LETTER TO JMG

Myopathy caused by *HRAS* germline mutations: implications for disturbed myogenic differentiation in the presence of constitutive HRas activation

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J Med Genet 2007;44:459–462. doi: 10.1136/jmg.2007.049270

Background: Rare reports on patients with congenital myopathy with excess of muscle spindles (CMEMS), hypertrophic cardiomyopathy and variable features resembling Noonan syndrome have been published, but the genetic basis of this condition is so far unknown.

Methods and results: We analysed *PTPN11* and *RAS* genes in five unrelated patients with this phenotype, and found *HRAS* mutations in four of them. Two disease-associated mutations, G12V and G12S, have previously been observed in patients with Costello syndrome (CS), and two other mutations, E63K and Q22K, are novel. All four mutations are predicted to enhance downstream HRas signalling, suggesting that CMEMS is a developmental consequence of sustained HRas activation in skeletal muscle.

Conclusion: This type of myopathy may represent a previously unrecognized manifestation of CS. However, some patients carrying *HRAS* mutations may exhibit prominent congenital muscular dysfunction, although features of CS may be less obvious, suggesting that germline *HRAS* mutations may underlie some cases of otherwise unclassified neonatal neuromuscular disorders.

Several groups have shown that Noonan syndrome (NS; OMIM 163950) and the related disorders cardio-facio-cutaneous syndrome (CFC; OMIM 115150) and Costello syndrome (CS; OMIM 218040) are caused by activating mutations in genes of the Ras–MAPK–ERK signalling cascade (reviewed by Gelb and Tartaglia¹). These disorders share a common pattern of congenital anomalies, including typical heart defects, craniofacial dysmorphism, short stature and skeletal anomalies.^{2–4} Although NS, CFC and CS represent separate entities, they have overlapping features that may hamper the clinical distinction between these three syndromes, particularly in young infants.

There have been some reports describing newborns or young infants with congenital myopathy with excess of muscle spindles (CMEMS) associated with hypertrophic cardiomyopathy and variable NS-like facial features.^{5–7} Therefore, we conducted a study to identify the genetic basis of this condition.

PATIENTS AND METHODS

This study was approved by the University of Erlangen-Nuremberg Ethics Committee, and informed consent for genetic analyses was obtained from parents. Our study population consisted of four previously reported patients^{5–7} and one newly ascertained case. All patients presented with congenital muscular weakness.

A muscle biopsy was taken from each patient, which revealed an excess of muscle spindles with otherwise minor and non-specific myopathic changes (fig 1A). Two patients were originally recognised as having NS-like facial features; in the other three patients, nonspecific facial anomalies were recorded. The clinical findings are summarized in table 1.

DNA samples were collected from blood, buccal swabs, fibroblasts and/or frozen or paraffin-embedded tissues. Mutational screening of *PTPN11*, *NRAS*, *KRAS* and *HRAS* was carried out as described previously.⁸ Primer pairs and PCR conditions are available on request. Sequence analysis was performed by bidirectional sequencing using a commercial sequencing kit (ABI BigDye Terminator Sequencing Kit V2.1; Applied Biosystems, Weiterstadt, Germany) and an automated capillary sequencer (ABI 3730; Applied Biosystems).

RESULTS AND DISCUSSION

We discovered heterozygous missense *HRAS* mutations in four of five patients (fig 1B, table 1). The respective mutation was detected in different tissue samples in three patients: patient 1 (blood, muscle biopsy), patient 3 (muscle, liver, thymus) and patient 5 (buccal cells, muscle biopsy), thus demonstrating that the mutations probably occurred in the germline. The G12V and G12S mutations have been described previously in patients with CS: G12V was reported in a single case with a severe fatal disease course⁹ and G12S has been identified as the most common mutation in patients with CS, representing approximately 85% of disease-associated alleles.^{9–12} Two novel mutations were identified: Q22K and E63K in exons 2 and 3, respectively. Both affect evolutionarily conserved amino acid residues identical in all Ras proteins (data not shown) and are de novo mutations. Notably, a Q22K exchange in K-Ras, the G domain of which is almost identical to HRas in its sequence and function, has been found in tumors (COSMIC database; <http://www.sanger.ac.uk/genetics/CGP/cosmic/>) and previously shown to transform NIH3T3 cells.¹³ The HRas mutant, E63K, has been demonstrated to abolish guanosine triphosphatase reaction stimulated by guanosine triphosphatase activating protein¹⁴ and to potentiate cellular transformation.^{15 16} Together, these data indicate that all four mutations lead to constitutive activation of HRas. Structural analysis suggests that these gain of function effects are mediated by different mechanisms (supplementary information online; available online at <http://jmg.bmj.com/supplemental>).

We conclude that activating *HRAS* mutations cause the syndrome of CMEMS, hypertrophic cardiomyopathy and variable NS-like facial features. The morphological changes

Abbreviations: CFC, cardio-facio-cutaneous syndrome; CMEMS, congenital myopathy with excess of muscle spindles; CS, Costello syndrome; NS, Noonan syndrome

Figure 1 Myopathy with excess of muscle spindles: histological presentation and genetic alterations. (A) Representative section from the quadriceps femoris muscle of patient 5 showing an excess of muscle spindles (white circles) (H&E staining, magnification $\times 100$). (B) Genomic representation of the *HRAS* gene and location of disease-associated *HRAS* mutations. A schematic drawing showing exon–intron structure of the *HRAS* gene is shown on the top of the panel. Exons are represented by filled boxes. Coding (parts of) exons are depicted in blue and non-coding regions in grey. Exon 5 (light blue) is alternatively spliced, generating two *HRas* isoforms. Electropherograms showing *HRAS* mutations (red arrows) in exons 2 and 3 are presented in the lower part of the figure, together with the normal sequence in letters and electropherograms from a control. Red lettering indicates the codon affected by the respective mutation.

with an excess of muscle spindles probably reflect developmental consequences of sustained *HRas* activation in skeletal muscle. This muscular phenotype possibly represents a previously unrecognized manifestation of CS and may, at least in part, be responsible for the muscular weakness and congenital contractures often observed in CS patients. However, skeletal muscle morphology has yet not been studied systematically in CS patients. Nevertheless, a review of clinical data on our patients found that they have some features consistent with the diagnosis of CS, such as the craniofacial findings (originally quoted as NS-like⁵), hypertrophic cardiomyopathy, lymphoedema and neuroblastoma⁶ (table 1). Additionally, dysmorphic features of CS were less obvious, possibly due to the young age of the patients. Skin abnormalities, such as increased palmar and plantar skin, were not noted. Other symptoms known as typical but less specific early manifestations of CS, such as polyhydramnios and feeding difficulties, are readily explained by the severe neurological phenotype. In comparison with a “classic” CS phenotype, however, our patients displayed more prominent muscular problems, including marked weakness, extended congenital contractures, neonatal ventilatory insufficiency, severe myocardial involvement and early lethality

attributable to their underlying muscular disease. We therefore speculate that, particularly in early lethal cases, clinicians may classify this disorder as a neuromuscular disease rather than a variant of CS.

One patient of our small cohort (patient 2) did not have an *HRAS* mutation, suggesting genetic heterogeneity. This patient had a similar phenotype, but in contrast to the other four cases, parental consanguinity was reported,⁵ thus raising the speculation that there might be an autosomal recessive form of this disorder.

Notably, when selecting patients for this particular type of myopathy, we detected two *HRAS* mutations that have not been encountered in any of the >100 reported cases of CS. In addition, the mutation G12V was previously described in only one child, who had an early lethal course of CS.⁹ In contrast, the G12S mutation largely predominating in CS^{9–12} was found only in one patient of our cohort, thus indicating that there might be a different mutational spectrum in patients with this phenotype compared with CS patients in general. However, whether this neonatal myopathic phenotype is a distinct entity caused by specific *HRAS* alleles is undetermined at this time.

Our observations, together with the high frequency of cardiomyopathy and rhabdomyosarcoma in CS patients and *in vitro* studies showing that *Ras* activation perturbs both the biochemical and morphological differentiation of myoblasts,^{17–19} highlights the particular role of *HRas* in striated muscle development. Moreover, transgenic mice overexpressing human *HRas* (*rasH2* mice) have been found to exhibit a skeletal myopathy.²⁰ It remains to be clarified whether the excess of muscle spindles itself plays the primary pathogenetic role in the condition or it represents the most prominent morphological sign of a more complex disorder of skeletal muscle development. Our patients did not exhibit consistent findings other than muscular weakness that would allow us to draw conclusions about the nature of the myopathic process (no elevated CK, no specific electromyographic abnormalities).^{5–7}

ELECTRONIC DATABASE INFORMATION

- <http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=gene> for *HRAS* cDNA (accession numbers NM_005343 and NM_176795.2) and *HRas* amino acid (accession numbers NP_005334 and NP_789765) sequences.
- *Catalogue of somatic mutations in cancer* (COSMIC) at <http://www.sanger.ac.uk/genetics/CGP/cosmic/> for somatic mutations observed in *HRAS* and *KRAS*.

KEY POINTS

- We identified two known activating and two novel germline *HRAS* mutations in four patients with an unusual form of congenital myopathy histologically characterized by an excess of muscle spindles.
- This myopathic disorder is probably a variant of Costello syndrome (CS), and musculoskeletal problems frequently observed in patients with CS may have the same underlying pathogenesis.
- These observations provide evidence that hyperactive *HRas* perturbs skeletal muscle development and function in a specific manner.

Table 1 Clinical features of patients with congenital myopathy and excess of muscle spindles (CMEMS)

Patient no	1	2	3	4	5
Reference	de Boode <i>et al.</i> , ⁵ case 1	de Boode <i>et al.</i> , ⁵ case 2	Selcen <i>et al.</i> ⁶	Stassou <i>et al.</i> ⁷	This report
Age at last follow-up	3 weeks	10 months	14 months	7 months	13 months
Sex	Male	Female	Male	Female	Male
Birth weight	1938 g (75th centile), preterm infant	3370 g (40th centile)	Not available	2460 g (75th centile), preterm infant	2765 g (<3rd centile)
Congenital heart defect	Progressive HOCM	Severe HOCM	HOCM, chaotic atrial tachycardia	HOCM	Mild (transient) HCM
Facial anomalies	Hypertelorism, epicanthal folds, broad nasal bridge, low-set, posteriorly rotated ears	Hypertelorism, broad nasal bridge, low-set, posteriorly rotated and dysplastic ears	Bifrontal hollowing, full cheeks, triangular mouth, high-arched palate	Hypertelorism, low-set ears	Low-set ears, triangular mouth
Short stature	3rd–10th centile	10th centile	Not available	<10th centile	<1st centile
Relative macrocephaly	Yes (98th centile)	No	Increased brain weight on autopsy	Yes (>90th centile)	No
Short/webbed neck	Webbed neck	Short neck	No	Short, webbed neck	Short neck
Lymphoedema	Fetal hydrops	No	No	Congenital generalized oedema	No
Ophthalmologic problems	Cataracta incipiens	No	No	No eye contact at age 3 months	Nystagmus
Neurological signs/development	Generalized hypotonia, absence of spontaneous movements	None mentioned	Generalized muscle weakness, areflexia, joint contractures, bilateral club feet	Flaccid quadriplegia, absent reflexes, contractures of hands, bilateral talipes equinovarus	Generalized hypotonia, delayed motor development
EMG	No abnormalities	Not available	No abnormalities	Myopathic pattern	Not available
Creatine kinase	Normal	Normal	Normal	Normal	Normal
Muscle histology	Excess of small fibres (type IIC), packed or centrally located nuclei, excess of muscle spindles*. Heart: myocardial disarray	Excess of muscle spindles, excess of type IIC fibres	Fibre atrophy with rounded, small-diameter muscle fibres, excess of formations resembling muscle spindles	Abnormally numerous muscle spindles, increased fibre diameter variation	Excess of muscle spindles; fibre atrophy with rounded small-diameter muscle fibres; type II fibre predominance
Others	Polyhydramnios, hepatosplenomegaly, periventricular haemorrhage with consecutive hydrocephalus, respiratory insufficiency	Polyhydramnios, feeding difficulties, parental consanguinity	Ventilatory dependence by age 3 months, mild enlargement of cerebral ventricles neonatal neuroblastoma	Polyhydramnios, postnatal respiratory insufficiency, osteopenia, poor sucking	Intrauterine growth delay, postnatal transitory respiratory insufficiency, poor sucking requiring tube feeding
Outcome	Died at age 3 weeks	Died at age 10 months from HOCM	Died at age 14 months from cardiorespiratory failure	Died at age 7 months from respiratory failure	Alive (14 months)
HRAS mutation	c.35-36GC→TT (G12V)	Negative	c.34G→A (G12S)	c.187G→A (E63K)	c.64C→A (Q22K)

H(O)CM, hypertrophic (obstructive) cardiomyopathy; ASD, atrial septal defect.

*Small fibres and excess of type IIC fibres were reported to be consistent with immaturity and hypotonia.

Muscle spindles are stretch receptors that consist of specialized encapsulated groups of myofibres (intrafusal muscle fibres) innervated by γ and β motor neurons from the anterior horn and receiving Ia and II sensory afferents. There are indications that unique populations of myogenic cells are destined to become intrafusal fibres.²¹ Muscle spindle formation is also dependent on trophic factors released by sensory Ia afferent neurons in a subpopulation of myotubules that receive sensory myoneural contacts and are followed by γ innervation. The exact mechanisms of muscle spindle development are incompletely understood, but there is evidence that growth factors such as neurotrophin-3,²² neuregulin-1,^{23, 24} and glial cell-derived neurotrophic factor²⁵ are involved in the process. Notably, signalling induced by these molecules has been shown to be at least partly dependent upon Ras.^{26–28} These processes may be perturbed in the presence of constitutively active HRas during skeletal muscle differentiation and eventually lead to excess muscle spindle formation. Further experimental studies are needed to elucidate the precise underlying mechanisms.

Rather than defining a distinct syndrome or separate entity, we propose that the skeletal muscle phenotype referred to as CMEMS is a variant of CS and thus belongs to the spectrum of abnormalities caused by activating mutations of *HRAS*. The finding of muscle spindle excess in a patient with a congenital myopathy should prompt an analysis of the *HRAS* gene. Whether Ras pathway inhibitors that are currently being

developed as anti-cancer agents may have beneficial effects for patients with germline mutations of *HRAS* and life-threatening symptoms such as myopathy is an important research question.

ACKNOWLEDGEMENTS

We are grateful to the patients and families who participated in this study. We thank Dr Mwe Mwe Chao for help with the preparation of the manuscript. Mohammad Reza Ahmadian received a DFG grant (AH 92/1-3).

Supplementary information can be viewed on the JMG website at <http://jmg.bmj.com/supplemental>

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Competing interests: None declared.

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Received 16 January 2007

Revised 13 March 2007

Accepted 19 March 2007

Published Online First 5 April 2007

REFERENCES

- 1 **Gelb BD**, Tartaglia M. Noonan syndrome and related disorders: dysregulated RAS-mitogen activated protein kinase signal transduction. *Hum Mol Genet* 2006;**15**(Suppl):R220–6.
- 2 **Allanson JE**. Noonan syndrome. *J Med Genet* 1987;**24**:9–13.
- 3 **Hennekam RC**. Costello syndrome: an overview. *Am J Med Genet C Semin Med Genet* 2003;**117**:42–8.
- 4 **Roberts A**, Allanson J, Jadico SK, Kavanama MI, Noonan J, Opitz JM, Young T, Neri G. The cardiofaciocutaneous syndrome. *J Med Genet* 2006;**43**:833–42.
- 5 **de Boode WP**, Semmekrot BA, ter Laak HJ, van der Burgt CJ, Draaisma JM, Lommen EJ, Sengers RC, van Wijk-Hoek JM. Myopathology in patients with a Noonan phenotype. *Acta Neuropathol (Berl)* 1996;**92**:597–602.
- 6 **Selcen D**, Kupsky WJ, Benjamins D, Nigro MA. Myopathy with muscle spindle excess: A new congenital neuromuscular syndrome? *Muscle Nerve* 2001;**24**:138–43.
- 7 **Stassou S**, Nadroo A, Schubert R, Chin S, Gudavalli M. A new syndrome of myopathy with muscle spindle excess. *J Perinat Med* 2005;**33**:179–82.
- 8 **Zenker M**, Lehmann K, Schulz AL, Barth H, Hansmann D, Koenig R, Korinthenberg R, Kreiss-Nachtshiem M, Meinecke P, Morlot S, Mundlos S, Quante AS, Raskin S, Schnabel D, Wehner LE, Kratz CP, Horn D, Kutsche K. Expansion of the genotypic and phenotypic spectrum in patients with KRAS germline mutations. *J Med Genet* 2007;**44**:131–5.
- 9 **Aoki Y**, Niihori T, Kawame H, Kurosawa K, Ohashi H, Tanaka Y, Filocamo M, Kato K, Suzuki Y, Kure S, Matsubara Y. Germline mutations in HRAS proto-oncogene cause Costello syndrome. *Nat Genet* 2005;**37**:1038–40.
- 10 **Estep AL**, Tidyman WE, Teitell MA, Cotter PD, Rauen KA. HRAS mutations in Costello syndrome: detection of constitutional activating mutations in codon 12 and 13 and loss of wild-type allele in malignancy. *Am J Med Genet A* 2006;**140**:8–16.
- 11 **Gripp KW**, Lin AE, Stables DL, Nicholson L, Scott, CI Jr, Doyle D, Aoki Y, Matsubara Y, Zackai EH, Lapunzina P, Gonzalez-Meneses A, Holbrook J, Agresta CA, Gonzalez IL, Sol-Church K. HRAS mutation analysis in Costello syndrome: genotype and phenotype correlation. *Am J Med Genet A* 2006;**140**:1–7.
- 12 **Kerr B**, Delrue MA, Sigaudy S, Perveen R, Marche M, Burgelin I, Stef M, Tang B, Eden OB, O'Sullivan J, De Sandre-Giovannoli A, Reardon W, Brewer C, Bennett C, Quarell O, McCann E, Donnai D, Stewart F, Hennekam R, Cave H, Verloes A, Philip N, Lacombe D, Levy N, Arveiler B, Black G. Genotype-phenotype correlation in Costello syndrome: HRAS mutation analysis in 43 cases. *J Med Genet* 2006;**43**:401–5.
- 13 **Tsukuda K**, Tanino M, Soga H, Shimizu N, Shimizu K. A novel activating mutation of the K-ras gene in human primary colon adenocarcinoma. *Biochem Biophys Res Commun* 2000;**278**:653–8.
- 14 **Nur EKMS**, Maruta H. The role of Gln61 and Glu63 of Ras GTPases in their activation by NF1 and Ras GAP. *Mol Biol Cell* 1992;**3**:1437–42.
- 15 **Fasano O**, Aldrich T, Tamanoi F, Taparowsky E, Furth M, Wigler M. Analysis of the transforming potential of the human H-ras gene by random mutagenesis. *Proc Natl Acad Sci U S A* 1984;**81**:4008–12.
- 16 **Quilliam LA**, Hisaka MM, Zhong S, Lowry A, Mosteller RD, Han J, Drugan JK, Broek D, Campbell SL, Der CJ. Involvement of the switch 2 domain of Ras in its interaction with guanine nucleotide exchange factors. *J Biol Chem* 1996;**271**:11076–82.
- 17 **Kong Y**, Johnson SE, Taparowsky EJ, Konieczny SF. Ras p21Val inhibits myogenesis without altering the DNA binding or transcriptional activities of the myogenic basic helix-loop-helix factors. *Mol Cell Biol* 1995;**15**:5205–13.
- 18 **Lassar AB**, Thayer MJ, Overell RW, Weintraub H. Transformation by activated ras or fos prevents myogenesis by inhibiting expression of MyoD1. *Cell* 1989;**58**:659–67.
- 19 **Olson EN**, Spizz G, Tainsky MA. The oncogenic forms of N-ras or H-ras prevent skeletal myoblast differentiation. *Mol Cell Biol* 1987;**7**:2104–11.
- 20 **Tsuchiya T**, Kobayashi K, Sakairi T, Goto K, Okada M, Sano F, Sugimoto J, Morohashi T, Usui T, Mutai M. Skeletal myopathy in transgenic mice carrying human prototype c-Ha-ras gene. *Toxicol Pathol* 2002;**30**:501–6.
- 21 **Soukup T**, Pedrosa-Domellof F, Thornell LE. Expression of myosin heavy chain isoforms and myogenesis of intramuscular fibres in rat muscle spindles. *Microsc Res Tech* 1995;**30**:390–407.
- 22 **Ernfors P**, Lee KF, Kucera J, Jaenisch R. Lack of neurotrophin-3 leads to deficiencies in the peripheral nervous system and loss of limb proprioceptive afferents. *Cell* 1994;**77**:503–12.
- 23 **Hippenmeyer S**, Schneider NA, Birchmeier C, Burden SJ, Jessell TM, Arber S. A role for neuregulin1 signaling in muscle spindle differentiation. *Neuron* 2002;**36**:1035–49.
- 24 **Jacobson C**, Duggan D, Fischbach G. Neuregulin induces the expression of transcription factors and myosin heavy chains typical of muscle spindles in cultured human muscle. *Proc Natl Acad Sci U S A* 2004;**101**:12218–23.
- 25 **Whitehead J**, Keller-Peck C, Kucera J, Tauterlotte WG. Glial cell-line derived neurotrophic factor-dependent fusiform neuron survival during development. *Mech Dev* 2005;**122**:27–41.
- 26 **Besset V**, Scott RP, Ibanez CF. Signaling complexes and protein-protein interactions involved in the activation of the Ras and phosphatidylinositol 3-kinase pathways by the c-Ret receptor tyrosine kinase. *J Biol Chem* 2000;**275**:39159–66.
- 27 **Goodearl A**, Viehaver A, Vartanian T. Neuregulin-induced association of Sos Ras exchange protein with HER2(erbB2)/HER3(erbB3) receptor complexes in Schwann cells through a specific Grb2-HER2(erbB2) interaction. *Dev Neurosci* 2001;**23**:25–30.
- 28 **Yamauchi J**, Miyamoto Y, Tanoue A, Shooter EM, Chan JR. Ras activation of a Rac1 exchange factor, Tiam1, mediates neurotrophin-3-induced Schwann cell migration. *Proc Natl Acad Sci U S A* 2005;**102**:14889–94.

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