Clinical report
Cardiofaciocutaneous syndrome, a Noonan syndrome related disorder: Clinical and molecular findings in 11 patients

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Genotype–phenotype correlation

A B S T R A C T
Objectives: To describe 11 patients with cardiofaciocutaneous syndrome (CFC) and compare them with 130 patients with other RAS-MAPK syndromes (111 Noonan syndrome patients [NS] and 19 patients with LEOPARD syndrome).

Patients and methods: Clinical data from patients submitted for genetic analysis were collected. Bidirectional sequencing analysis of PTPN11, SOS1, RAF1, BRAF, and MAP2K1 focused on exons carrying recurrent mutations, and of all KRAS exons were performed.

Results: Six different mutations in BRAF were identified in 9 patients, as well as 2 MAP2K1 mutations.

Short stature, developmental delay, language difficulties and ectodermal anomalies were more frequent in CFC patients when compared with other neuro-cardio-faciocutaneous syndromes (p < .05). In at least 2 cases molecular testing helped reconsider the diagnosis.

Discussion: CFC patients showed a rather severe phenotype but at least one patient with BRAF mutation showed no developmental delay, which illustrates the variability of the phenotypic spectrum caused by BRAF mutations. Molecular genetic testing is a valuable tool for differential diagnosis of CFC and NS related disorders.

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Síndrome cardiofaciocutáneo, un trastorno relacionado con el síndrome de Noonan: hallazgos clínicos y moleculares en 11 pacientes

R E S U M E N
Objetivos: Describir los hallazgos clínicos y moleculares de 11 pacientes españoles con síndrome cardiofaciocutáneo (CFC), y compararlos con una serie de 130 pacientes con otros trastornos neurocardiofaciocutáneos (111 pacientes con síndrome de Noonan [SN] y 19 con síndrome LEOPARD).

Pacientes y métodos: Se obtuvieron datos clínicos de los pacientes remitidos para estudio genético. Se estudiaron los genes PTPN11, SOS1, RAF1, BRAF y MAP2K1 mediante secuenciación bidireccional de los exones donde se localizan las mutaciones más recurrentes, y todos los exones del gen KRAS.

Palabras clave:
Síndrome de Noonan
Síndrome cardiofaciocutáneo
Síndrome LEOPARD
Estenosis pulmonar valvular
Miocardiopatía hipertrófica
Vía RAS-MAPK

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Introduction

Cardiofaciocutaneous Syndrome (CFC, OMIM 115150) is a disorder characterized by characteristic craniofacial malformations, ectodermal abnormalities, congenital cardiopathies, gastrointestinal disorders, growth retardation, intellectual disability and neurological disorders, among other symptoms. The incidence of this syndrome is unknown, and almost 60 cases have been documented in medical literature, although there are more than 100 instances recorded by international support organizations.

Many of its clinical manifestations are considerably overlapped with those of other entities, such as Noonan syndrome (NS, OMIM 163950) or Costello Syndrome (CS, OMIM 218040). These and other associated disorders, such as LEOPARD syndrome (LS, OMIM 151100) or neurofibromatosis type 1 (OMIM 162200) stem from germline disorders in terms of RAS-MAPK (Fig. 1) intracellular signalling cascade regulation, and they have been jointly called “Neurocardiofaciocutaneous syndromes”. RAS-MAPK disorders or RASopathies. Molecular studies in CFC patients have made it possible to identify mutations in Braf, Ras, MAP2K1, and an isolated case in KRAS.

We hereby present the phenotypic description of a series of patients with CFC diagnosis, characterized by a genetic mutational study of genes Braf and MAP2K1, and the statistical comparison with a wide series of patients carrying other neurocardiofaciocutaneous syndromes with confirmed mutation of genes PTPN11, SOS1, Braf and Raf1.

Patients and method

Clinical data was collected and mutation analysis was conducted subject to the statements of Carcavilla et al. and Ezquieta et al. The status was assessed in standard deviations regarding the reference population, and it was considered to be small when it was below –2 standard deviations for a certain age and gender. We included study results for genes Braf and MAP2K1 collected from studies on patients with a CFC clinical diagnosis, conducted by 2 other research centres. These patients were diagnosed with NS (n = 111), LS (n = 19) and CFC, according to clinical criteria. Among CFC patients, there was a case who was referred under clinical suspicion of having another neurocardiofaciocutaneous syndrome, and in 5 other patients it was difficult to establish the type of RASopathy they had, from a clinical point of view.

Results

The study included 11 patients, 5 of whom were females, with ages ranging from 7 months to 7 years of age at the time of clinical assessment. These cases were referred by 6 hospitals from 4 different Spanish communities. Table 1 summarizes clinical characteristics and identified mutations. The most frequent manifestations were congenital cardiopathy, short stature and psychomotor retardation, all of which were present in 10 out of the 11 patients. Referring physicians regarded psychomotor retardation as severe in one case, moderate in 2 cases, and mild in the remaining ones. There was the case of a female patient whose psychomotor development was considered to be normal, except for a speech retardation which was diagnosed at her assessment when she was 6 years old, and there were other 2 cases whose retardation in psychomotor milestones acquisition was mainly attributed to motor retardation and hypotonia. Speech retardation was observed in all patients older than 3 years of age at the time of assessment.

Central nervous system (CNS) malformations were identified in 4 patients (malformative encéphalopathy with pachygyria and retardation in myelination pattern, subependymal nodular heterotopia, myelination disorder with diffuse corticosubcortical atrophy, and Chiari malformation I). In 6 out of 9 patients, we obtained a description of ectodermal alterations showing typical disorders (scarce, friable and curly hair, absence of eyebrows).

In 3 patients, deep palmoplantar furrows were described, and another patient had articular hypermobility with redundant skin. Among the documented cases, there were 3 patients with polyhydranmios and one with gestational nuchal fold, and anthropometric perinatal data were within normal ranges, as described in Table 1.

Table 2 describes the characteristics of genetic findings in patients from our series, and Fig. 2 illustrates the mutational distribution identified in gene Braf. Those with mutations previously associated with cancer in the somatic line were 7 months, 2, 3 and 5 years of age at the time the study was conducted, and they have not developed any cancer to date. The referring clinician regarded all cases as sporadic. In 2 cases (7 and 8), DNA samples from both parents were obtained to prove it was a de novo mutation. In 2 other cases (2 and 8), a prenatal test was performed on both siblings, obtaining a negative result.

Results from the statistical comparison with the 130 patients suffering from other neurocardiofaciocutaneous syndromes are summarized in Table 3. Clinical characteristics of these patients are described in detail in other publications (Carcavilla et al., Ezquieta et al., Carcavilla et al., underway). As far as the molecular characterization of these patients, mutation of PTPN11 was identified in 81 patients with NS and in 16 patients with LS, mutation of RAF1 was detected in 8 patients with NS and in 2 patients with LS, and mutation of SOS1 was found in 22 patients with NS. The Braf study also identified a p.Gln257Arg mutation in a patient with an SL clinical diagnosis that had been previously described. KRAS gene has been studied in 27 patients with severe neurocardiofaciocutaneous syndrome and significant psychomotor retardation, not having found mutations in any patient. Every identified alteration had been previously described in association with the mentioned symptoms.

Discussion

CFC was originally described in 1986, arising considerable controversy as to whether it really was a differentiated syndromic entity or just an NS variant. Its clinical peculiarity resides in

Rasopathia

PTPN11

Braf

MAP2K1

Correlación genotipo-fenotipo

Resultados: Se identificaron 6 mutaciones en Braf en 9 pacientes, y 2 mutaciones en MAP2K1. La talla baja, el retraso psicomotor, los trastornos del lenguaje y las anomalías ectodérmicas fueron más frecuentes en el CFC que en el resto de los síndromes (p<0.05). En al menos 2 casos el estudio genético contribuyó a reorientar el diagnóstico.

Discusión: Los pacientes con CFC muestran un fenotipo más grave, si bien se describe un paciente sin retraso psicomotor, lo que ilustra la variabilidad del espectro fenotípico asociado a las mutaciones en Braf. El estudio genético es una herramienta útil en el diagnóstico diferencial del CFC y de los trastornos relacionados con el SN.
that it is a sporadic disorder, less prevalent than NS, with high frequency of intellectual disability, facies similar to NS, but rougher, and frequent skin conditions.

In our sample, it was not possible to make a family screening for all cases, even though they were all considered sporadic cases by the referring clinicians, and in those cases where progenitors were screened, mutation was proved to appear de novo in the patient.

Cutaneous manifestations described in medical literature include follicular hyperkeratosis with eyebrow alopecia, and scarce, friable and curly hair with hairless areas. These characteristics on the CFC patients herein described had not been documented under the first observations, and they, subsequently, proved having a high specific value to allow the identification of patients with CFC.
Table 2
Genetic findings in 11 patients with cardiofaciocutaneous syndrome.

<table>
<thead>
<tr>
<th>Gene</th>
<th>Exon</th>
<th>Nucleotide change</th>
<th>Amino acid change</th>
<th>Previous description</th>
<th>Number of affected subjects</th>
<th>Parental study</th>
<th>Mutation of the same residue in other CFCs</th>
<th>Association with cancer in somatic line</th>
</tr>
</thead>
<tbody>
<tr>
<td>BRAF</td>
<td>6</td>
<td>c.736G→C</td>
<td>p.Ala246Pro</td>
<td>Rodriguez-Viciana et al., Nava et al.</td>
<td>1</td>
<td>Negative parents</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>14</td>
<td>c.1741A→G</td>
<td>p.Asn581Asp</td>
<td>Rodriguez-Viciana et al., Nava et al.</td>
<td>1</td>
<td>–</td>
<td>No</td>
<td>Colon, lung, ovary</td>
<td></td>
</tr>
<tr>
<td>MAP2K1</td>
<td>2</td>
<td>c.199G→A</td>
<td>p.Asp67Asn</td>
<td>Narumi et al., Nava et al.</td>
<td>1</td>
<td>–</td>
<td>p.Pro124Leu</td>
<td>Melanoma</td>
</tr>
<tr>
<td>3</td>
<td>c.371C→A</td>
<td>p.Pro124Gln</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

CFC: cardiofaciocutaneous syndrome.

Moreover, and in line with cases published in international medical literature,1 the patients who were studied had normal weight and length at birth, and, afterwards, they had growth disorders, typically associated with feeding problems (60% of our sample). Although this manifestation was significantly more frequent among patients with CFC in comparison with NS patients and with patients suffering from other neurocardiofaciocutaneous syndromes altogether, in this sample it did not attain statistical significance.

Short stature has been described in almost 80% of patients,2 although we do not have access to studies where growth and development patterns are assessed in detail. All of the cases that constitute this series but one presented short stature, but there were no data on the development, and the eldest patient was assessed at 7 years of age.

Approximately 75% of patients described have congenital cardiopathy, and although pulmonary stenosis has been reported in 40% of the cases, hypertrophic cardiomyopathy is, at least, equally frequent, and almost 40% of patients have other types of cardiopathies.2 In this sample, there were no overt differences between the frequency of pulmonary stenosis or hypertrophic cardiomyopathy in CFC and the remaining syndromes, and only one of the cases described presented another cardiopathy, a coarctation of the aorta with atrial septal defect.

The CFC patients from this series, in general, presented a more severe neuropsychological phenotype, with a higher incidence of psychomotor retardation and speech disorders. There was also a higher incidence of CSN anatomic disorders, similar to that previously documented.21 However, we shall describe a patient with CFC caused by p.Glu501Lys mutation of BRAF, who presented motor retardation but whose intellectual development was within normal ranges, which illustrates the phenotypical expression variability of BRAF mutations.

CFC patients in this study presented a high incidence of hearing loss (30%), higher than patients with other neurocardiofaciocutaneous syndromes and than patients with NS, even though the last comparison attained statistical significance (p = 0.059 and p = 0.048, respectively). However, the series described do not identify hearing loss as a CFC distinctive trait. Our research findings may translate a finding that has not been described to date, or reflect the limited nature in size of the studied sample; in any case, ampler series of patients are required to clarify this end.

The differential diagnosis of neurocardiofaciocutaneous syndromes has suffered considerable changes throughout the last decade, with the arrival of the molecular tool as a confirmation diagnosis aid. In this study, at least 5 out of the 11 patients were referred with an NS diagnosis, with some CFC manifestations, and one of them was suspected of having CS. The molecular result and the clinical reassessment made it possible to reorient the diagnosis. Differentiation between CFC and CS, in particular, has received special attention from most publications dedicated to assessing these syndromes, typically reaching the conclusion that despite the fact that some manifestations are more frequent in some cases than others, overlapping tends to be usual.20,22 In this sample of patients with CFC, there were some clinical characteristics typical of CS, such as prenatal polyhydramnios (which was so severe in one of the patients that he was suspected of having oesophagus atresia), or deep palmoplantar furrows and articular hypermobility with redundant skin. In spite of its similarities with CS, CFC has been historically considered an exception to the increased risk of developing cancer in patients with neurocardiofaciocutaneous syndromes. Nonetheless, there are some cases of neoplasias described in CFC patients,23 and recently the CFC cancer incidence has been reported to be similar to that of NS.24 None of the patients from this sample had developed cancer at the time of data collection, despite the fact that some of the mutations identified at
Table 3
Comparison of clinical data among with cardiofaciocutaneous syndrome and other neurocardiofaciocutaneous syndromes, as well as among patients with cardiofaciocutaneous syndrome and Noonan syndrome.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>CFC</th>
<th>Other</th>
<th>p</th>
<th>Noonan</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Females/males</td>
<td>5/6</td>
<td>53/77</td>
<td>0.76</td>
<td>47/64</td>
<td>0.81</td>
</tr>
<tr>
<td>Index/familial</td>
<td>11/0</td>
<td>122/8</td>
<td>0.4</td>
<td>106/5</td>
<td>0.48</td>
</tr>
<tr>
<td>Growth</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Short stature (less than –2 SD), %</td>
<td>90.9</td>
<td>59.1</td>
<td>0.049</td>
<td>63.5</td>
<td>0.096</td>
</tr>
<tr>
<td>Stature, medium ± SD</td>
<td>–2.44 ± 1.33</td>
<td>–2.41 ± 1.6</td>
<td>0.32</td>
<td>–2.55 ± 1.7</td>
<td>0.53</td>
</tr>
<tr>
<td>Weight, medium ± SD</td>
<td>–1.7 ± 1.3</td>
<td>–1.63 ± 0.82</td>
<td>0.36</td>
<td>–1.7 ± 0.86</td>
<td>0.48</td>
</tr>
<tr>
<td>BMI, medium ± SD</td>
<td>–0.41 ± 1.3</td>
<td>–0.53 ± 0.85</td>
<td>0.48</td>
<td>–0.53 ± 0.9</td>
<td>0.49</td>
</tr>
<tr>
<td>CP, medium ± SD</td>
<td>–0.77 ± 1.5</td>
<td>–0.95 ± 1.73</td>
<td>0.63</td>
<td>–1.2 ± 1.61</td>
<td>0.37</td>
</tr>
<tr>
<td>Cardiopathy, %</td>
<td>90.9</td>
<td>93.5</td>
<td>0.55</td>
<td>94.3</td>
<td>0.51</td>
</tr>
<tr>
<td>Pulmonary stenosis</td>
<td>63.6</td>
<td>62.8</td>
<td>0.96</td>
<td>67.9</td>
<td>0.81</td>
</tr>
<tr>
<td>Hypertrophic cardiomyopathy</td>
<td>36.4</td>
<td>27.7</td>
<td>0.51</td>
<td>20.7</td>
<td>0.27</td>
</tr>
<tr>
<td>Septal defect</td>
<td>9.1</td>
<td>16.2</td>
<td>0.53</td>
<td>18.2</td>
<td>0.69</td>
</tr>
<tr>
<td>Mitral abnormalities</td>
<td>0</td>
<td>3.8</td>
<td>0.51</td>
<td>4.5</td>
<td>0.35</td>
</tr>
<tr>
<td>Other cardiopathies</td>
<td>9.1</td>
<td>16.3</td>
<td>0.27</td>
<td>11</td>
<td>0.61</td>
</tr>
<tr>
<td>Haematological abnormalities, %</td>
<td>0</td>
<td>15.2</td>
<td>0.53</td>
<td>17</td>
<td>0.21</td>
</tr>
<tr>
<td>Easy bruising</td>
<td>0</td>
<td>6.8</td>
<td>0.37</td>
<td>8</td>
<td>0.33</td>
</tr>
<tr>
<td>Cryptorchidism, %</td>
<td>83.3</td>
<td>62</td>
<td>0.41</td>
<td>70.8</td>
<td>0.66</td>
</tr>
<tr>
<td>Nefrourological abnormalities, %</td>
<td>25</td>
<td>12.7</td>
<td>0.3</td>
<td>17.5</td>
<td>0.84</td>
</tr>
<tr>
<td>Pterygium coll, %</td>
<td>0</td>
<td>18.5</td>
<td>0.21</td>
<td>17.1</td>
<td>0.21</td>
</tr>
<tr>
<td>Thoracic abnormalities</td>
<td>27.3</td>
<td>35.9</td>
<td>0.75</td>
<td>35.7</td>
<td>0.75</td>
</tr>
<tr>
<td>Pectus excavatum</td>
<td>18.2</td>
<td>20.6</td>
<td>0.86</td>
<td>19.6</td>
<td>0.92</td>
</tr>
<tr>
<td>Pectus carinatum</td>
<td>10</td>
<td>6.6</td>
<td>0.6</td>
<td>10.8</td>
<td>0.69</td>
</tr>
<tr>
<td>Tunnel chest</td>
<td>19.1</td>
<td>12.3</td>
<td>0.76</td>
<td>15.8</td>
<td>0.8</td>
</tr>
<tr>
<td>Thyroid abnormalities, %</td>
<td>0</td>
<td>5.3</td>
<td>0.44</td>
<td>6.3</td>
<td>0.4</td>
</tr>
<tr>
<td>Cafe au lait spots, %</td>
<td>10</td>
<td>23.2</td>
<td>0.68</td>
<td>7.8</td>
<td>0.81</td>
</tr>
<tr>
<td>Ectodermal abnormalities, %</td>
<td>60</td>
<td>14.5</td>
<td>0.004</td>
<td>19.6</td>
<td>0.02</td>
</tr>
<tr>
<td>Feeding disorders, %</td>
<td>60</td>
<td>26.1</td>
<td>0.009</td>
<td>31.4</td>
<td>0.15</td>
</tr>
<tr>
<td>Hearing disorders, %</td>
<td>30</td>
<td>7.2</td>
<td>0.059</td>
<td>5.9</td>
<td>0.048</td>
</tr>
<tr>
<td>Speech disorders, %</td>
<td>45.5</td>
<td>8.4</td>
<td>0.003</td>
<td>8.9</td>
<td>0.04</td>
</tr>
<tr>
<td>ADHD, %</td>
<td>0</td>
<td>17.9</td>
<td>0.35</td>
<td>20.8</td>
<td>0.44</td>
</tr>
<tr>
<td>Psychomotor retardation, %</td>
<td>90</td>
<td>43.5</td>
<td>0.007</td>
<td>43.6</td>
<td>0.013</td>
</tr>
</tbody>
</table>

CFC: cardiofaciocutaneous syndrome; SD: standard deviations for age and gender (Carrascosa et al.19); BMI: body mass index; Other: 111 patients with Noonan syndrome and 19 patients with LEOPARD syndrome; CP: cephalic perimeter; ADHD: attention deficit hyperactivity disorder.

The statistical analysis was conducted by means of Fisher’s exact test and the Mann-Whitney U test for group comparison. It was considered statistically significant p < 0.05.

(Values with p below 0.05 are highlighted in bold on the text.)

References

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Conflict of interest
The authors declare that there are no conflicts of interest.

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a germin al level had been previously described in relation to cancer at a somatic level. In any case, the small number of patients and their early ages make it risky to affirm that they shall not develop any neoplasia as they grow older.


