ARTICLE

Spectrum of *MEK1* and *MEK2* gene mutations in cardio-facio-cutaneous syndrome and genotype–phenotype correlations

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Cardio-facio-cutaneous syndrome (CFCS) is a rare disease characterized by mental retardation, facial dysmorphisms, ectodermal abnormalities, heart defects and developmental delay. CFCS is genetically heterogeneous and mutations in the *KRAS*, *BRAF*, *MAP2K1* (*MEK1*) and *MAP2K2* (*MEK2*) genes, encoding for components of the RAS–mitogen activated protein kinase (MAPK) signaling pathway, have been identified in up to 90% of cases. Here we screened a cohort of 33 individuals with CFCS for *MEK1* and *MEK2* gene mutations to further explore their molecular spectrum in this disorder, and to analyze genotype–phenotype correlations. Three *MEK1* and two *MEK2* mutations were detected in six patients. Two missense *MEK1* (L42F and Y130H) changes and one in-frame *MEK2* (K63_E66del) deletion had not been reported earlier. All mutations were localized within exon 2 or 3. Together with the available records, the present data document that *MEK1* mutations are relatively more frequent than those in *MEK2*, with exons 2 and 3 being mutational hot spots in both genes. Mutational analysis of the affected *MEK1* and *MEK2* exons did not reveal occurrence of mutations among 75 patients with Noonan syndrome, confirming the low prevalence of *MEK* gene defects in this disorder. Clinical review of known individuals with *MEK1/MEK2* mutations suggests that these patients show dysmorphic features, ectodermal abnormalities and cognitive deficit similar to what was observed in *BRAF*-mutated patients and in the general CFCS population. Conversely, congenital heart defects, particularly mitral valve and septal defects, and ocular anomalies seem to be less frequent among *MEK1/MEK2* mutation-positive patients.

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Introduction

Cardio-facio-cutaneous syndrome (CFCS) is a sporadic multiple congenital anomaly/mental retardation disorder characterized by failure to thrive, severe feeding problems, developmental delay, reduced growth, distinctive dysmorphic face, ectodermal abnormalities and heart defects.¹ CFCS is genetically heterogeneous, with mutations in the

KRAS, *BRAF*, *MAP2K1* (*MEK1*) and *MAP2K2* (*MEK2*) genes occurring in approximately 62.5–90% of affected individuals.^{2–5} CFC disease genes encode for signal transducers participating in the RAS–MAPK pathway, which is known to play a crucial role in development and oncogenesis.⁶ Remarkably, dysregulated function of these and other proteins implicated in this signaling cascade has been recently shown to represent the molecular cause underlying a group of disorders clinically related to CFC, including Noonan syndrome (NS), LEOPARD syndrome and Costello syndrome.^{5,7–14} On the basis of the shared pathogenic mechanism and clinical overlap, these disorders are now grouped in the recently coined neuro-CFC family.¹⁵

In approximately 20% of cases, CFC is caused by mutations affecting the *MEK1* and *MEK2* genes.^{3,4,16–18} *MEK1* and *MEK2* are dual-specificity protein kinases, which work as effectors of the serine/threonine kinase RAF family members by phosphorylating and activating ERK proteins.¹⁹ MEK proteins are composed of a regulatory domain at the N-terminus, a single kinase domain and a C-terminal

tail (Figure 1). Available mutation data support the view that the vast majority of CFC-causing mutations are missense changes affecting residues located in the regulatory region and the N-terminal portion of the catalytic domain. Until now, mutations in the *MEK1* and *MEK2* genes have been detected in 58 patients with CFC, and in three patients exhibiting a phenotype apparently fitting NS.¹⁶ Although these findings would be suggestive of phenotypic heterogeneity, the clinical features associated with mutations in the *MEK1* and *MEK2* genes have not been delineated exhaustively. The presence of normal cognitive abilities in a minor percentage of individuals seems to suggest that *MEK1/MEK2* mutation-positive patients show a milder clinical phenotype than what is observed in patients with *BRAF* mutations or in the general CFC population.

To further characterize the molecular spectrum of *MEK1* and *MEK2* gene mutations and the phenotypic features associated with these molecular defects, we screened cohorts including patients with CFC and NS, and carried out a detailed clinical characterization of mutation-

Figure 1 *MEK1* and *MEK2* mutations in cardio-facio-cutaneous syndrome. Genomic characterization of the *MEK1* and *MEK2* genes. Exons are represented by bars and numbered. Orange boxes indicate regions coding for the protein kinase domain (residues 68–361 in *MEK1* and 72–369 in *MEK2*). Earlier identified mutations are represented as blue squares,^{2–4,16–18,20,21} whereas those identified in this study are colored yellow. Colour figure can be viewed in the online issue, available at www.nature.com/ejhg.

positive cases. To explore possible genotype–phenotype correlations, we also compared the phenotype of *MEK1/MEK2* mutation-positive CFC cases with those associated with *BRAF* mutations, as well as with the general CFC population.

Material and methods

Patients

Two cohorts were included in the study. A first group included 33 sporadic patients with a full-blown CFC phenotype. Clinical diagnosis of CFC was based on the presence of clinical features such as craniofacial dysmorphisms, cardiac anomalies, characteristic ectodermal and musculoskeletal anomalies, postnatal growth deficiency, hypotonia and developmental delay.¹ A second group comprised 75 patients with NS or exhibiting a phenotype that was suggestive for the disorder. Informed consent was obtained for all the patients included in the survey. Mutations in the *PTPN11*, *SOS1* and *RAF1* genes had been earlier excluded by denaturing high-performance liquid chromatography (DHPLC) analysis and by direct sequencing of whole coding regions in all cases. Although no *KRAS* mutations have been detected, 15 missense *BRAF* mutations were identified in 17 CFC patients (17/33) (our unpublished data).

Mutation analysis

The patient's genomic DNA was extracted from circulating leukocytes according to the standard procedures. Mutation screening of the entire *MEK1* and *MEK2* coding regions and of the flanking intron–exon boundaries (CFC cohort), or *MEK1* exons 2, 3 and 6, and *MEK2* exons 2, 3 and 7 (NS cohort), was carried out by DHPLC analysis (3100 WAVE DNA fragment analysis system, Transgenomic). Primer pairs, PCR and DHPLC analysis settings are available upon request. PCR products showing an abnormal DHPLC profile were reamplified and processed through PCR purification columns (Qiagen, Hilden, Germany) and sequenced using the ABI BigDye Terminator Sequencing Kit v.1.1 (Applied Biosystems, Foster City, CA).

Results

Mutation analysis

The molecular analysis identified five mutations in 6 out of 33 patients with CFC, whereas no *MEK1* or *MEK2* gene variant was observed in the NS cohort (Figure 1, Table 1). Among the identified mutations, three missense changes, including the novel L42F and Y130H amino acid substitutions, affected *MEK1* and occurred in four patients. One missense change and an earlier unreported short in-frame deletion (K63_E66del) in *MEK2* gene were identified in two patients. Parental DNAs were available for five of the six sporadic cases, and genotyping confirmed the *de novo* origin of each mutation (Table 1).

MEK1 and MEK2 mutation-associated features

All patients with a mutated *MEK1* or *MEK2* allele exhibited the full-blown phenotype of the disorder (Table 2).¹ Pregnancies were complicated by polyhydramnios in four cases. Weight at birth was over the 97th percentile in two of them. Recurrent facial features included macrocephaly, downslanting palpebral fissures, hypertelorism, epicanthal folds, palpebral ptosis, low set ears with thickening of the helix, flat nasal bridge, thick lips and macrostomia (Table 2, Figure 2). Ectodermal anomalies were detected in all these patients, with sparse eyebrows (6/6), sparse and/or curly hair (4/6) and keratosis pilaris (2/6) occurring most commonly. Similarly, short stature was a common feature (4/6), but the bone age was delayed in only one patient. Cardiac defects were observed in four cases, with pulmonary valve stenosis documented in three patients, and mild hypertrophic cardiomyopathy occurring in one patient. Tetralogy of Fallot with abnormal pulmonary venous return was diagnosed in one patient. Ocular abnormalities, in particular strabismus and cataract, were observed in single patients. All patients showed a variable degree of mental retardation, with the exception of patient no. 4, who was evaluated in the neonatal period (Table 2).

Discussion

Consistent with the available records,^{2–4,16–18,20–22} these data indicate that *MEK1* and *MEK2* gene mutations

Table 1 *MEK1* and *MEK2* gene mutations identified and cohorts screened in this study

Disorder and gene	No. of cases	Exon	Nucleotide change	Amino acid change	Status
CFC	33				
MEK1	1	2	c.124C>T	Leu42Phe	De novo
	2	3	c.389A>G	Tyr130Cys	De novo
	1	3	c.388T>C	Tyr130His	De novo
MEK2	1	2	c.170T>C	Phe57Cys	Controls
	1	2	c.186_197del	Lys63_Glu66del	De novo
NS	75 ^a				

^aThis cohort was screened for *MEK1* exons 2, 3 and 6 and *MEK2* exons 2, 3 and 7.

Table 2 Clinical features of MEK1/MEK2 mutation-positive CFCS patients included in the study, and comparison with published data

Features	CFCS patients with MEK1/MEK2 gene mutations from this study						Present cohort	Literature ^{a,b}	Total CFCS with MEK mutations (%)	BRAF-positive patients ^c (%)	CFCS index ^d (%)
Patients	N.1	N.2	N.3	N.4	N.5	N.6	6	55	61	32	54
Gene	MEK1	MEK1	MEK1	MEK1	MEK2	MEK2					
Nucleotidic/amino acid change	c.124C>T L42F	c.389A>G Y130C	c.389A>G Y130C	c.388T>C Y130H	c.170T>C F57C	c.186_197del K63_E66del	4 MEK1/2 MEK2	37 MEK1/18 MEK2	41 MEK1/20 MEK2	32 BRAF	
Sex	F	F	F	F	M	M	2 M, 4 F/6	7 M, 10 F/17	9 M, 14 F/23	14 M, 18 F/32	
Years	6.6	5.11	9y	Neonate	4	2.5	4.6/6	7.5/26	6.9/32		
Polyhydramnios	Yes	No	Yes	Yes	Yes	No	4/6	16/26	20/32 (62)	19/32 (60)	
Fetal macrosomia	No	No	Yes	Yes	No	No	2/6	11/19	13/25 (52)	31/32 (97)	
Short stature (<3rd centile)	Yes	Yes	Yes	No	No	Yes	4/6	26/32	30/38 (79)		78
Macrocephaly	Yes	Yes	Yes	Yes	Yes	Yes	6/6	20/28	26/34 (76)		78
Failure to thrive	Yes	Yes	Yes	NA	Yes	Yes	4/4	25/31	29/35 (83)	19/30 (63)	
<i>Facial anomalies</i>	Yes	Yes	Yes	Yes	Yes	Yes	6/6	30/30	36/36 (100)		
Prominent supraorbital ridge	No	No	No	No	No	No	0/6	9/14	9/20 (45)		
Sparse eyebrows	Yes	Yes	Yes	Yes	Yes	Yes	6/6	29/32	35/38 (92)	24/29 (83)	63
Downslanting palpebral fissures	Yes	Yes	Yes	Yes	Yes	Yes	6/6	15/23	21/29 (72)	8/17 (47) ^e	61
Hypertelorism	Yes	Yes	Yes	Yes	Yes	Yes	6/6	17/24	23/30 (77)	12/17 (71) ^e	46
Epicanthal folds	Yes	No	Yes	Yes	Yes	No	4/6	10/20	14/26 (54)		59
Palpebral ptosis	Yes	Yes	Yes	Yes	Yes	Yes	6/6	12/21	18/27 (67)		52
Low-set ears/thickened helix	Yes	Yes	Yes	Yes	Yes	Yes	6/6	21/24	27/30 (90)		76
Large, thick ear lobe	No	Yes	Yes	Yes	Yes	Yes	5/6	16/18	21/24 (87)		30
Flat nasal bridge	Yes	Yes	Yes	Yes	Yes	Yes	6/6	4/6	10/12 (83)		89
Thick lips/macrostomia	Yes	Yes	No	No	Yes	Yes	4/6	7/13	11/19 (58)	8/17 (47) ^e	
Long/prominent philtrum	No	No	Yes	Yes	No	Yes	3/6	13/20	16/26 (62)		39
Micrognathia	Yes	Yes	No	Yes	No	No	3/6	8/17	11/23 (48)		24
<i>Congenital heart defect</i>	Yes	Yes	No	Yes	No	Yes	4/6	21/33	25/39 (64)	23/32 (72)	78
Pulmonary valve stenosis	Yes	Yes	No	No	No	Yes	3/6	14/36	17/42 (40)	11/27 (41)	
Pulmonary valve dysplasia	No	No	No	No	No	Yes	1/6	0/27	1/33 (3)		
Supravalvular PVS	No	No	No	No	No	Yes	1/6	0/27	1/33 (3)		
Atrial/ventricular septal defect	No	No	No	No	No	No	0/6	7/32	7/38 (18)	9/27 (33)	
HCM	No	No	No	No	No	Mild	1/6	13/36	14/42 (33)	11/28 (40)	
Other		ST		AVPR ToF	No	No	1/6 AVPR, ToF; 1/6 ST	1/27 BAV 1/27 SVT		9/27 MVD	
<i>Skin anomalies</i>	Yes	Yes	Yes	Yes	Yes	Yes	6/6	37/38	43/44 (98)		
Keratosis pilaris facies	No	No	No	No	Yes	Yes	2/6	12/26	14/32 (44)	16/22 (73)	37
Cavernous angiomata	No	No	Yes	No	No	No	1/6	3/6	4/12 (33)	11/27 (41)	24
Nevi	No	No	Yes	No	No	No	1/6	10/28	11/34 (32)	23/31 (74)	
Café-au-lait spots	Yes	No	No	No	No	No	1/6	4/24	5/30 (17)	7/27 (26)	9
Sparse hair	Yes	Yes	No	No	Yes	Yes	4/6	29/43	33/49 (67)	24/29 (83)	85
Curly hair	Yes	Yes	Yes	Yes	No	No	4/6	32/37	36/43 (84)	29/32 (91)	72
Others	No	No	No	No	No	No		3/15 PP; 7/26 EZ; 8/24 DePP; 3/3 ES, 3/3 DS		5/21 EZ	
<i>Musculoskeletal abnormalities</i>	Yes	Yes	Yes	Yes	Yes	Yes	6/6	26/28	32/34 (94)		
Short webbed neck	Yes	Yes	Yes	Yes	Yes	Yes	6/6	20/21	26/27 (96)		50
Pectus excavatum	No	No	Yes	Yes	Yes	Yes	4/6	19/25	23/31 (74)	14/23 (61)	
Joint limitations/hyperextension	No	No	Yes	No	No	No	1/6	13/20	14/26 (54)	14/24 (58)	13
Others	No	PC	No	No	No	No	1/6 PC	1/3 DSD; 1/5 UD			

Table 2 (Continued)

Features	CFCS patients with MEK1/MEK2 gene mutations from this study						Present cohort	Literature ^{a,b}	Total CFCS with MEK mutations (%)	BRAF-positive patients ^c (%)	CFCS index ^d (%)
Patients	N.1	N.2	N.3	N.4	N.5	N.6	6	55	61	32	54
Gene	MEK1	MEK1	MEK1	MEK1	MEK2	MEK2					
Nucleotidic/amino acid change	c.124C>T L42F	c.389A>G Y130C	c.389A>G Y130C	c.388T>C Y130H	c.170T>C F57C	c.186_197del K63_E66del	4 MEK1/2 MEK2	37 MEK1/ 18 MEK2	41 MEK1/ 20 MEK2	32 BRAF	
Cubitus valgus	No	No	Yes	Yes	No	No	2/6	1/2	3/8 (37)		
Thoracic anomalies	Yes	Yes	Yes	Yes	Yes	Yes	6/6	18/23	24/29 (82)		
Undescended testes					Yes	Yes	2/2	3/3	5/5 (100)	4/14 (29)	43
Neurological	Yes	Yes	Yes	NA	Yes	Yes	5/5	42/44	47/49 (96)		
Mental retardation	Yes	Yes	Yes	NA	Yes	Yes	5/5	38/41	43/46 (93)		91
CNS abnormality	No	No	No	No	NA	No	0/5	7/10	7/15 (47)		
Seizures	No	No	No	NA	No	No	0/5	16/39	16/44 (36)	15/31 (48)	15
Development delay	Yes	Yes	Yes	NA	DBA	Yes	5/5	38/40	43/45 (96)		81
Hypotonia	Yes	Yes	Yes	Yes	Yes	Yes	6/6	34/39	40/45 (89)	29/31 (94)	28
Ocular abnormalities	No	No	No	No	Yes	Yes	2/6	17/30	19/36 (53)		
Photophobia	No	No	No	NA	No	No	0/5	3/6	3/11 (27)		
Nystagmus	No	No	No	No	Yes	No	1/6	6/14	7/20 (35)		30
Strabismus	No	No	No	No	No	No	0/6	17/31	17/37 (46)	25/30 (83)	33
Myopia	No	No	Astigmatism	No	No	No	0/6	6/21	6/27 (22)	18/23 (78)	
Optic nerve hypoplasia	No	No	No	No	No	No	0/6	3/13	3/19 (16)	9/20 (45)	
Cataract (bilateral)	No	No	No	No	No	Yes	1/6	1/7	2/13 (15)		
Others								1/20 HB; 3/3 HT			

Abbreviations: AVPR, partial anomalous venous pulmonary return; BAV, bicuspid aortic valve; DBA, delayed bone age; DPP, deep palmar/plantar creases; DS, dry skin; DSD, diffuse skeletal demineralization; ES, excessive sweating; EZ, eczema; HB, hepatoblastoma; HT, heat intolerance; MVD, mitralic valve dysplasia; NA, not available; PC, pectus carinatum; PP, papilloma;

ST, sinus tachycardia; SVT, supraventricular tachycardia; ToF, tetralogy of Fallot; UD, ulnar deviation.

^aThis column includes three cases with phenotype apparently fitting NS (see Nava *et al.*¹⁶).

^bReferences: Rodriguez-Viciano *et al.*⁴ Niihori *et al.*² Nava *et al.*¹⁶ Narumi *et al.*³ Yoon *et al.*²⁰ Armour *et al.*²² Gripp *et al.*²¹ Schulz *et al.*¹⁷ Nyström *et al.*¹⁸

^cAccording to Armour and Allanson.²²

^dAccording to Kavamura *et al.*²³

^eAs facial dysmorphisms are not reported among Armour and Allanson's cohort, we compared the MEK-mutated CFCS cohort with our unpublished clinical data of 17 CFC BRAF-mutated positive patients.

Figure 2 Dysmorphic features of subjects heterozygous for a *MEK1* or *MEK2* mutation. Pictures of cases N.2 (a and c) and N.6 (b and d). Note the thin and sparse hair (b), curly hair (a and c), absence of eyebrows (a, b and c), hyperkeratosis and wrinkled palm with wide squared tips (d).

underlie a significant fraction of CFCS, accounting for approximately 20% of cases. Of note, *MEK1* mutations seem to recur more frequently compared with *MEK2* defects (observed ratio 2:1), but the molecular basis for such a differential prevalence of mutations requires further investigation. Although the majority of mutations are missense changes, ours and earlier data indicate that short in-frame deletions occur in approximately 5% of cases. In our cohort, all mutations were found to affect exons 2 and 3 of both genes, confirming earlier observations indicating that the regulatory region and the adjacent N-terminal portion of the catalytic domain are mutation hot spots, possibly because of the specific perturbing effect of mutations on the autoinhibitory mechanism controlling

protein activation. Nevertheless, based on the identification of rare mutations outside this region (E203Q in *MEK1*¹⁸ and K273R in *MEK2*³), *MEK1* and *MEK2* mutation screening should not be limited to these exons. Our data also confirmed that mutations affecting the tyrosine residue at codon 130 of *MEK1* represent the most common event underlying CFCS, and that mutations involving residue F57 are the most frequent lesions occurring in *MEK2*. The perturbing role of mutations affecting these residues has been shown.⁴ Of note, mutations affecting the corresponding residues in the paralogs (F53 in *MEK1* and Y134 in *MEK2*) also recur in CFCS, further supporting the relevance of these amino acids in the modulation of protein function. The novel C-to-T transition at position

c.196 (L42F) in *MEK1* and the in-frame c.186_197 (K63_E66 deletion) in *MEK2* involve residues not found to be mutated so far. Two lines of evidence support their pathogenicity. Firstly, their *de novo* origin was shown for both the mutations. Secondly, multiple mutations have been documented to affect adjacent and equivalent residues in both the proteins.

To delineate more precisely the phenotypic features of patients with *MEK1* and *MEK2* mutations and to explore possible genotype–phenotype correlations, the clinical data of the present cohort were compared with those referred to patients with *MEK* or *BRAF* gene mutations recently provided by Armour and Allanson [21] and the CFCS cohort originally described by Kavamura *et al*²³. From the analysis it is apparent that *MEK1* and *MEK2* mutation-positive patients from the present cohort show a ‘classical’ CFCS phenotype, particularly regarding presence of dysmorphisms, short stature, ectodermal features and cognitive deficits (Table 2, Figure 2). We also observed that patients with *MEK* and *BRAF* mutations share most of the clinical features, although some differences are recognizable. Specifically, downslanting palpebral fissures is more characteristic of patients with *MEK* mutation than of *BRAF*-positive patients ($P < 0.001$). Conversely, ocular abnormalities and the presence of naevi are less common than in the *BRAF*-positive cohort ($P < 0.001$) (Table 2). All of our patients and 77% of all *MEK*-positive patients show hypertelorism, a figure similar to that observed in *BRAF*-mutated patients (71%), but significantly higher ($P < 0.01$) from the prevalence observed in the Kavamura’s²³ cohort (46%). These results are in disagreement with earlier studies pointing to a lower prevalence of hypertelorism among *MEK1/MEK2* mutation-positive patients (73%) than of those with *BRAF* mutation (91%).¹⁶ Cryptorchidism has been described in the five male patients heterozygous for a *MEK1/MEK2* mutation, although additional observations are necessary to support this association.

Nava *et al*¹⁶ suggested that patients heterozygous for a *MEK1/MEK2* mutation show a milder phenotype than those with a *BRAF* mutation who display a higher frequency of mental retardation. In contrast, all *MEK1/MEK2* mutation-positive patients in this cohort and 93% of all *MEK1* or *MEK2* mutation-positive patients exhibited developmental delay, which is similar to that observed among *BRAF* mutation-positive patients (Table 2).

Congenital heart defects (CHDs) were slightly less common in patients with mutated *MEK1* or *MEK2* (64%) compared with those with a mutation in *BRAF* (72%),²² confirming the data reported by Nava *et al*.¹⁶ Of note, mitral valve and septal defects seem less frequent in patients with *MEK1/MEK2* mutations than in those with *BRAF* mutations (Table 2). Interestingly, one patient with *MEK1* mutation had tetralogy of Fallot with abnormal pulmonary venous return. Tetralogy of Fallot is included among CHDs reported in clinical series of patients with

NS,²⁴ although molecular studies have documented this CHD only in cases with *RAF1* mutations.^{10,11} To our knowledge, the association of tetralogy of Fallot and abnormal pulmonary venous return, which is suggestive of a partial expression of a cardiac laterality defect, has never been described in patients with mutations in the Ras/MAPK pathway.

The risk of cancer is reported to be rare in CFCS patients.² None of our patients showed malignancy, and only a single patient with *MEK1* mutation (Y130C) has been reported with a hepatoblastoma.²⁵ Consistent with this finding, no somatic mutation in *MEK1* gene has been observed in hepatoblastoma or in any other solid tumors (<http://www.sanger.ac.uk/genetics/CGP/cosmic/>). Similarly, the somatically acquired V600E amino acid substitution of *BRAF* occurs at high frequency in cancer, but it has not been observed among patients with CFCS. Accordingly, only two CFCS patients with *BRAF* mutations have been reported with malignancy.^{4,26,27} These data would indicate that CFCS syndrome should not be regarded as a cancer-predisposing condition.

In conclusion, our data confirm that mutations in *MEK1* gene are more frequent than those in *MEK2*. CFCS patients heterozygous for *MEK1* or *MEK2* mutation genes display a phenotype well overlapping that observed in the general CFCS population and in patients with *BRAF* mutations, including dysmorphic facial features, mental retardation, development delay and ectodermal abnormalities. However, the frequency of CHDs, particularly mitral valve and septal defects, and of ocular anomalies seems lower among *MEK1/MEK2* mutation-positive patients compared with patients carrying a *BRAF* gene mutation.

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