

LYMPHODYSPLASIA AND KRAS MUTATION: A CASE REPORT AND LITERATURE REVIEW

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ABSTRACT

Cardio-facio-cutaneous (CFC) syndrome is a very rare and sporadic disease whose characteristics include dysmorphic facial appearance, ectodermal abnormalities, cardiac abnormalities, growth retardation and neuro-developmental delay. This syndrome is classified as one of the RAS syndromes which are caused by altered signal transduction of the RAS/MAPK (mitogen activated protein kinase) pathway due to the mutation of genes including BRAF, MEK1/2, HRAS and KRAS. Other RAS syndromes, such as Costello syndrome and Noonan syndrome, share clinical features with CFC. Moreover, patients with the same clinical phenotype may have different molecular diagnoses. Clinical diagnosis is the starting point for correct classification. We describe the clinical data of one case of CFC syndrome, genetically determined by KRAS mutation, that involved chylothorax, lymphedema, sinus pericranii, craniosynostosis, and seizures. This is the second case report of the literature.

Keywords: Cardio-facio-cutaneous syndrome, RAS syndrome, lymphodysplasia, KRAS mutation

Cardio-facio-cutaneous syndrome (CFC,

OMIM #115150) is a very rare and sporadic disease that is characterized by dysmorphic facial appearance, ectodermal abnormalities, cardiac abnormalities, growth retardation and neuro-developmental delay (1). This syndrome, together with Costello syndrome (CS, OMIM #218040) and Noonan Syndrome (NS, OMIM #163950), is usually classified as one of the RAS/MAPK syndromes. It is caused by altered signal transduction of the RAS/MAPK pathway due to the mutation of genes including BRAF, MEK1/2, HRAS, and KRAS (2,3).

Since CFC shares clinical features with CS and NS, these syndromes have been tentatively classified on the basis of their peculiar findings (4). Recent insights obtained through RAS/MAPK molecular studies (2,3) have resulted in further disease challenges.

We describe a female CFC patient presenting a KRAS mutation which has only been reported once in the literature. She displayed peculiar clinical findings including chylothorax, ascites, lymphatic dysplasia, heart disease, sinus pericranii, cranio-synostosis, seizures, and GH deficiency.

CASE REPORT

Our patient is a 10 year old female with CFC whose KRAS gene analysis revealed a

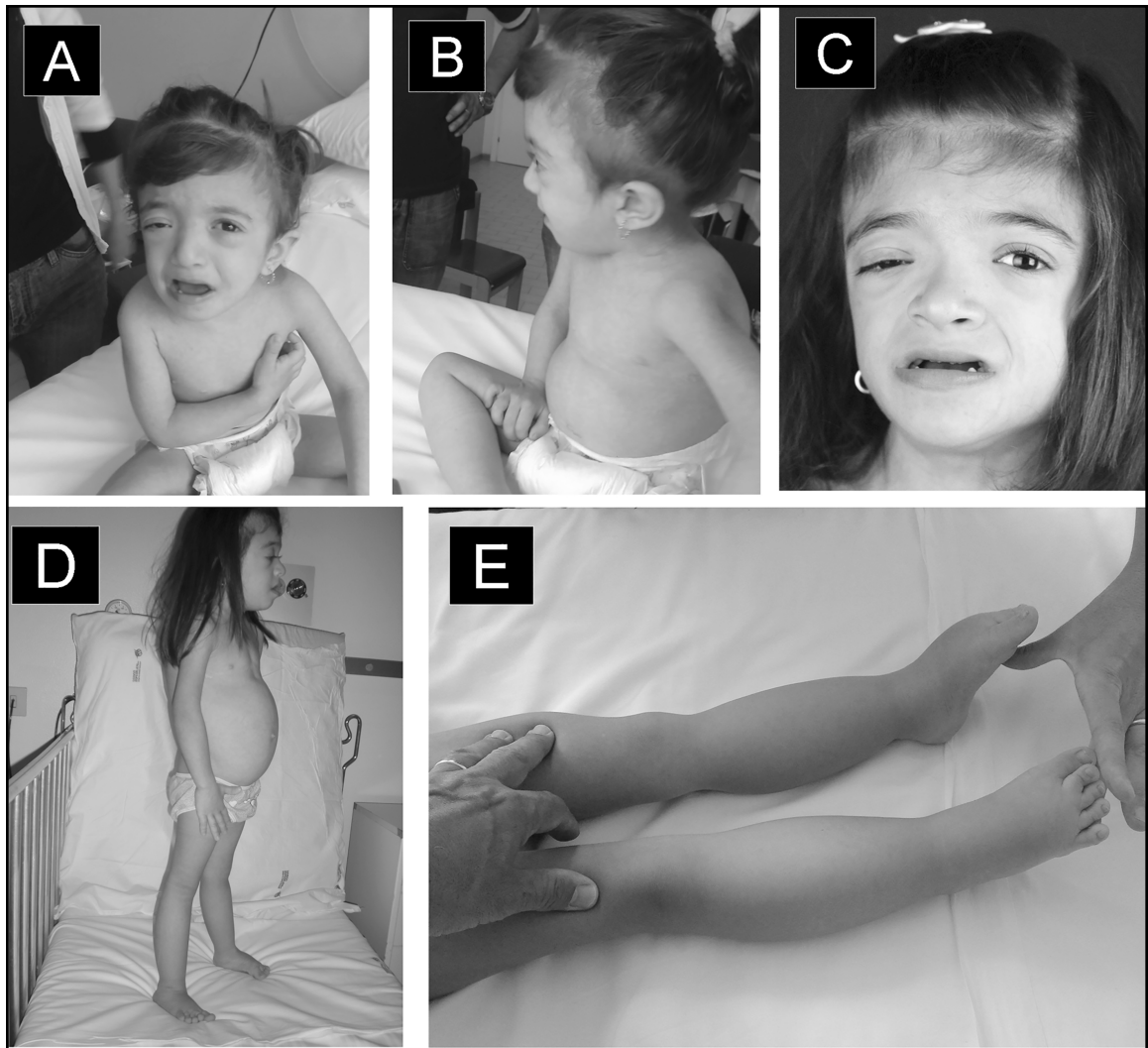


Fig. 1. Proband's features. Panels A and B: the patient at 30 months of age; Panel C. age 8 years and 11 months. First admission evaluation, peculiar craniofacial features showed "coarse face", curly hair, prominent forehead, downslanting palpebral fissures (more to the right), short nose, broad nasal tip, low-set posteriorly angulated ears, short and webbed neck, chest deformity. Panel D: At the last follow up (8 y 11 m), facial features were largely unchanged. Panel E: limb lymphedema.

101 C->G mutation with a P34R substitution. She is the first child of healthy, non-consanguineous Italian parents (mother 25 years old, father 39 years old), and has a healthy brother who is now 2 years old.

Pregnancy was complicated by polyhydramnios (diagnosed at 30 weeks). The child was delivered at 35 weeks of gestational age by cesarean section for suspected aortic

coarctation diagnosed at 33 weeks, her birth weight was 2,960 g and she showed mild respiratory distress (Apgar score 6/8). She was first admitted to our Institute at 30 months of age because of acute respiratory distress and chylothorax. Peculiar craniofacial features included coarse face, sparse and thin hair, prominent forehead, downslanting palpebral fissures (more to the right),

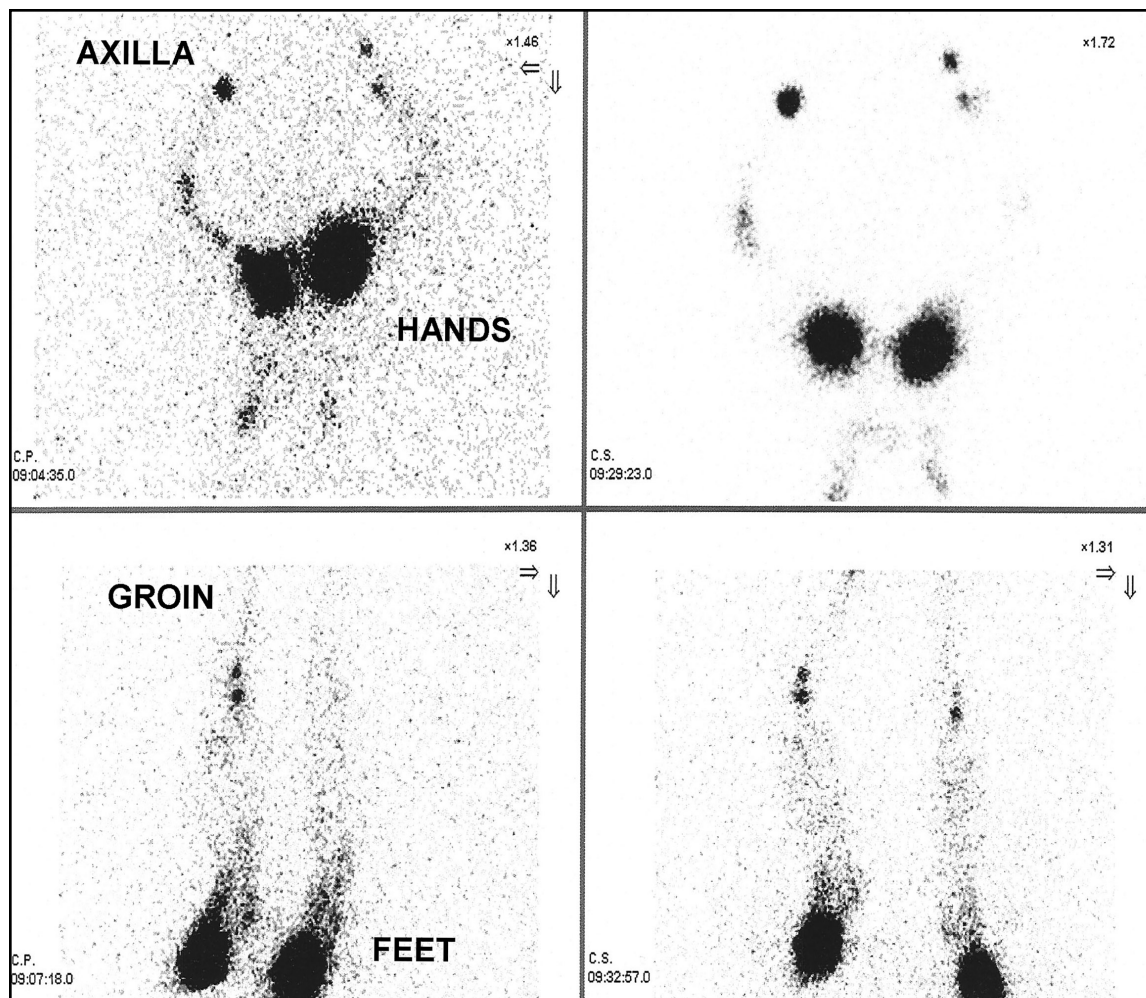


Fig. 2. Lymphoscintigraphic study. Lymphoscintigraphy displayed lack of tracer transport at the upper segments of the lower limbs.

short nose and broad nasal tip, low-set posteriorly angulated ears, short and webbed neck, chest deformity (Fig. 1, panels A, B, and D), and abnormal skin (a café au lait spot, wrinkled palms with deep palmar and plantar creases). Neurological evaluation showed hypotonia and inability to speak or walk. Sitting position was acquired by 11 months.

Karyotype test and Array-CGH were normal, PTPN11, FGFR2, and RECQL4 gene analysis were all negative.

Ultrasound cardiac evaluation showed mild to moderate tricuspid regurgitation,

dilated right cavities and pulmonary artery. Thoracic drainage analysis demonstrated typical chyle effusion. On the basis of both the clinical picture and instrumental studies, bilateral pleural decortication was performed, followed by closure of lymphatic ectasia and pleurodesis using biological glue which resulted in resolution of the chylothorax. Treatment with diuretic drugs was started and low sodium intake was ordered due to cardiac problems (not for lymphedema treatment) in an effort to decrease central venous pressure. Although fecal alpha-1-

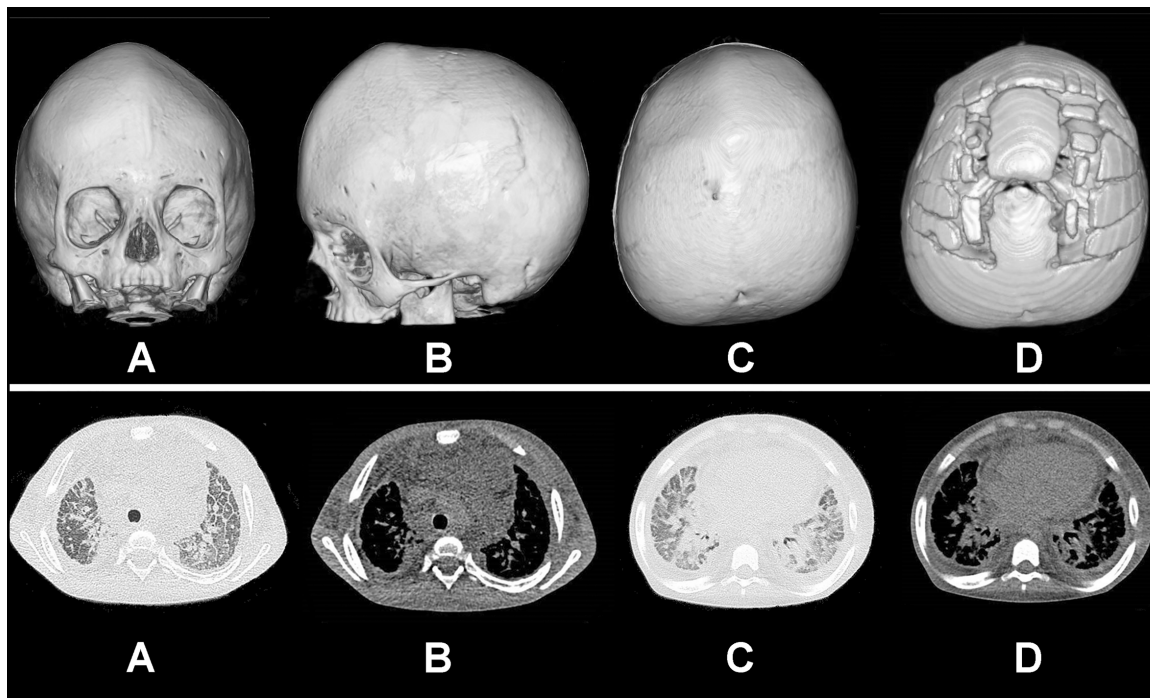


Fig. 3. Upper Panel. CT Volume rendering of the Skull. Craniosynostosis, asymmetry of the parietal bone. Panel A: frontal view. Panel B: lateral view. Panels C and D: vertex views. Sinus pericranii along the course of the sagittal suture in the lambda region. Panel D: outcome of surgery. Lower Panel. CT scan of the chest. Axial view. Panels A and B. Scans (3-mm collimator) obtained at the level of the aortic arch. Panel A (lung window): thickening of the interlobular septa with subtle bilateral parenchymal bands. Panel B (mediastinal window): peri-bronchovascular thickening most evident at the level of the lower lobes; areas of ground glass and condensation (dysventilation as well) prevailing at the lower posterior lung fields. Panels C and D. Scans (1-mm collimator) obtained at the level of the heart. Panel C: lung window. Panel D: mediastinal window. In addition to the findings already described in Panels A and B, diffuse pleural thickening (arrow) and a layer of pericardial effusion (arrowheads) are evident.

antitrypsin excretion was within normal limits, a fat-free and mild-chain triglyceride-rich (MCT) diet was started owing to the persistence of mild chylous pleural effusion; MCT diet probably prevented chylous ascites formation. Onset of lower limb lymphedema occurred at the age of 4 years and 2 months (*Fig. 1, panel E*), which was treated by physical therapy. Lymphoscintigraphy displayed reduced tracer transport to the upper segments of the lower limbs (*Fig. 2*). Thoracic CT demonstrated the presence of pulmonary lymphangiectasia (5-8) (*Fig. 3, bottom*). Brain MRI showed decreased myelination and sinus pericranii in the median-paramedian right side of the lambda

area. Craniosynostosis had been characterized by CT study at the age of 6 years and 3 months; biparietal craniotomy was then performed three months later (*Fig. 3, top*).

At the age of 7 years and 9 months, Growth Hormonal deficiency was diagnosed. At 8 years and 5 months, she had her first seizure (tonic-clonic). Treatment was started with valproic acid per os.

Her last follow-up was at the age of 8 years and 11 months: her facial features were unchanged (*Fig. 1, panel C*). Due to the persistence of seizures, Clobazam was also prescribed.

DISCUSSION

RAS proteins are central signal transduction molecules which act as molecular switches and cycle between an active GTP-bound state and an inactive GDP-bound state (9). A functional study reported that the P34R KRAS protein shows normal, intrinsic GTPase activity but reduced response to neurofibromin and to p120 GAP (10). Gremer et al (11) showed that the P34R KRAS mutation resided in a GTP-bound activated state and resisted in vitro GTP hydrolysis induced by RAS-GAP similar to the oncogenic G12V RAS mutation. Moreover, they demonstrated that the P34R KRAS mutation showed increased levels of phosphorylated MEK1/2, ERK1/2, and AKT compared to RAS wild type. As expected, accumulation of the KRAS mutant in its active state as a consequence of increased nucleotide exchange and impaired interaction with GAP sustained the activation of effectors and cellular signal transduction.

RAS/MAPK syndromes include patients who may present a very similar clinical phenotype, but a peculiar genetic asset. These conditions are caused by different molecular study results in the presence of wide phenotypic overlap. Regardless of these considerations, in the case of NS-CFC continuum, there are no obvious reasons to abandon a clinically based diagnosis (12). On the other hand, molecular definition is appropriate when the prognosis and risks for some complications (with implications for daily care) depend upon the genotype more than on the phenotype. This is typically the case for CS, for which cancer risk and the risk of arrhythmia or vascular anomalies is clearly genotype-dependent. Thus, Nava et al (13) strongly recommend limiting the diagnosis of CS exclusively to patients carrying the HRAS mutation, while patients with BRAF, KRAS, MEK1, or MEK2 mutations should be diagnosed as NS or CFC, regardless of their phenotype.

Kratz et al (14) showed that 8/19 patients with NS and myelodysplasia or Juvenile Myelo-Monocytic Leukemia (JMML) carried

a single T73I PTPN11 substitution, a mutation that confers a much higher risk of leukemia than other alterations of PTPN11, even though the developmental anomalies are similar to those observed with other mutations. Considering the disorders in terms of gene-specific phenotype may not suffice to correctly define the clinical picture and then to provide a proper prognosis. This is why Nava et al (13) proposed classifying each patient in terms of mutation-specific complications.

We performed a literature overview on this topic by carrying out a Pubmed database search covering the period between 2006, (the year of the first reports of KRAS mutation in patients with CFC) (15,16) and March 31, 2015, using these key words: “KRAS,” “Cardiofaciocutaneous syndrome,” “Noonan syndrome,” and “Costello syndrome”. We then selected the papers reporting patients with a mutated KRAS gene. Among these papers, we only took into consideration publications that included a description of patients. We finally selected 16 publications which included a total of 39 patients. We found only one paper which described a patient bearing the same mutation as our subject, i.e., P34R (15) (*Table*).

Among the 39 patients who presented KRAS mutations, patient number 5, reported by Schubbert et al (15) is the only one carrying the P34R mutation. Our patient, like the one reported by Schubbert et al, showed CFC phenotype, cardiopathy, short stature, short neck, thorax deformity, ptosis, developmental delay, skin anomalies and one café-au-lait spot. Moreover, both our patient and the patient reported by Schubbert presented severe lymphatic dysplasia (lymphedema and chylothorax). Some of the other reported subjects showed polyhydramnios, and only one patient reported by Quaio et al (17), Bertola et al (18,19), and one patient reported by Nosan et al (20) showed lymphedema. The P34R mutation would appear to play a role in the development of severe lymphodysplasia.

TABLE
Clinical Features of Patients Carrying the KRAS Mutation and Lymphatic Dysplasia

Features	Proband	Schubbert et al, 2006 (15) Case # 5
K-Ras alteration	P34R	P34R
Age at last follow up	8 years and 11 months	13 years
Sex	Female	Female
Congenital heart defect	Tricuspid regurgitation	PS, left ventricle hypertrophy
Facial features	Prominent forehead, short nose, broad nasal tip, low-set posteriorly angulated ears	Noonan
Short Stature	Yes	Yes, <1 st centile
Webbed neck	Short and webbed neck	Short neck
Thorax deformity	Yes	Broad thorax
Lymphodysplasia	Lymphedema, chylothorax, lung lymphangiectasia	Lymphedema, chylothorax
Ophthalmologic problems	Ptosis, bilateral (more to the right)	Ptosis, left
Developmental delay/mental retardation	Yes, severe	Yes, severe
Cerebral alterations	Reduced myelination and sinus pericranii	Agenesis of corpus callosum, large cerebral ventricles
Cranial alterations	Craniosynostosis	Macrocephaly
Seizures	Yes	Not reported
Skin anomalies	Curly hair, wrinkled palms, deep palmar and plantar creases	Wrinkled skin
Café-au-lait spots	One	One
Diagnosis	CFC	CFC
The table shows our patient's features compared with patient # 5 described by Schubbert et al (15). PS: pulmonary stenosis; CFC: cardio-facio-cutaneous syndrome.		

The T58I mutation is shared by patient number one reported by Schubbert et al (15) and patient number 1 reported by Kratz et al (21). Both of these subjects show Noonan phenotype, heart disease, short stature, mental retardation, and craniosynostosis.

The V14I mutation as well as Noonan phenotype and short stature are characteristics shared by: patients 2 [case 2 Zenker et al (22)], 3 and 4 reported by Schubbert et al (15), one patient reported by Croonen et al (23), one patient reported by Ko et al (24). Four cases out of 5 also have mental retardation/development delay.

CONCLUSION

Currently, the actual effect of KRAS mutations on lymphatic development can only be hypothesized. We found a strong correlation between the clinical features described by Schubbert et al (15) and those of our own patient, including features of lymphatic dysplasia. This confirms the possibility of a genotype-phenotype correlation among the RAS/MAPK syndromes, or possibly a mutation-phenotype correlation. More studies and further reports of patients would be useful to better define this condition.

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