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Phenotypic Spectrum of Costello Syndrome Individuals Harboring the Rare *HRAS* Mutation p.Gly13Asp

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Abstract

Costello syndrome is part of the RASopathies, a group of neurocardiofaciocutaneous syndromes caused by deregulation of the RAS mitogen-activated protein kinase pathway. Heterozygous mutations in *HRAS* are responsible for Costello syndrome, with more than 80% of the patients harboring the specific p.Gly12Ser variant. These individuals show a homogeneous phenotype. The clinical characteristics of the Costello syndrome individuals harboring rarer *HRAS* mutations are less understood, due to the small number of reported cases. Here we describe the phenotypic spectrum of five additional individuals with *HRAS* c.38G>A; p.Gly13Asp, including one with somatic mosaicism, and review five previously described cases. The facial and hair abnormalities of the *HRAS* p.Gly13Asp individuals differ from the typical pattern observed in those showing the common *HRAS* (p.Gly12Ser) mutation, with less coarse facial features and slow growing, sparse hair with abnormal texture, the latter resembling the pattern observed in Noonan syndrome-like disorder with loose anagen hair and individuals harboring another amino acid substitution in *HRAS* (p.Gly13Cys). Although some individuals with *HRAS* p.Gly13Asp developed papillomata and vascular proliferation lesions, no malignant tumors occurred, similar to what was reported for individuals harboring the *HRAS* p.Gly13Cys. The fact that no malignant tumors were described in these individuals does not allow definitive conclusions about the risk for cancer development. It remains to be determined if substitutions of amino acid 13 in *HRAS* (p.Gly13Asp and p.Gly13Cys) increase the risk of tumor development.

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Keywords

Costello syndrome; HRAS; mutation p.Gly13Asp; ectodermal; cancer

Introduction

A group of clinically overlapping neurocardiofaciocutaneous syndromes, known as RASopathies, are caused by heterozygous germline mutations in genes belonging to the RAS mitogen-activated protein kinase (MAPK) pathway, known for cellular proliferation, differentiation and survival [Aoki et al., 2016]. Among these disorders, Costello syndrome (CS) is a rare disorder caused by mainly de novo, heterozygous mutations in *HRAS*. It is characterized by failure-to-thrive in infancy, short stature, characteristic facial features, curly or sparse hair, papillomata, osteoporosis, cardiovascular malformations such as pulmonic stenosis and hypertrophic cardiomyopathy, and rhythm disturbances such as multifocal atrial tachycardia; neurological abnormalities including intellectual disability, a friendly outgoing personality, Chiari I malformation, syringomyelia and hydrocephalus, and a predisposition to malignancies, especially embryonal rhabdomyosarcoma [Gripp et al., 2006; Gripp et al., 2012; McCormick et al., 2013].

More than 90% of the mutations in CS patients are clustered in codons 12 and 13 (p.Gly12Ala/Ser/Val/Cys/Asp/Glu and p.G13Cys/Asp), constituting a mutational hotspot [Giannoulatou et al., 2013]. Phenotypic characterization has a bias towards individuals harboring the specific p.Gly12Ser mutation, present in approximately 85%. These individuals tend to show a homogeneous phenotype [Zampino et al., 2007]. Recently, Gripp et al. [2011] analyzed clinical data in 12 individuals showing the rare p.Gly13Cys *HRAS* mutation and observed lower rates of neurological abnormalities requiring surgery, lack of multifocal atrial tachycardia and papillomata, and long eyelashes requiring trimming, termed dolichocilia, when compared to individuals with the most frequent mutation (p.Gly12Ser). Interestingly, two of these 12 individuals showed loose anagen hair (LAH), an ectodermal condition characterized by easily pluckable, sparse, thin, and slow growing hair with abnormal hair bulb. This hair abnormality is considered a hallmark of another RASopathy – Noonan syndrome-like disorder with loose anagen hair (NSLAH) [Cordeddu et al., 2009].

We report on the clinical findings of five CS individuals harboring a rarer mutation in codon 13 (p.Gly13Asp) and review the phenotype described in another five individuals reported in the literature, in order to delineate the phenotypic spectrum.

Patients and Methods

Patient 1 was identified clinically and consent was obtained to share the information and images. To perform the molecular analysis (Sanger sequencing), the patient was enrolled in an ongoing clinical and molecular study of individuals with RASopathies, approved by the local institutional review board (Hospital das Clínicas da Faculdade de Medicina da Universidade de São Paulo – CAPpesq # 0843/08). Patients 2-5 were enrolled in an IRB approved research study (Nemours #2005-051). Molecular studies were completed in a clinical diagnostic laboratory or performed as previously published [Gripp et al., 2006].

Clinical data were obtained through parent interview and documentation was obtained as possible. Signed consent was obtained in order to publish images.

We reviewed the phenotypic description of CS individuals with the *HRAS* p.Gly13Asp mutation reported in the English language literature, as well as available photographs.

Clinical Reports

Patient 1—This 13 year-old girl (Fig.1A-D) was the first child of healthy and non-consanguineous parents. She had a younger healthy sister. As a neonate, she developed respiratory distress requiring mechanical ventilation, and hypoglycemic episodes, which resolved with glucose infusion. She was released from the hospital after 13 days. An echocardiogram revealed pulmonary hypertension, patent ductus arteriosus and patent foramen ovale. She had swallowing difficulties and slow weight gain, requiring tube feeding for 5 months. At the age 1 year, her weight was 6 kg (well below the 5th centile). She had motor developmental delay with sitting unsupported at 1 year, walking independently and saying first words at 2 6/12 years. She attended a regular school, with learning difficulties only in Mathematics. She has been evaluated by a cardiologist and her most recent echocardiogram and electrocardiogram showed no abnormalities. She never had arrhythmias. At age 3 years, she had an abnormal increase of her OFC and cranial CT scan showed hydrocephaly, requiring ventriculostomy. At age 12, brain MRI disclosed Chiari I abnormality, syringomyelia and microgyria in the occipital region. No abnormal EEG discharges have been observed. Two other surgeries were performed: Achilles tendon release at 6 and correction of the palpebral ptosis at 7 years; the latter, without resolution of the ptosis. Ophthalmologic evaluation disclosed, besides palpebral ptosis, optic nerve hypoplasia, nystagmus and myopia. She had short stature, but never received growth hormone therapy. At age 10 she developed lower limb edema and a vascular evaluation showed greater saphenous vein insufficiency. She used compression stockings. At age 9 she developed a hemangioma in her neck, which was surgically excised and, at 12, she developed perinasal and external ear canal papillomata. A rapid, progressive scoliosis developed at adolescence. She always had slow growing hair, not requiring a haircut. Long eyelashes were evident in infancy and childhood, but never required trimming. She had a hoarse voice. Ophthalmologic evaluation disclosed myopia, nystagmus, and optic nerve atrophy.

A clinical diagnosis of Costello or cardiofaciocutaneous syndrome was made at 5 years. G-banded karyotype was normal.

At the age 13, when evaluated at our center, her clinical features were compatible with the diagnosis of a RASopathy. Among the distinct disorders within this group, the presence of slow growing hair, associated with hoarse voice and Chiari I in cranial MRI in this patient, favored the diagnosis of Noonan-like/LAH. Initially the recurrent p.Ser2Gly mutation in *SHOC2* was tested and excluded. Following the investigation, the heterozygous variant c.38G>A (p.Gly13Asp) in *HRAS* was found and the diagnosis of Costello syndrome established.

Patient 2—This 28 year-old man (Fig. 1E, F) was born vaginally after an uncomplicated pregnancy to a 33-year-old mother of Japanese ancestry and a 32-year-old father of Croatian ancestry. His neonatal course was complicated by hypoglycemia requiring intravenous glucose supplementation for one week. Maternal hyperparathyroidism resulted in hypocalcemia and hypomagnesemia and patient 2 had two brief hypocalcemic seizures at days 8 and 9. No seizure occurred subsequently. Brain imaging studies including brain and spinal MRI had normal results. He had gastroesophageal reflux and feeding difficulties, but never required a feeding tube. He had failure-to-thrive and short stature. Echocardiograms showed mild pulmonary stenosis with mild right ventricular outflow tract obstruction, moderate right and left ventricular and septal hypertrophy, and a dilated aortic root measuring 3.5 cm at age 27 years. Progressive thoracic scoliosis was first noted at 3 years and resulted in spinal fusion at age 18 years and 19 years. His development was delayed and intellectual disability was diagnosed. He required special education and attended a life skills program in college. During his college years he lived in a dormitory with a resident advisor. Anxiety resulted in frequent emesis and need for medication. He was very social and enjoyed his work in a grocery store. This individual was included in the cohorts reported in Detweiler et al. [2013], McCormick et al. [2013] and Schwartz et al. [2013].

Patient 3—This 6 year-old girl (Fig. 1I, J) was born by repeat cesarean at 35 6/7 weeks gestation after a pregnancy complicated by polyhydramnios and non-immune fetal hydrops to a 45-year-old mother and a 47-year-old father. She required intubation with significant support including high frequency jet ventilation for respiratory distress and pleural effusions. Cardiac echography showed biventricular hypertrophy in the neonate and propranolol use resulted in improvement by age 18 months. Feeding difficulties resulted in placement of a gastric feeding tube, which was still used at age 6 years to supplement her fluid intake. Her motor development was delayed (Table I), but she was very social and had mild intellectual and learning disabilities. At age 6 years, foot position abnormalities and tight Achilles tendons were becoming more prominent. She had papillomata in her external ear canal and below her nose and hyperkeratosis on her soles. Hair remained very slow growing and short.

Patient 4—This girl (Fig. 1K, L) was born to 35-year-old mother and a 32-year old father of Scandinavian ancestry. The pregnancy was complicated by polyhydramnios and severe maternal pre-eclampsia, resulting in delivery by cesarean at 37 weeks gestation. Pleural effusion resolved without intervention. A facial hemangioma was treated with propranolol. Mild hypertrophic cardiomyopathy and a narrow aortic root were noted on the neonatal echocardiography but resolved without intervention. A weak suck and persistent feeding difficulties resulted in placement of a g-tube at age 13 months. Brain and spine imaging studies had normal results. Her development was delayed with standing at 22 months and walking independently at 2.5 years. At age 3 8/12 years she used short sentences with difficulties in pronunciation and remained almost exclusively G-tube fed.

Patient 5—This girl was born after a pregnancy complicated by late polyhydramnios to a 32-year-old mother with a history of childhood epilepsy and a 34-year-old father with rheumatoid arthritis, both parents were of North European ancestry. Two older and one younger sibling were in good health. Several tufts of darker hair were noted in the neonate

and considered to present hyperpigmented moles. A weight loss of about 450 gr occurred postnatally. Otherwise, she had a normal perinatal course and fed without difficulties. A cardiac murmur led to a cardiac echography at age 2 months, with normal results. A re-evaluation through cardiology at age 13 months resulted in her being discharged from further re-evaluations. At age 4 months, failure-to-thrive with difficulty gaining weight developed. Endocrinology evaluation reportedly showed delayed bone age, and a low growth hormone level at age 1 year which normalized subsequently. She never received supplemental feeding through a feeding tube, growth hormone or other medication for her mild failure to gain weight. During adenoidectomy and pressure equalizing tube placement at age 2 years a bifid uvula was noted. Her motor and cognitive development was age appropriate and she was completely toilet trained at age 22 months. At age 3 years 11 months her OFC was 51.5 cm, height 103 cm and weight 14.6 kg.

Sparse scalp hair described as brittle and slow growing, with few interspersed regions of longer, straight and typical appearing brown hair (Fig. 2A,D,E), led to dermatology evaluations and the local use of minoxidil starting at age 3 years without significant improvement of scalp hair growth. Hyperkeratosis of knees, palms and soles (Fig 2 B, C) occurred at age 3 years. Areas of irregular skin hypo- or hyperpigmentation (Fig 2E) suggested the possibility of somatic mosaicism.

Laboratory evaluations performed clinically included a chromosomal microarray and karyotype, both were nondiagnostic. Whole exome analysis on blood derived DNA samples from the patient and her parents was performed through Baylor Genetics and showed a de novo *HRAS* c.38G>A; p.Gly13Asp variant, first reported as heterozygous. Upon a requested reanalysis, the variant was reported to be present in less than 50% of alleles (variant: reference read depth 39:166) and considered mosaic. After consenting into the above referenced research study, Sanger sequencing of cheek swab derived DNA on the patient and her parents and on the patient's hair root cells was performed. Blond and brown hair roots were collected and analyzed separately.

Molecular analysis of buccal and hair root samples—Buccal and hair root samples were collected in 600ul cell lysis and processed for DNA using the Puregene Tissue kit (Qiagen) following the buccal brush protocol. Hair samples were supplemented with an additional 10ul proteinase K and 30ul 1M DTT during lysis at 55°C. Sample DNA was PCR amplified for a 649bp discrete region of *HRAS* containing exon 2 with primers 5'-ACCTGTTCTGGAGGACGGTAA-3' and 5'-CCTCTAGAGGAAGCAGGAGACA-3', using an annealing temperature of 62°C. Sanger sequencing was performed in both directions using the BigDye Terminator v3.1 Cycle Sequencing Kit (Thermo Fisher Scientific) and analyzed on an ABI3130XL Genetic Analyzer.

Sequencing results confirmed the *HRAS* mutation reported by Baylor Genetics and was consistent with somatic mosaicism in the buccal sample, which showed more wild-type alleles than mutant (Fig. 2F). DNA isolated from blond hair was heterozygous for the mutation while DNA from brown hair was only wild-type (Fig. 2F). The mutation was not found in parental samples.

Literature review—We identified five individuals reported in the literature harboring the p.Gly13Asp [Aoki et al., 2005; Digilio et al., 2008; Limongelli et al., 2008; Takahashi et al., 2013]. The female patient described by Aoki et al. [2005] was reevaluated at age 18 years [Abe et al., 2012]. Photographs were available for the patients reported by Digilio et al., 2008 (age 3 months) and Limongelli et al., [2008] (age 15 years). The clinical features were considered present in these individuals only if mentioned by the authors or depicted by photographs.

Results

Phenotypic characteristics in our cohort and the five individuals reported in literature are detailed in Table I, displaying absolute and relative frequencies of each variable. Facial photographs of the four patients reported here in detail are depicted in figure 1 and patient 5, in figure 2.

Comparing the clinical findings in individuals with *HRAS* p.Gly13Asp to those with *HRAS* p.Gly13Cys and p.Gly12Ser (Table I), we observe that several cardinal characteristics of CS showed a frequency of 50% or more in all three groups, such as prenatal (polyhydramnios) and perinatal complications (feeding problems, often requiring tube feeding), high birth weight and OCF, failure to thrive, developmental delay/hypotonia and cardiac abnormality. On the other hand, the facial features, associated with ectodermal abnormalities, especially the hair pattern, seem to be more uniform in individuals harboring mutations in the *HRAS* residue 13 (p.Gly13Cys and p.Gly13Asp), although sparse hair is frequently observed in the three groups. Interestingly, no malignant tumors have been reported in these two groups of individuals, contrasting to the increased prevalence (12%) in individuals harboring the *HRAS* p.Gly12Ser.

Discussion

RASopathies constitute a group of disorders sharing phenotypic overlap. In some of these disorders, genetic heterogeneity is evident, as is the case of Noonan syndrome, the most prevalent RASopathy associated with more than ten different genes. On the other hand, CS and NSLAH are caused by mutations in *HRAS* and *SHOC2*, respectively [Aoki et al., 2016]. Before the employment of next-generation sequencing (NGS) in a diagnostic setting, the precise delineation of the phenotype was essential for a more directed molecular analysis. In the last decade, NGS allowed the simultaneous study of gene panels, such as genes responsible for the RASopathies, identifying the molecular basis of a specific RASopathy more easily and faster. Still, phenotypic delineation of each RASopathy and its genotype-phenotype correlation is important in order to most accurately tailor medical care, therapeutic intervention and genetic counseling to the individuals needs.

The mutational spectrum in CS is narrow. The vast majority of mutations affect the residue p.Gly12, mainly p.Gly12Ser (71%), followed by p.Gly12Ala (9%) and the residue p.Gly13, especially p.Gly13Cys (6%). Other mutations reported in more than two individuals in residue 12, includes p.Gly12Cys (2.5%), p.Gly12Asp (2%) and p.Gly12Val (2%) [Giannoulidou et al., 2013]. Rarer mutations in other residues have been described

occasionally and the associated phenotype could differ from that observed in individuals harboring the p.Gly12Ser *HRAS* mutations. Amino acid substitutions in residue 12 other than the common p.12GlySer have been associated with a more severe phenotype, with lethal outcome caused by cardiac compromise, whereas mutations in other residues, such as p. Thr58 and p.Gly60, often cause an attenuated phenotype [Lorenz et al., 2012; Gripp et al., 2012; Gripp et al., 2015].

The small number of individuals harboring *HRAS* p.Gly13Asp mutation and lack of a systematically exploration of the clinical features in all reports preclude a robust analytical approach comparing our cohort and the groups of individuals harboring the other *HRAS* p.Gly13Cys and p.Gly12Ser mutations. Nevertheless, Table I shows that the patients with *HRAS* p.Gly13Asp frequently present with perinatal abnormalities (data from seven individuals, note somatic mosaicism in patient 5): Polyhydramnios and/or fetal hydrops (4/6), premature labor (3/7), high birth weight (4/7) and head circumference (3/4), hypoglycemic episodes (4/6), and feeding difficulties (5/6), commonly requiring nasogastric tube and/or gastrostomy (4/5). Perinatal abnormalities can be observed in all RASopathies, but are more prevalent in CS, with hypoglycemic episodes having the greatest specificity for CS [Myers et al., 2014]. Failure-to-thrive was universal and short stature (Z -score of $>2SD$) was observed in 6 out of 8 individuals, one of them with growth hormone deficiency. The craniofacial features described as typical in CS include relative macrocephaly, coarse facial features with curly and sparse hair, prominent epicanthal folds, long eyelashes, full nasal tip, fleshy ear lobes, and a wide mouth with full lips [Gripp et al., 2012]. We identified a different facial profile in the individuals harboring the *HRAS* p.Gly13Asp, which is most evident in individuals 1 and 3 (Fig.1), and recognizable in the photographs of the patient reported by Limongelli et al. [2008]. The craniofacial features include macrocephaly (absolute in 2/7 and of prenatal onset in 3/4); very short, uncombable, sparse hair; less coarseness of the facial features, although full lips and a wide mouth remain; wrinkled inferior palpebral skin, probably secondary to the skin laxity. The decreased coarseness of the facial features and the hair abnormality was also noted by Gripp et al. [2011] in the patients harboring the *HRAS* p.Gly13Cys mutation. Among the ectodermal findings, the hair in the typical CS individuals is usually sparse and curly. Although poor hair growth has been reported in these individuals, the extent observed here is not common [Siegel et al., 2012]. The effect of this mutation on hair is very clearly demonstrated through the analysis of the roots of the long brown “typical” appearing hair and its comparison to the short, blond and sparse appearing slow growing hair in patient 5 (Fig. 2). The typical brown hair with normal growth did not show the mutation in its roots, whereas the mutation accounts for approximately 50% of the alleles in the blond hair roots. This demonstrates the somatic mosaicism within the patient, as well the direct correlation between the hair structure and the mutation. Notably slow growing hair, not requiring haircuts, has been occasionally described in CS, mainly associated with *HRAS* p.Gly13Cys mutations [Gripp et al., 2011]. The hair was described as less curly than typical for most CS individuals, sparse and uncombable, similar to what was observed in some patients reported here. Recently, Gripp et al. [2016] described mutations in a novel gene (*PPP1CB*) associated with a Noonan-like phenotype also presenting slow growing hair, with unruly texture. Thus, slow growing hair, frequently not requiring haircuts, seems to be more widespread among the RASopathies, and not

restricted to NSLAH. Interestingly, one of our patients (patient 1) was screened initially for mutations in *SHOC2*, as the clinical impression lead to the suspected diagnosis of NSLAH. Another ectodermal finding is palmo-plantar hyperkeratosis (Fig. 1G,H; Fig 2B,C), a characteristic shared by CS and cardiofaciocutaneous syndrome. No dolichocilia, as described in patients with p.Gly13Cys *HRAS* mutations, was observed in this cohort. The cardiac abnormalities were typical of CS, with a high prevalence of hypertrophic cardiomyopathy (6/10) and arrhythmias 4/10. Global developmental delay was present in all, but patient 5, with variable cognitive impairment (5/7), ranging from mild to severe. On the other hand, CNS abnormalities requiring surgical intervention were observed only in patient 1 in our cohort. Macrocephaly was prevalent in this group, but was not associated with crowded posterior fossa. As the number of individuals that had a formal CNS evaluation is small, it is too early to conclude that this group shows fewer CNS abnormalities. It is important to consider the somatic mosaicism in patient 5 in the context of her normal developmental milestones. Among the skeletal abnormalities, severe scoliosis was observed in the two older patients in our cohort, an orthopedic problem that should be closely monitored.

A serious medical concern in CS individuals is the increased risk for cancer. *HRAS* is a proto-oncogene, in which somatic mutations usually affecting codons 12 and 13, are responsible for several types of cancers. Among the RASopathies, CS shows the highest malignancy risk, especially for embryonal rhabdomyosarcoma, leading to the establishment of a tumor screening protocol [Gripp et al., 2005; Kratz et al., 2015]. Kerr et al. [2006] showed a higher tumor risk in individuals harboring *HRAS* p.Gly12Ala, rather the commonest p.Gly12Ser. Biological assays suggest that the transforming potential of *HRAS* is dependent of the location of the amino acid substitution [Fasano et al., 1984]. Benign tumors, such as papillomas and/or vascular proliferative lesions, have been observed in five individuals harboring the p.Gly13Asp *HRAS* mutation. The prevalence of papillomas is similar to the individuals with p.Gly12Ser *HRAS*. On the other hand, no patient reported or reviewed here with a mean age of approximately 9 years, presented a malignant neoplasm, similar to the individuals harboring the p.Gly13Cys mutations in *HRAS* [Gripp et al., 2011]. Although a preliminary impression favors the possibility that individuals with mutations in residue p.Gly13 have a lower cancer risk compared to those with mutations affecting the p.Gly12, the number of reported individuals (N=21) is too small and the cohort is young to draw a definitive conclusion. Description of further patients, with long-term follow-up, is required. In the meantime, we suggest that the screening tumor protocol proposed for CS should be applied to all individuals with this diagnosis, independently of the identified mutation.

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Figure 1.

Clinical features of patients 1-4. Facial features at different ages observed in patients 1 (A – infancy, B – childhood and C and D – adolescence), 2 (E – infancy and F – adulthood) and 3 (I – infancy and J – childhood). Frontal and lateral view of patient 4 is depicted in K and L. Note the short, sparse hair with abnormal texture, especially in individuals 1 and 3, perinasal papilloma in patient 2(D), and the prominent hyperkeratosis in the hands of patient 2 (G,H).

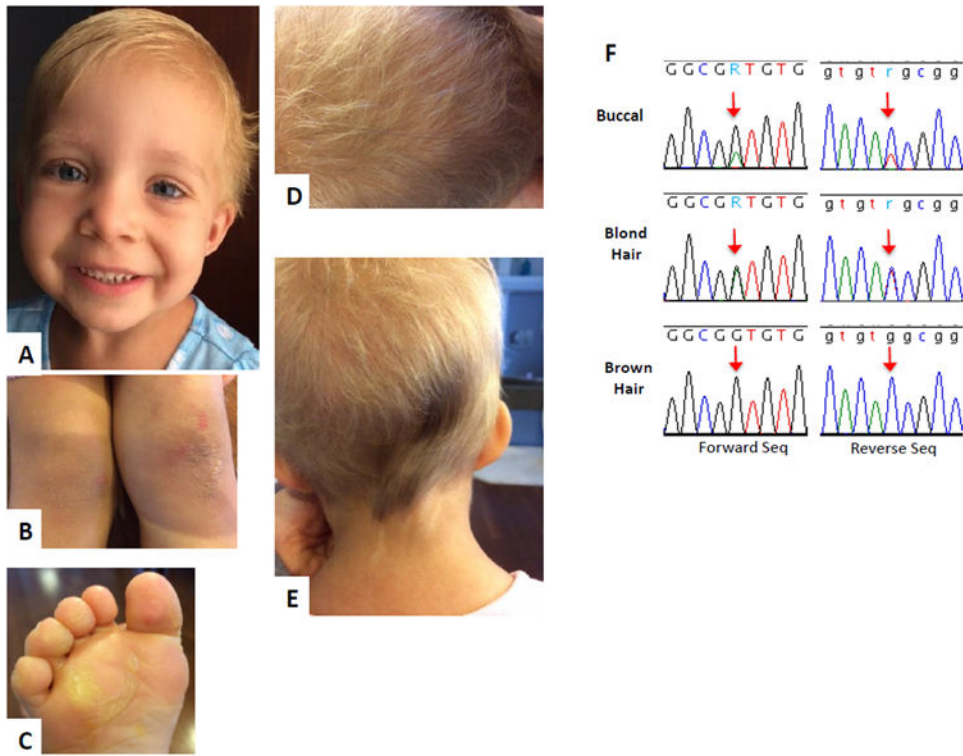


Figure 2. Clinical features of patient 5 and chromatogram of *HRAS* sequencing. A- Facial features at age 3 years 11 months, note high forehead and short blond hair, B- Hyperkeratosis on her knees, C-hyperkeratosis on her foot, D- scalp hair with a majority of short blond hairs and very few long brown hairs, E- scalp hair over her occiput showing the majority of blond hair with a patch of darker, longer and typical appearing hair, as well as streaky skin hypopigmentation compared to the relatively hyperpigmented surrounding skin of the neck. F- chromatogram of *HRAS* sequencing of the probands cheek swab derived DNA (labeled buccal) showing the more prominent wild type and the lesser mutant allele (arrow); the chromatogram for hair root cell derived DNA (labeled blond and brown, respectively) showing 50% mutant allele (blond hair, arrow) or only wild type allele (brown hair, arrow), respectively.

Table 1 Clinical findings of the individuals presented here (patients 1 to 5) and those previously described in the literature with the *HRAS* p.Gly13Asp mutation, compared to findings in individuals with the *HRAS* p.Gly13Cys and p.Gly12Ser mutations

Clinical Findings	Patient 1 Present report	Patient 2 Present report	Patient 3 Present report	Patient 4 Present report	Patient 5 Present report	Aoki et al., 2005 (COS30); Abe et al., 2012 (NS30)	Aoki et al., 2005 (COS44)	Digilio et al., 2008 (Case 3)	Limongelli et al., 2008	Takahashi et al., 2013 (patient 4)	Total <i>HRAS</i> p.G13D (N=9)	Gripp et al., 2011 <i>HRAS</i> p.G13C (N=12)	Gripp et al., 2011 <i>HRAS</i> p.G12S
Demographics													
Sex	F	M	F	F	F	F	M	M	F	F	7F/3M		
Age	13y3m	28y	6y	3y 8 mo	3y 11mo	8y3mo/18y	3mo	6mo	15y	7y			
Origin	Brazil	Japan Europe	North Europe Turkey/Lebanon	Northern Europe	North Europe	Japan	Japan	Europe	Europe	Japan			
Prenatal													
Polyhydramnios/fetal hydrops	-	-	+	+	+			+			4/6 (66%)	7/11 (64%)	7/197 (73%) ^a
Neonatal													
Gestational age (weeks)	40	38	35 6/7	37	38		32	36			Preterm 3/7 (43%)	Preterm 6/12 (50%)	preterm ^a 19/48 (40%)
BW (g)	2860 (10-25%)	3170 (50-75%)	4260 (>90%)	3220 (75-90%)	3690 (>90%)		2500 (<90%)	2970 (50-75%)			BW>75% 4/7 (57%)	BW>75% 10/12 (83%)	BW>90% ^a 28/48 (58%)
BL (cm)	45.5 (<10%)	50 (75%)	48.3 (75-90%)	48 (50%)	51 (75-90%)		43 (25-50%)				BL>75% 2/6 (33%)	BL>75% 5/12 (42%)	BOFC>90% 25/48 (52%)
BOFC (cm)	35 (50-75%)	35.5 (75-90%)	37 (>90%)	?			34 (>90%)				BOFC >75% 3/4	BOFC >75% 5/6 (83%)	BOFC>90% 25/48 (52%)
Hypoglycemia	+	+	+	-	-		+	+			4/6 (67%)	1/?	8/18* (44%)
Feeding problems	+	+	+	+	-		+	+			5/6 (83%)	12/12 (100%)	36/48 (75%) ^a
Feeding tube NG/gastrostomy	+	-	+	+			+	+			4/5 (80%)	7/12 (58%)	
Growth													
Failure to thrive	+	+	+	+	+		+	+			8/8 (100%)	11/12 (92%)	33/33 (100%)
Height (cm) (Z-score)	135.5 (13y5mo) (-3.38)	153 (-3.15)	83 (2y8mo) (-2.23)	83 (2y8mo) (-2.23)	103 (3y 11mo) (0.64)		62 (-2.35)	154 (-1.22)			6/8 (75%)	5/12 (42%)	19/19 (100%)
Short stature	+	+	+	+	-		+	-			1/3 (33%)	2/8 (25%)	14/30 (47%)
GH deficiency	NA	-			Low normal						0/5 (0%)	1/12 (8%)	10/30 (33%)
GH treatment	-	-											
Craniofacial													
Macrocephaly (absolute or relative)	absolute	+	relative	relative	-	relative	relative	relative		absolute	OFC >98% 2/7 (28.5%)	OFC >98% 6/12 (50%)	OFC >98% 9/30 (30%)
Coarse facial appearance	-	-	-	-	-		+	+		distinct	3/10 (30%)		Typical ^b 29/29 (100%)
Thick lips	+	+	+	-	-		+	+		+	8/10 (80%)		
Ophthalmologic abnormalities	+	+	+	-	-		-	-			4/7 (57%)		
Refractory error/strabismus	myopia	Strabismus	Mild myopia		NA								

Clinical Findings	Patient 1 Present report	Patient 2 Present report	Patient 3 Present report	Patient 4 Present report	Patient 5 Present report	Aoki et al., 2005 (COS30); Abe et al., 2012 (NS30)	Aoki et al., 2005 (COS44)	DiGilio et al., 2008 (Case 3)	Limongelli et al., 2008	Takahashi et al., 2013 (patient 4)	Total HRAS p.G13D (N=9)	Gripp et al., 2011 HRAS p.G13C (N=12)	Gripp et al., 2011 HRAS p.G12S
Optic nerve atrophy	+				NA						3/4 (75%)	4/12 (33%)	13/30 (43%)
Nystagmus	+	+		+	-								
Cardiac													
Structural anomaly	PDA, PFO, PH neonatal	mild PS; DAR	PDA		-	ASD	-	-	-	-	ASD 1/10 (10%)	ASD 3/12 (25%)	ASD 3/30 (10%)
Hyperrophic cardiomyopathy	-	moderate	Mild, stable to improving	mild	-	+	-	-	Severe myectomy	+	6/10 (60%)	8/12 (67%)	15/33 (45%)
Arythmias	-	-	-	-	-	-	+	+	+		4/10 (40%)	1/12 (8%)	15/33 (45%)
CNS/developmental milestones													
Hypotonia/delayed milestones	+	+	delayed	delayed	-	+		delayed	delayed		7/8 (87.5%)	12/12 (100%)	Hypotonia 22/30 (73%) ^c
Walked unassisted	2y6m	2y9m	2y	2y6m	16m	Cane-assisted gait							
First words	2y6m	2y	18m	2y	10m; Sentences at 18 months								
Intellectual disability	-	+	mild	NA	-	severe	?		moderate	+	5/7 (71%)	3/7 (43%)	11/14 (78.5%) ^d
Seizures	-	hypocalcaemia	-	-	-						1/5 (20%)	2/12 (17%)	
Hydrocephalus	+	-	-	-	NA			-	-		1/5 (20%)	1/12 (8%)	3/30 (10%)
Crowded posterior fossa	-	-	-	-	NA			-	-		0/5 (0%)	7/7 (100%)	21/22 (95%)
Chiari I	+	-	-	-	NA			-	-		1/5 (20%)	1/8 (12.5%)	7/22 (32%)
CNS surgical procedure	+	-	-	-	-						1/5 (10%)	1/12 (8%)	11/22 (50%)
Ectodermal													
Slow growing hair	+	+	+	+	+						5/5 (100%)	4/10 (40%)	
Uncombable hair	+	+	+	NA	+						4/5 (80%)	2/10 (20%)	
Sparse hair/loose anagen hair	+	slightly	+	+	+		+	-	+		8/9 (89%)	6/10 (60%)	Sparse 5/8 (62.5%) ^e
Curly hair	-	+	-	-	-		+	-	-		3/9 (33%)		7/8 (87.5%) ^f
Thin hair					+			+	+		3/3 (100%)		
Dolichocilia	-	-	-	-	-						0/5 (0%)	8/10 (80%)	
Freckles	+		+		-						2/2 (100%)		
Deep palmar/plantar creases	-		palms		-	+	?	palms			3/5 (60%)		
Palmo-plantar hyperkeratosis	+	+	+	+	+			-	+		5/6 (83%)		5/8 (62.5%)
Skeletal													
Hyperextensible fingers	-				-						1/5 (20%)		
Kyphosis/scoliosis	+ severe	+ spinal fusion	?	?	-						2/3 (66%)	2/12 (17%)	
Limited extension of the elbows	+	?	-	?	-						1/4 (25%)		
Ulnar deviation	-	-	mild	-	-						1/5 (20%)	0/12 (0%)	24/30 (80%)

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Abnormal foot position	-	?	Developing age 6 years	?	Toe walking	+	?			+	4/5 (80%)		
Tight Achilles tendon	+	?	+	+	+						4/4 (100%)	2/12 (17%)	
Osteoporosis	N/A	osteopenia						+			2/2 (100%)	1/?	
Tumors													
Papillomata	+	-	+	-	-	+	-	-	+		4/9 (44%)	0/12 (0%)	16/33 (48%)
Vascular anomalies	hemangioma	-	hemangioma	hemangioma	-	Renal angioma					3/6 (50%)	2/10 (20%)	
Cancer	-	-	-	-	-	-	-	-	-	-	0/9 (0%)	0/12 (0%)	4/33 (12%)
Other	venous insufficiency	anxiety	ovarian cyst		Bifid uvula, somatic mosaicism	galbladder; polyps		LM; EK; FLA; OSA		oral cavity anomalies			

NA: not assessed; ? unknown; PDA: patent duct arteriosus; PFO: patent foramen ovale; PH: pulmonary hypertension; PS: pulmonary stenosis; DAR: dilated aortic root; ASD: atrial septal defect; LM: laryngomalacia; EK: ectopic kidney; FLA: frontal lobe atrophy; OSA: obstructive sleep apnea; a. Myers et al., 2014; b. Kerr et al., 2006; c. Gripp et al., 2006; d. Axelrad et al., 2007; e. Morice-Picard et al., 2013