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CLINICAL REPORT

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A sibling pair with cardiofaciocutaneous syndrome (CFC) secondary to *BRAF* mutation with unaffected parents—the first cases of gonadal mosaicism in CFC?

Sarah Geoghegan[1*] | Graham King[1*] | Jennifer Henchliffe[2] | Simon C. Ramsden[2] | Raymond J. Barry[3] | Andrew J. Green[4] | Susan M. O'Connell[1]

[1]Department of Paediatrics and Child Health, Cork University Hospital, Cork, Ireland

[2]St. Mary's Hospital, Manchester Academic Health Science Centre, Manchester Centre for Genomic Medicine, Manchester, United Kingdom

[3]Department of Paediatrics and Child Health, Mercy University Hospital, Cork, Ireland

[4]Department of Clinical Genetics, Our Lady's Hospital Crumlin, School of Medicine and Medical Science, University College Dublin, Dublin, Ireland

Correspondence
Graham King, Department of Paediatrics and Child Health, Cork University Hospital, Wilton, Cork T12 DC4A, Ireland.
Email: gking@coombe.ie

Cardiofaciocutaneous (CFC) syndrome is a RASopathy characterized by intellectual disability, congenital heart defects, a characteristic facial appearance, gastro-intestinal complications, ectodermal abnormalities and growth failure. The RASopathies result from germline mutations in the Ras/Mitogen-activated-protein-kinase (MAPK) pathway. CFC is associated with mutations in *BRAF*, *KRAS*, *MEK1* and *MEK2*. CFC has been considered a "sporadic" disorder, with minimal recurrence risk to siblings. In recent years, vertical transmission of CFC has been seen in mutations involving the *MEK2* and *KRAS* genes, but has not previously been reported with *BRAF* mutations. Two brothers with clinical features of CFC and mutations in *BRAF* (c.770A > G, p.Gln257Arg) are described. Neither parent (both phenotypically normal) had the *BRAF* mutation in their leukocyte DNA. Although this mutation is one of the most common mutations in CFC, to our knowledge, this is the first molecularly confirmed *BRAF* mutation causing CFC in siblings. This observation also likely represents the first description of germ cell mosaicism in CFC and so it is important to provide optimal genetic counselling to families regarding the risk of reoccurrence.

KEYWORDS
BRAF, cardiofaciocutaneous syndrome, germ cell mosaicism, rasopathy, siblings, recurrence risk

1 | INTRODUCTION

Cardiofaciocutaneous (CFC) syndrome was first reported in 1986; eight patients with a previously undefined multiple congenital anomalies and intellectual disability syndrome were designated the CFC syndrome which includes congenital heart defects, characteristic facial appearance, ectodermal abnormalities, and growth failure. Cardiac defects were variable, the most common being pulmonary stenosis and atrial septal defect. Typical facial characteristics were high forehead with bitemporal narrowing, underdevelopment of supraorbital ridges, downslanting palpebral fissures, hypertelorism, epicanthic folds, depressed nasal bridge, sparse and friable hair, and posteriorly rotated ears with prominent helices (Reynolds et al., 1986). In the same year, Baraitser and Patton (1986) described four cases presenting with a similar phenotype and suggested that they represented a distinctive previously unreported syndrome.

*Both authors contributed equally to the manuscript.

There have now been over a hundred reports of CFC in the literature (Rauen, 2016). Since the initial case reports, further detailing of the phenotype includes: polyhydramnios and preterm delivery, sensorineural deafness, seizures, hydrocephalus and other brain defects, failure to thrive, gastro-esophageal reflux disease and infant feeding problems, hydronephrosis, and cryptorchidism (Armour & Allanson, 2007).

CFC is one of the RASopathies, which result from germline mutations in the Ras/MAPK pathway. This MAPK pathway is essential in the regulation of the cell cycle, differentiation, growth and cell senescence, all of which are critical to normal development (Tidyman & Rauen, 2009). It is now known that CFC is associated with mutations in *BRAF* (Niihori et al., 2006; Rodriguez-Viciana et al., 2006), *KRAS* (Niihori et al., 2006), and *MEK1* and *MEK2* genes (Rodriguez-Viciana et al., 2006). BRAF is a serine/threonine protein kinase and one of the direct downstream effectors of Ras. Activated Ras leads to the activation of Raf (ARAF, BRAF, and/or CRAF) the first MAPK kinase kinase of the pathway (Tidyman & Rauen, 2009). Heterozygous *BRAF*

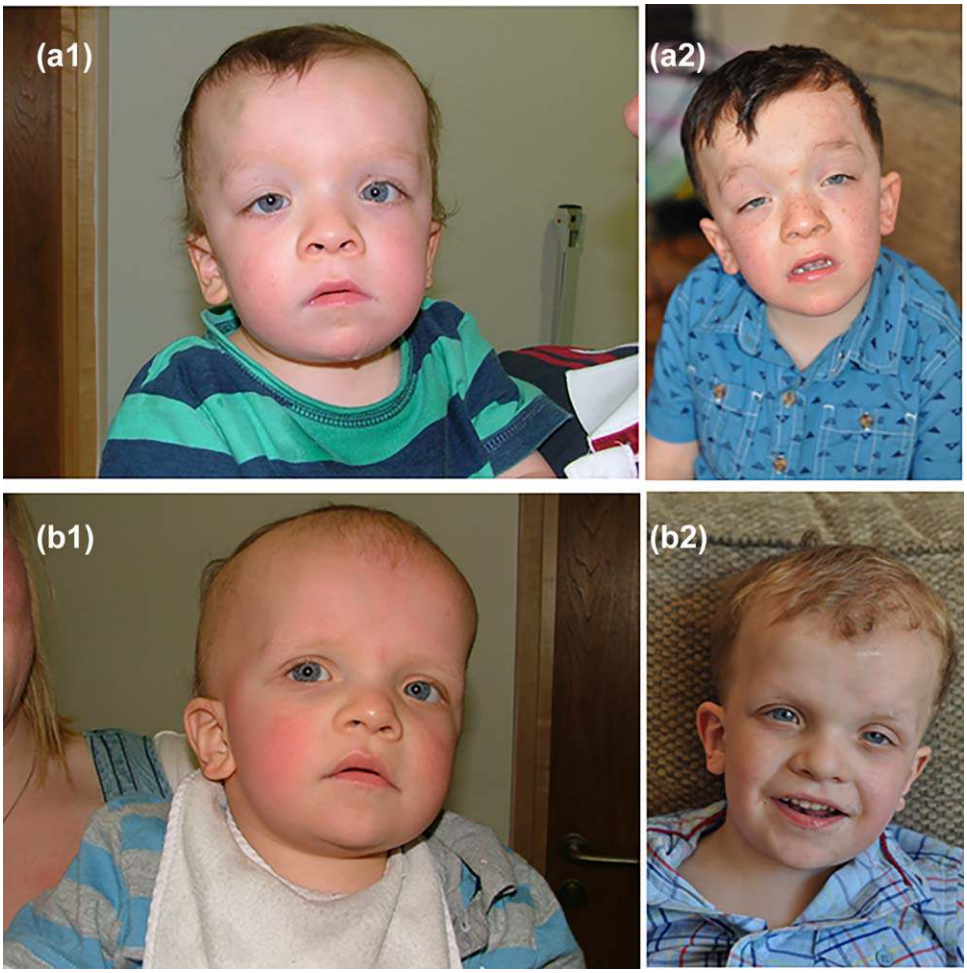

FIGURE 1 (a1) Index case with a high forehead, bitemporal narrowing, underdeveloped supraorbital ridges, ocular hypertelorism, downslanting palpebral fissures, ptosis, a broad nasal bridge, low set and posteriorly rotated ears, and prominent ear lobe creases. (a2) Index case now aged 9 years with more obvious ptosis and marked sparse head and eyebrow hair. (b1) Younger brother with similar early features as his brother (as listed above). (b2) Younger brother aged 7 years with marked left-sided ptosis and sparsity of hair of head and eyebrows (Note: photos submitted with parental consent) [Color figure can be viewed at wileyonlinelibrary.com]

mutations are found in approximately 75% of mutation-positive CFC individuals. The majority of *BRAF* mutations cluster in the cysteine rich domain in exon 6 and in the protein kinase domain (Tidyman & Rauen, 2009).

2 | MATERIALS AND METHODS

2.1 | Clinical reports

We present the case of two brothers with CFC. The index case was a boy born to a 28-year-old mother and 30-year-old father, at 38 weeks gestation by emergency caesarean section for fetal distress. Birth weight was 3.27 kg (50th centile). The pregnancy was complicated by maternal hypertension, fetal polyhydramnios, right-sided hydronephrosis, and short femur length noted on antenatal scans.

The first year of life was complicated by feeding problems, gastroesophageal reflux disease, developmental delay and failure to thrive. The dysmorphic features noted initially led to a clinical diagnosis of Noonan syndrome (Figure 1a1). There was a systolic murmur present. Echocardiogram showed pulmonary stenosis and a small persistent ductus arteriosus and patent foramen ovale. Renal ultrasound confirmed right-sided hydronephrosis. Audiological assessment revealed mixed conductive and sensorineural hearing loss due to bilateral middle ear effusions. Magnetic resonance imaging (MRI) brain was normal.

One year later his brother was born full term and with a birth weight of 3.6 kg (>50th centile). He was a poor feeder from birth and started supplemental nasogastric feeding at 8 weeks of age and subsequently had a percutaneous gastrostomy inserted. He was noted to have very similar dysmorphic features to his older sibling including a left-sided divergent strabismus (Figure 1b1). Echocardiogram revealed concentric left ventricular hypertrophy and mild pulmonary stenosis. He also had bilateral sensorineural hearing loss. MRI brain showed benign external hydrocephalus.

The index case, now 9 years old, is on the third centile for height and weight. He wears a hearing aid for right-sided sensorineural hearing loss. The younger brother is now nearly 8 years old and is on the 0.4th centile for height and weight. Both brothers have sparse eyebrow and head hair (Figure 1a2,b2). Both boys have moderate

learning disability. They are prone to hypoglycemia during intercurrent illnesses which requires management with early intravenous dextrose infusions. Both brothers suffer from constipation. Surveillance echocardiography is conducted every 2 years. The younger brother also has focal epilepsy with secondary generalization (onset was at 6 years old) managed with levetiracetam.

2.2 | Molecular analysis

High resolution microarray chromosome analysis was normal. Bidirectional fluorescent Sanger sequencing analysis of the *PTPN11*, *MEK1* and *MEK2* genes were normal. Bidirectional fluorescent sequencing analysis of the *BRAF* gene showed heterozygosity for a pathogenic mutation in *BRAF* c.770A > G (p.Gln257Arg) in both brothers compatible with a diagnosis of CFC. The identical mutation has been described in other patients with CFC (Niihori et al., 2006). The mutation is not found in the EXAC exome sequencing database of 60,000 individuals (accessed 06/07/2017). Neither parent (both phenotypically normal) had the *BRAF* mutation in DNA extracted from peripheral blood leukocytes. The analysis has the capacity to detect a level of mosaicism in the *BRAF* gene of approximately 30% for a heterozygous change on one chromosome.

The siblings' parents were offered the opportunity to provide further tissue/cell sampling to identify any possible somatic mosaicism but deciding that their family was complete, they declined. Specific paternity testing was not undertaken.

3 | DISCUSSION

These siblings were originally thought to have many of the clinical features of a phenotype consistent with a diagnosis of Noonan syndrome. However, molecular genetic analysis indicated a diagnosis of CFC. CFC is most strongly linked phenotypically to Noonan syndrome and Costello syndrome (Der Kaloustian et al., 1991), and as a result this can often make clinical distinction difficult. Well-characterized individuals with CFC have been shown not to have a mutation in *PTPN11* (Ion et al., 2002; Kavamura et al., 2003) which can be seen in approximately 50% of cases of Noonan syndrome (Tartaglia et al., 2001), nor the *HRAS* gene which causes Costello syndrome (Estep et al., 2006).

Many of the RASopathies exhibit autosomal dominant inheritance (although de novo mutations certainly do occur). In contrast, CFC syndrome is generally considered a "sporadic" disorder, with minimal recurrence risk to siblings (Stark et al., 2012). However, in recent years, vertical transmission of CFC has been seen in mutations involving the *MEK2* and *KRAS* genes. In 2010, Rauen et al. (2010) described the vertical transmission of a pathogenic *MEK2* mutation with complete penetrance through four generations including the proband's mother and half sibling. Stark et al. (2012) showed vertical transmission of a fully penetrant pathogenic *KRAS* mutation from mother to son. To our knowledge, there have been no previous reports of vertical transmission of a *BRAF* mutation.

We identified a c.770A > G mutation in the *BRAF* gene, predicting a p.Gln257Arg amino acid substitution in both these siblings. Although this mutation (*BRAF* c.770A > G) is the most common *BRAF* mutation in CFC (Pierpont et al., 2014), this case documents, to our knowledge, the first molecularly confirmed *BRAF* mutation causing CFC in siblings.

The presence of the *BRAF* c.770A > G mutation in both siblings in the absence of identifiable mosaicism in parental blood derived DNA, suggests the following possible origins: (A) two mutations arising independently from each other, (B) somatic mosaicism for the mutation in one parent below a level detectable in peripheral blood lymphocytes, or (C) mosaicism for the mutation in one or other parent's germ cells.

Somatic mosaicism in the RASopathies is seen in a number of conditions including: keratinocytic epidermal nevi (& syndrome); sebaceous nevi; Shimmelpenning syndrome; and segmental neurofibromatosis type 1. With regard to germline mosaicism in RASopathies, Gripp et al. (2011) have already described a case of germ cell mosaicism (*HRAS* mutation) in siblings with Costello syndrome. Another case report described a father who had a mosaic *HRAS* G12S somatic mutation in buccal cells. The father had some (but not all) clinical findings of Costello syndrome. He had an offspring with an *HRAS* G12S germline mutation and full clinical findings of Costello syndrome, suggesting a father-to-son transmission of the mutation (Sol-Church et al., 2009). Expert opinion to date has been that no instance of germline mosaicism in a parent has been reported in the CFC cohort (Rauen, 2016).

Current practice to investigate the genetic etiology of a clinically suspected case of CFC is to commence with multigene Ras/MAPK pathway panel testing (Pierpont et al., 2014). Whole genome sequencing or whole exome sequencing may subsequently be used. Until now if parental samples (leukocyte DNA) were negative for the same mutation as the affected child, and the parents' exams showed no phenotypic features of CFC, it was concluded that this likely represented a de novo mutation in the proband and as a result the risk to siblings was presumed to be low (Rauen, 2016). However, the current clinical description of *BRAF* c.770A > G mutations in two brothers with CFC (but clinically unaffected parents who are mutation negative in leukocyte DNA) represents the first reported instance of probable germ cell mosaicism in CFC. It also highlights the importance of counseling the unaffected parents of a child with CFC that the risk to future offspring may indeed be higher than previously suspected. As a result, prenatal diagnosis of subsequent pregnancies should be made available.

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CONFLICT OF INTEREST

None.

ORCID

Graham King http://orcid.org/0000-0002-2894-9360

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