially non-dysmorphic and not coarse; (G,H) Patient 2 showing maximal extension of his elbows.

FIG. 3. Facial photographs of Patient 3 at age 6 years (A) and 9 years (B), note tall forehead, straight hair, and absence of coarse features.

FIG. 4.

Chromatogram showing sequencing results for the regions around SNP rs41258054 (G > A) and *HRAS* c.173C > T in the respective family members. The amino acid change predicted to result from *HRAS* c.173C > T is indicated below the chromatogram. Note heterozygous presence of the SNP in individuals III-1, II-1, and I-1; and heterozygosity for *HRAS* c.173C > T change in III-1 and II-1.

TABLE I

Phenotypic Findings in Individuals with HRAS p.T58I, Compared to Those With More Common Germline HRAS Mutations

	Patient 1	Patient 2	Patient 3 [Gripp et al., 2008]	Typical Costello syndrome HRAS p.G12S	Costello syndrome HRAS p.G13C
Polyhydramnios	+/-	N/A	N/A	+	+/-
Failure to thrive	-	"no body fat"	+	+	+
Macrocephaly	+	+	+	+	+
Height less than -2SD below mean for age	-	-	-	+	+/-
Hypertrophic cardiomyopathy	+	-(top normal)	-	+	+
Other cardiac findings	Double-chambered right ventricle	-	Mitral valve prolapse	Pulmonic stenosis; ASD; multifocal atrial tachycardia	ASD; no multifocal atrial tachycardia
Limited range of motion in elbows	-	+	+	+	-
Ligamentous laxity	+/-	-	+/-	Small joints, thumb	+/-
Deep palmar creases	+/-	-	+	+	+/-
Developmental delay	-	N/A	+	ID	+/-
Hypotonia	-	N/A	+/-	+	+
Neurologic and behavior issues	Very active; poor fine motor skills	Possible ADD; hand tremors	Severe behavior problems	Sleep disturbance; anxiety	Anxiety
Eye findings	Strabismus	Myopia	Strabismus, nystagmus	Strabismus; nystagmus	Strabismus; nystagmus
Papillomata	-	-	-	+	-
Malignant tumor	-	-	-	+/-	-
Other	-	-	Pyloric stenosis	Coarse facial features; curly hair	Loose anagen hair; dolichocilia