MAP2K2 (MEK2) in cardiofaciocutaneous (CFC) syndrome - research roadmap

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Aim

The main aim of this research roadmap is to select and recommend the most promising research directions which stimulate cure development for cardiofaciocutaneous (CFC) syndrome and CFC4 in particular.

NOTE: MAP2K2 and MEK2 are synonyms and will be used interchangeably.

Contents

1. State of the art

1.1. Introduction on disease

Maria was born in 2024 with *MAP2K2* gene mutation (c.619G>A p.Glu207Lys) resulting in cardiofaciocutaneous (CFC) syndrome. *MAP2K2* gene (or *MEK2* - synonyme) mutations are rare and this particular mutation is ultra-rare with unspecified true prevalence.

CFC syndrome is a multiple congenital anomaly disorder in which individuals have characteristic craniofacial features, cardiac defects, ectodermal anomalies, gastrointestinal dysfunction, and neurocognitive delay (Rauen et al., 2010). CFC syndrome belongs to a family of RASopathies (together with other diseases such as Noonan syndrome (NS) and Costello syndrome (CS)). RASopathies are caused by mutations in the RAS/MAPK pathway. Mutations in this pathway are predominantly studied in cancer. RASopathies' symptoms include: congenital heart defects, craniofacial abnormalities, skin abnormalities and intellectual disability.

CFC syndrome can be caused by mutations in 4 different genes: a) *BRAF* (most common mutation present in ~75% of CFC individuals with an identified gene mutation) - CFC1, b) *KRAS* - CFC2, c) *MAP2K1* - CFC3, d) *MAP2K2* - CFC4.

The *MAP2K2* gene encodes the MAP2K2 enzyme, which is part of the RAS/MAPK pathway. MAP2K2 is a dual specificity protein kinase. MEK2 protein phosphorylates (activates) MAPK1 (aka ERK2) and MAPK3 (aka ERK1). When the *MEK2* gene is mutated the pathway can get stuck in an "ON" position.

In CFC pathogenic variants are mainly missense with a gain-of-function mechanism on proteins of the pathway, leading to ERK1-2 hyperactivation (Scorrano et al., 2023).

1.2. Prevalence

Data from the Japanese population estimates CFC syndrome prevalence as 1 in 810 000 (Abe et al., 2012). Currently there is no worldwide data. CFC International (leading

patients' advocacy organization) claims it is probably more common. The true prevalence and incidence of CFC syndrome are still unknown.

Disease affects both females and males equally.

1.3. Clinical symptoms

CFC syndrome is characterized by a range of symptoms, with the most characteristic: cardiac defects (cardio-), craniofacial features (-facio-), and skin anomalies (-cutaneous). There are also other symptoms, which will be described below and their frequency will be compared in **Table 1**.

CFC requires multidisciplinary care from specialists and international consensus on management guidelines was published in 2014 (Piermont et al., 2014).

Feature	Frequency			Comment
	Nearly all	Common	Less frequent	
Prenatal polyhydramnios	•			
Characteristic facial features	•			
Cardiac issues	•			
Feeding difficulties	•			
Poor growth	•			
Skin issues	•			
Neurocognitive delays	•			Ranging from mild to profound
Eye anomalies	•			
Musculoskeletal abnormalities	•			
Hypotonia & motor developmental delay	•			
Seizures		•		
Behavioral issues		•		
Neonatal chylothorax &/or lymphedema		•		
Otolaryngologic issues		•		Most commonly recurrent otitis media
Urogenital anomalies		•		Most commonly cryptorchidism in males
Malignancy			•	
Hematologic issue			•	
Immunologic issues			•	

Table 1. Frequency comparison of the CFC clinical features. Based on: Rauen 2007.

1.3.1. Cardiac issues

Present in 75-80% of individuals. Most abnormalities (like pulmonic stenosis, atrial septal defects and/or ventricular septal defects, mitral valve dysplasia, tricuspid valve dysplasia, and bicuspid aortic valve) **present at birth** but hypertrophic cardiomyopathy and rhythm disturbances may manifest later.

1.3.2. Dysmorphic features

The face is triangular in shape and overall may be more coarse than in Noonan syndrome (a clinically similar condition often confused with CFC syndrome), but usually not as coarse as is typically seen in Costello syndrome.

1.3.3. Ectodermal (skin) issues

Present in all individuals. Dryness of skin and follicular hyperkeratosis tend to improve with time. Palmoplantar hyperkeratosis and lymphedema may become more severe with time. Severe skin infections can develop. Skin findings can include: xerosis, hyperkeratosis of arms, legs and face, keratosis pilaris, ichthyosis, Ulerythema ophryogenes, eczema, hemangiomas, Café au lait macules, erythema both on the face or generalized, pigmented moles that may be progressive in number.

1.3.4. Gastrointestinal issues

Most patients have severe issues that lead to poor growth. Many children need tube feeding (nasogastric or gastrostomy). Oral feeding is typically achieved in early childhood. Issues include aspiration and swallowing problems (may improve with age), constipation (recurrent issue), gastroesophageal reflux disease or malrotation leading to recurrent vomiting, oral aversion, dysmotility, umbilical and inguinal hernia.

1.3.5. Poor growth

Affects most of the patients. Parameters may be normal at birth, but weight and height can drop to <5 percentile. Head circumference typically remains normal - relative macrocephaly (size of the head in relation to the body seems overly big).

1.3.6. Developmental delay and intellectual disability

Present in majority or all individuals. Delays range from mild to profound (however some patients might have IQ within normal range). The majority of patients have hypotonia due to skeletal muscle myopathy (that results in motor delays). Many never achieve waking, those that do reach this milestone on average by ~3 years. A “significant number of individuals” remain nonverbal. In those that develop verbal skills, the first word is spoken on average around the second year of life.

1.3.7. Seizures

Occur in >50% patients. Most seizures begin in infancy/early childhood. Typic seizures: complex partial seizures, generalized tonic-clonic seizures, absence seizures, and/or infantile spasms.

1.3.8. Neurobehavioral issues

These issues are common. May include irritability, short attention span, stubbornness, obsessive and/or aggressive behaviors, anxiety, autism.

1.3.9. Musculoskeletal issues

Are present in the majority of individuals. Issues include paucity of muscle mass, skeletal myopathy, lax joints, pectus deformity, pes planus, hip dysplasia, scoliosis, kyphosis, gait disturbances, joint contractures of elbows, knee and hips, reduced bone density.

1.3.10. Neonatal lymphatic issues

Chylothorax and lymphedema have been reported at birth, Peripheral edema.

1.3.11. Otolaryngologic issues

Recurrent otitis media, narrow external auditory canals, Hyperacusis and hearing loss. Many require pressure equalization tubes.

1.3.12. Renal/urogenital anomalies

Occur in up to 33% individuals. Cryptorchidism (males) is the most common, renal cysts and stones, hydronephrosis, bladder, uterine and cervical (females) abnormalities.

Phenotype correlations by gene

Individuals with MAP2K2-related CFC syndrome (CFC4) have a lower risk of severe neurodevelopmental delay and epilepsy than individuals with MAP2K1-related CFC syndrome (CFC3). They also presented lower risk of epilepsy (30%) than those with MAP2K1 mutation (61%) (Pierpont et al., 2022).

1.4. Pathophysiology of the disease

Based on Rauen 2007: “The four genes currently known to be associated with cardiofaciocutaneous (CFC) syndrome are in the Ras/mitogen-activated protein kinase (MAPK) signaling cascade. The MAPK signaling cascade of dual-specificity kinases (Rauen et al 2011) is highly conserved among eukaryotic organisms and is critically involved in cell proliferation, differentiation, motility, apoptosis, and senescence. The Ras/Raf/MEK/ERK signal transduction pathway is activated by extracellular stimuli. Activated Ras recruits Raf, the first kinase of the cascade, to the cell membrane. Activated Raf phosphorylates MEK1 (encoded by MAP2K1) and/or MEK2 (encoded by MAP2K2), which then phosphorylates ERK1 and/or ERK2 (aka MAPK). (...) CFC syndrome is associated with pathogenic variants in BRAF, MAP2K1, and MAP2K2. Because KRAS pathogenic variants were identified in individuals clinically diagnosed with CFC syndrome or with Noonan syndrome (Niihori et al 2006, Schubbert et al 2006), the role of its protein product, GTPase KRas (KRAS), in CFC syndrome warrants further study.
Mechanism of disease causation: The vast majority of pathogenic variants are missense or small in-frame deletions that cause a gain-of-function activation of the protein product BRAF, MEK1, MEK2, or KRAS that leads to activation of the Ras/MAPK pathway. This results in increased phosphorylation and, thus, activation of ERK1 and/or ERK2.”

1.5. CFC4 Genetics

1.5.1. *MAP2K2* gene

MAP2K2 (mitogen-activated protein kinase kinase 2) gene is located on chromosome 19 (19p13.3). There are 54 unique public DNA variants reported for this gene (https://databases.lovd.nl/shared/genes/MAP2K2). c.619G>A p.Glu207Lys variant present in the patient is reported as “likely pathogenic” (https://databases.lovd.nl/shared/variants/0000649984#00011741 https://www.ncbi.nlm.nih.gov/snp/rs727504382) (Duzkale et al., 2013). European network on Noonan Syndrome and Related Disorders reports 53 entries regarding MEK2 (https://nseuronet.com/php/statistic.php?choice=general&genotype=10&phenotype=4) and specifies that they result from 22 mutations in this gene.

This gene region is highly conserved (**Fig. 1**) with high potential for pathogenic outcome:

- PolyPhen score = “probably_damaging”- A result of probably damaging indicates that the variant is likely to affect protein function.
- CADD score (variant effect predictor (VEP) Ensembl) = 32 - 0.00063 of all genome substitutions are considered more damaging.

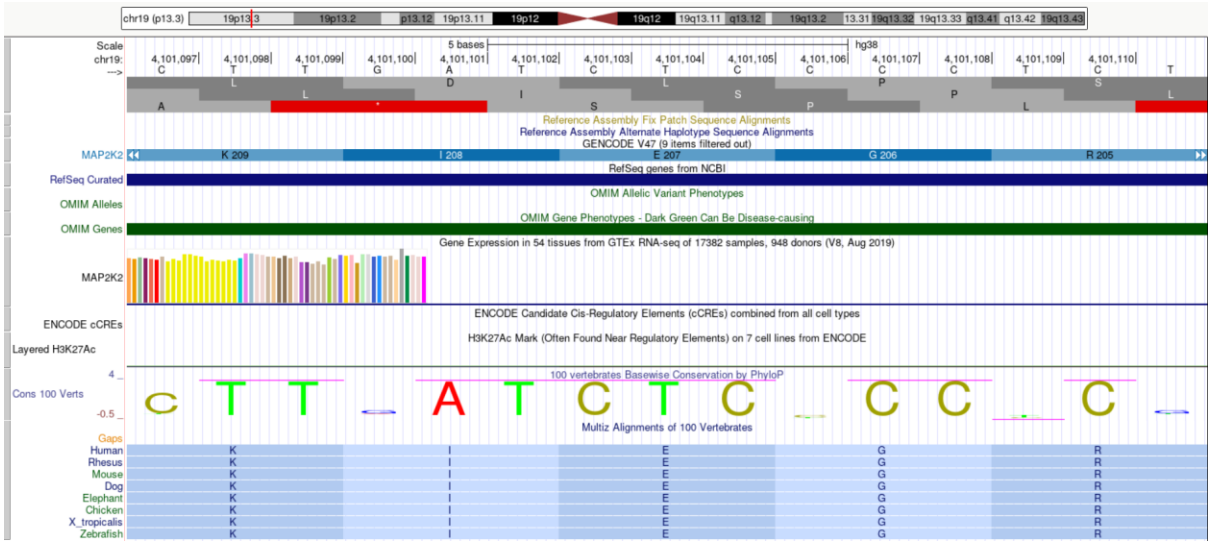

Fig 1. E207 region in *MAP2K2* gene. Based on: https://genome.ucsc.edu/

This gene variant is also reported in cancer genomic databases where it is related to melanomas (skin cancer):
https://cancer.sanger.ac.uk/cosmic/mutation/overview?id=106735399
Both *MAP2K1* and *MAP2K2* mutations were found in melanomas (Nikolaev et al., 2012).

1.5.2. MAP2K2 enzyme

The *MAP2K2* gene encodes the MAP2K2 enzyme, which is part of the RAS/MAPK pathway. MAP2K2 is a dual specificity protein kinase and is built from 400 amino acids. MEK2 protein phosphorylates (activates) MAPK1 (aka ERK2) and MAPK3 (aka ERK1).

Effect of mutations in *MAP2K2* gene on the protein function is not fully known but for some variants (e.g. VAR_069782 in position 128) increased kinase activity is observed with typical CFC4 symptoms (Rauen et al., 2010).

Alphafold and SMR (X-ray Diffraction, 3.90Å) 3D structures for the canonical MEK2 are known (https://alphafold.ebi.ac.uk/entry/P36507
https://swissmodel.expasy.org/repository/uniprot/P36507?csm=3401D522515C30A5).

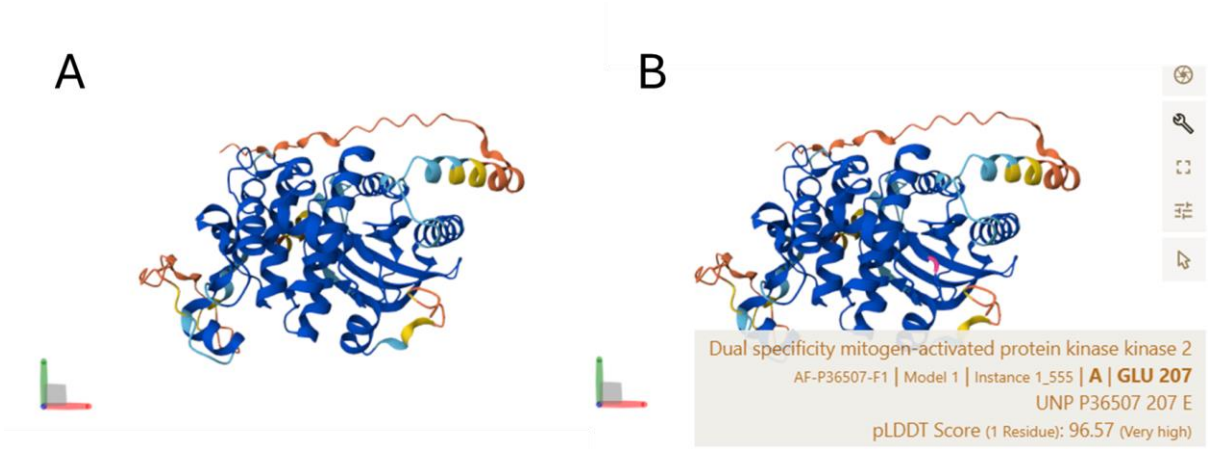

Fig. 2. Alphafold structure of MAP2K2 protein A) whole protein B) E207 site.

2. Ongoing research

Major research groups studying RASopathies are associated within the RASOPATHIES network (https://rasopathiesnet.org/rasopathies/research/researchers/).

There is not too much research going on CFC4 in particular. We recommend to focus on supporting the most promising research in the similar research fields and stimulate it to move the research resources toward CFC4 research. There are 3 groups working or RASopathies whose involvement would be very beneficial:

1. Ross Cagan group / University of Glasgow, Scotland - genetic and drug screening aimed at cancer and rare genetic diseases (mainly RASopathies) using fruit flies (*Drosophila melanogaster*). This group collaborates with research groups performing rodent experiments and clinical trials. Currently they focus on Neurofibromatosis Type 1 (*NF1* gene) - similarly to CFC the MAPK pathway has increased activity in this syndrome.
2. Bruce Gelb group / Icahn School of Medicine at Mount Sinai, NY, USA - this group focuses on Noonan Syndrome. They use human induced pluripotent stem cells to model RASopathies aspects like hypertrophic cardiomyopathy and juvenile myelomonocytic leukemia. Together with Cagan lab they are screening drug and chemical libraries to find potential therapeutics for HCM and neurocognitive impairment in Noonan Syndrome. They are working on fruit flies and human induced pluripotent stem cells. The lab is part of Pediatric Cardiac Genomics Consortium that performs genomics research on congenital heart defects.
3. Ype Elgersma group / Erasmus MC, Netherlands - laboratory studying both Angelman syndrome and 3 RASopathies (Costello syndrome, Neurofibromatosis and Legius syndrome). This laboratory uses mouse models of those disorders. They study cognitive and social impairments on the behavioral level of those models. In their research they are suggesting MEK inhibitors and HCN (hyperpolarization-activated cyclic nucleotide-gated) channels as potential RASopathies therapeutics. The laboratory is a part of EURAS consortium.

Taken together these groups have the full potential to perform advanced studies on CFC4 syndrome from basic, through preclinical to clinical registration level.

3. Therapeutic tracks

In our opinion the best studied potential therapy strategy involves MEK inhibition. Multiple MEK (1 and 2) inhibitors were developed as anti-cancer drugs, as at least 40% of human cancers are associated with aberrant ERK pathway activity. MEK inhibitors have been tested *in vivo* in zebrafish during embryonic development. However MEK activity is crucial for normal development. High doses of PD0325591 has a deleterious effect on developing organs. A study showed that a continuous administration of low levels of this inhibitor “is sufficient to prevent the effects of both kinase-active and kinase-impaired BRAF CFC alleles” without much impact of normal development. However, even small amounts of this inhibitor causes embryonic heart defects in zebrafish (Anastasaki et al., 2012).

In vitro CFC MEK mutations are also responsive to RAF inhibition (Senawond et al. 2008).

Another line of therapies involves treating individual symptoms of CFC syndrome. The focus is on the life-threatening symptoms (cardiac) or those affecting quality of life the most (neurological, gastrointestinal, motor, behavioral, communication).

MEK inhibitor Trametinib was used in patients diagnosed with Noonan syndrome to treat hypertrophic cardiomyopathy (Kiai, Tzemos. 2019).

C-type natriuretic peptide (CNP), a FGFR3-RAF1-MEK/ERK signaling inhibitor has been successfully used to increase body and tail lengths in BRAF CFC mouse model (Inoue et al., 2019).

Those results show that small molecule inhibitors could be used to treat progressive phenotypes of CFC.

“Genetic studies show that the germline pathogenic variants found in RASopathies overlap minimally with the somatic mutations observed in cancer. Biochemically, this usually corresponds to a stronger activation of the cascade in cancer as compared to RASopathies, making the latter one’s attractive targets for MEKi as monotherapy.” (Gelb et al., 2022).

MEK inhibitors were also used to treat seizures in a few patients with RASopathies (BRAF CFC, NF1).

Because MEK inhibitors (especially Trametinib and Selumetinib) have a good safety and efficacy profile in RASopathies, they are good candidates for drug repurposing for CFC4 treatment (Gazzin et al., 2024).

Our primary recommendation would be to employ the Cell Painting method using MEK2 patients’ fibroblasts to perform a drug repurposing screen on kinase-oriented drug library.

Cell Painting is a high-content, multiplexed image-based assay used for cytological profiling (evaluation of the cells’ shape and content). In a Cell Painting assay, up to six fluorescent dyes are used to label different components of the cell including the nucleus, endoplasmic reticulum, mitochondria, cytoskeleton, Golgi apparatus, and RNA.

Drug repurposing is a method of using drug libraries containing registered drugs or preclinically tested drug candidates to find treatment in other diseases.

4. Recommended research paths

Following this research roadmap analysis we would like to recommend the strategic four research paths to be supported by the Foundation:

4.1. Guidelines implementation

Because CFC requires multidisciplinary care from specialists and international consensus on management guidelines was published (Piermont et al., 2014) we recommend implementation of these standards in Polish hospitals in collaboration with Center for Rare Disorders at Medical University of Warsaw. This will secure the current wellbeing of the patients and enable collecting high quality data for the future clinical trials.

4.2. Bioinformatic modelling

3D modelling of the pathogenic variant coded protein structure is a fast way to predict good drug candidates and optimise drug libraries. *In silico* methods might give answers about the gain-of-function character of this mutation. Because the mutated domain is highly conserved there are methods based on the structural similarity between MAP2K2 and other kinases which could be employed here.

4.3. Cell Painting followed by drug repurposing screen

One of the primarily affected tissues in CFC syndrome is skin. Hence we suspect to observe a strong cellular phenotype in fibroblasts. Fibroblasts collection, and assay development phases could benefit from PACS2 Research Foundation experiences with Cell Painting performed by Charles River Laboratories. Taking into account the research progress done in the field of MEK inhibitors we recommend adjusting the drug library to contain more of the kinase inhibitors and similar compounds. This process should be advised by clinical pharmacologists and potentially supported by data from bioinformatic modelling.

4.4. Advanced research using animal models

Animal models are needed for complex therapy development. Creation and validation of the model is a laborious process. To speed up the development of these research tools we recommend collaborating with groups working on *Drosophila* and mouse models of

RASopathies (see section 2). Development a *Drosophila* than mouse model would open a new venue for research.

5. Funding model

We suggest to divide funding into 3 strategic directions:

5.1. Fellowships

Personal stipends directed toward MEK2 researchers to stimulate research paths: 4.1. Guidelines implementation and 4.2. Bioinformatic modelling. We suggest funding at least one yearly stipend for a senior fellow and one (preferably two) for junior fellows.

5.2. Contracted research

Implementation of research path 4.3. “Cell Painting followed by drug repurposing screen” by the CRO (Contract Research Organization), such as Charles River Laboratories.

5.3. Research grants

Calls for research grants for advanced research paths (point 4.4) should be preceded by discussions with the group leaders mentioned in section 2. After that either a contracted or open call should be defined.

6. Literature

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