



**HAL**  
open science

## Dissection of contiguous gene effects for deletions around ERF on chromosome 19

E. Calpena, S. J. McGowan, F. B. Kelly, E. Boudry-Labis, Anne Dieux, R. Harrison, D. Johnson, K. Lachlan, J. E. V. Morton, H. Stewart, et al.

► **To cite this version:**

E. Calpena, S. J. McGowan, F. B. Kelly, E. Boudry-Labis, Anne Dieux, et al.. Dissection of contiguous gene effects for deletions around ERF on chromosome 19. *Human Mutation*, 2021, *Human Mutation*, 42 (7), pp.811-817. 10.1002/humu.24213 . hal-04235739

**HAL Id: hal-04235739**

**<https://hal.univ-lille.fr/hal-04235739v1>**

Submitted on 11 Oct 2023



**HAL** is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



Distributed under a Creative Commons Attribution 4.0 International License

# Dissection of contiguous gene effects for deletions around *ERF* on chromosome 19

Eduardo Calpena<sup>1</sup>  | Simon J. McGowan<sup>2</sup> | Fiona Blanco Kelly<sup>3</sup> |  
Elise Boudry-Labis<sup>4</sup> | Anne Dieux-Coeslier<sup>5</sup> | Rachel Harrison<sup>6</sup> |  
Diana Johnson<sup>7</sup> | Katherine Lachlan<sup>8,9</sup> | Jenny E. V. Morton<sup>10</sup> | Helen Stewart<sup>3</sup> |  
Pradeep Vasudevan<sup>11</sup> | Genomics England Research Consortium |  
Stephen R. F. Twigg<sup>1</sup> | Andrew O. M. Wilkie<sup>1,12</sup> 

<sup>1</sup>Clinical Genetics Group, MRC Weatherall Institute of Molecular Medicine, University of Oxford, Oxford, UK

<sup>2</sup>Centre for Computational Biology, MRC Weatherall Institute of Molecular Medicine, University of Oxford, Oxford, UK

<sup>3</sup>Oxford Centre for Genomic Medicine, Nuffield Orthopaedic Centre, Oxford University Hospitals NHS Foundation Trust, Oxford, UK

<sup>4</sup>UF Cytogénomique des Déficiences Intellectuelles et Anomalies du Développement, Institut de Génétique Médicale, Hôpital Jeanne de Flandre, CHRU Lille, France

<sup>5</sup>CHU Lille, Clinique de Génétique "Guy Fontaine", Lille, France

<sup>6</sup>Nottingham Regional Genetics Service, City Hospital Campus, Nottingham University Hospitals NHS Trust, Nottingham, UK

<sup>7</sup>Sheffield Regional Genetics Services, Sheffield Children's NHS Trust, Sheffield, UK

<sup>8</sup>Wessex Clinical Genetics Service, University Hospital Southampton, Princess Anne Hospital, Southampton, UK

<sup>9</sup>Human Development and Health, Faculty of Medicine, University of Southampton, Southampton, UK

<sup>10</sup>West Midlands Regional Clinical Genetics Service and Birmingham Health Partners, Birmingham Women's and Children's Hospitals NHS Foundation Trust, Birmingham, UK

<sup>11</sup>Leicestershire Genetics Centre, University Hospitals of Leicester NHS Trust, Leicester Royal Infirmary NHS Trust, Leicester, UK

<sup>12</sup>Craniofacial Unit, Oxford University Hospitals NHS Trust, John Radcliffe Hospital, Oxford, UK

**Correspondence:** Andrew O. M. Wilkie,  
Clinical Genetics Group, MRC Weatherall  
Institute of Molecular Medicine, University of  
Oxford, John Radcliffe Hospital, Oxford OX3  
9DS, UK.  
Email: [andrew.wilkie@imm.ox.ac.uk](mailto:andrew.wilkie@imm.ox.ac.uk)

## Funding information

NIHR Oxford Biomedical Research Centre;  
VTCT Foundation; WIMM Strategic Alliance  
awards, Grant/Award Number: G0902418 and  
MC UU 12025

## Abstract

Heterozygous intragenic loss-of-function mutations of *ERF*, encoding an ETS transcription factor, were previously reported to cause a novel craniosynostosis syndrome, suggesting that *ERF* is haploinsufficient. We describe six families harboring heterozygous deletions including, or near to, *ERF*, of which four were characterized by whole-genome sequencing and two by chromosomal microarray. Based on the severity of associated intellectual disability (ID), we identify three categories of *ERF*-associated deletions. The smallest (32 kb) and only inherited deletion included two additional centromeric genes and was not associated with ID. Three larger deletions (264–314 kb) that included at least five further centromeric genes were associated with moderate ID, suggesting that deletion of one or more of these five genes

A list of authors from the Genomics England Research Consortium is provided in the Supplementary Information.

This is an open access article under the terms of the Creative Commons Attribution License, which permits use, distribution and reproduction in any medium, provided the original work is properly cited.

© 2021 The Authors. *Human Mutation* published by Wiley Periodicals LLC

causes ID. The individual with the most severe ID had a more telomerically extending deletion, including *CIC*, a known ID gene. Children found to harbor *ERF* deletions should be referred for craniofacial assessment, to exclude occult raised intracranial pressure.

#### KEYWORDS

CNV, craniosynostosis, *ERF*, haploinsufficiency, intellectual disability, mosaicism

## 1 | BRIEF REPORT

The gene *ERF*, first described in 1995, is located on chromosome 19q13.2 and encodes a member of the ETS family of transcription factors that acts as a key negative regulator of ERK1/2, effectors of the RAS-MAP kinase pathway (von Kriegsheim et al., 2009; Lavoie et al., 2020; Le Gallic et al., 2004; Polychronopoulos et al., 2006; Sgouras et al., 1995). Disease-causing heterozygous loss-of-function variants of *ERF* were first described in 2013, in 12 families segregating features of a newly recognized syndrome (termed *ERF*-related craniosynostosis or craniosynostosis type 4, OMIM# 600775), characterized by premature fusion of the cranial sutures (craniosynostosis), hypertelorism, and mild midface hypoplasia (Twigg et al., 2013). Confirmatory case reports have followed (Chaudhry et al., 2015; Korberg et al., 2020; Lee et al., 2018; Provenzano et al., 2021; Timberlake et al., 2017; Tønne et al., 2020; Yoon et al., 2020), and the clinical features of the disorder were further delineated and summarized in 16 additional families by Glass et al. (2019). In addition to craniosynostosis and facial dysmorphism, additional frequently associated features included Chiari-1 malformation, speech and language delay, poor gross and/or fine motor control, hyperactivity, and poor concentration. Importantly, craniosynostosis was often postnatal in onset, insidious, and progressive with subtle effects on head morphology, resulting in late median age at presentation of 42 months among the probands and, in some instances, permanent visual impairment occurred owing to unsuspected raised intracranial pressure (ICP) (Glass et al., 2019).

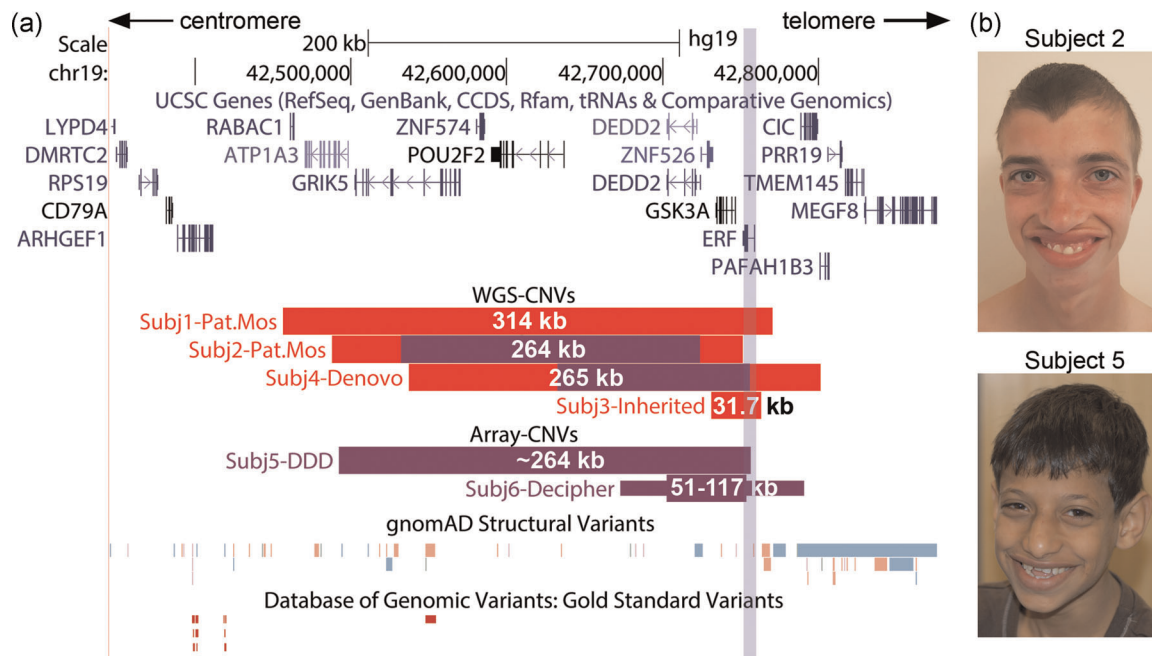
To our knowledge 26 different heterozygous variants in 39 unrelated probands/families have been described in *ERF*-related craniosynostosis. The pattern of *ERF* variants (eight frameshifts, three nonsense, three splice-site, three disrupting the initiation codon, and nine missense localized to the highly conserved DNA-binding domain) is strongly suggestive of a haploinsufficiency mechanism, and this is supported by functional studies of two of the missense variants that demonstrated loss of DNA binding (Twigg et al., 2013). Consistent with this, *ERF* is depleted of loss-of-function variants in the gnomAD database, with an observed/expected ratio of 0.06 (confidence interval 0.02–0.26) and a probability of loss-of-function intolerance (pLI) score of 0.99 (Karczewski et al., 2020).

Although partial or complete heterozygous deletions of *ERF* would be predicted to be associated with a similar pathogenic effect, none has previously been specifically reported. Neither the analysis

of *ERF* dosage using multiplex ligation-dependent probe amplification (MLPA) in 276 samples (Twigg et al., 2013) nor the capture-based targeted resequencing in an additional 156 samples from craniosynostosis cases without a genetic diagnosis (SRFT, unpublished data) identified any pathogenic copy number variant (CNV) affecting *ERF*, indicating either that such deletions are not a frequent cause of craniosynostosis, or that they could produce a more complex/severe syndrome. A few patients have been reported with large chromosome 19q13.2 deletions apparently including *ERF*, although the phenotype was often confounded by the inclusion of *RPS19*, which lies approximately 375 kb centromeric to *ERF*, in patients with Diamond-Blackfan anemia (Farrar et al., 2011; Kuramitsu et al., 2012; Quarello et al., 2008; Yuan et al., 2016) or *ATP1A3*, approximately 250 kb centromeric to *ERF*, in a case with a neurological disorder (Kessi et al., 2018); the names and positions of genes around *ERF* are given in Figure 1a and Table S1. The majority of individuals with large ( $\geq 333$  kb) deletions were reported to have combinations of facial dysmorphism and/or macrocephaly, but “mild craniosynostosis” was noted in one case (Yuan et al., 2016). Here, we describe the identification of six smaller (32–314 kb) deletions at the *ERF* locus, four of them characterized by whole-genome sequencing (WGS) at base-pair resolution, and two by array comparative genomic hybridization (aCGH).

The research elements of the genetic studies were approved by respective Research Ethics Committees (RECs): London–Riverside REC (09/H0706/20 for Genetic Basis of Craniofacial Malformations), East of England–Cambridge South REC (14/EE/1112 for 100,000 Genomes Project [100kGP]).

As part of a broader investigation into the genetic causes of craniosynostosis, we first analyzed the CNV calls (generated by Canvas and Manta; Chen et al., 2016; Roller et al., 2016) from Illumina paired-end read data available from WGS of 128 affected individuals (from 114 families) with craniosynostosis (as the primary phenotype) available in the Research Environment (main programme v10; RR65) of the Genomics England 100kGP. This revealed an apparent heterozygous 314 kb deletion, including *ERF*, in a proband with syndromic multisuture synostosis (Subject 1; Table 1; Figures 1a and S1A); the deletion was also detectable in his clinically unaffected father in a mosaic state; quantification by comparing the numbers of reads within, compared with outside the deletion on chromosome 19 (Figure S1E), indicated that approximately 75% of blood cells harbored the deletion. Previous array CGH in this patient had not



**FIGURE 1** Deletions of 19q13.2 encompassing *ERF*, and associated phenotype. (a) At the top, genes are represented in the UCSC Genome Browser with hg19 coordinates and directions of centromere and telomere indicated. In middle, custom tracks show the positions of deletions characterized by WGS (“WGS-CNVs,” in red) or by aCGH (“Array-CNVs,” in purple). Where aCGH findings were extended by WGS, this is shown by flanking red coloring. For Subject 6, the minimal deleted region is indicated by the thicker bar and the first flanking nondeleted probes with the thinner bar. The bottom two tracks show control population copy number variation (deletions in orange/red, duplications in blue) observed in the gnomAD (Structural Variants, v2.1) and DGV (Gold Standard Variants) databases. The pale blue vertical bar shows the position of *ERF* relative to all tracks. (b) Facial appearance of Subject 2 aged 20 years (above) and Subject 5 aged 10 years (below)

detected the chromosome 19 deletion; however, two other imbalances (one inherited from each parent) had been reported (Table 1; see Supplementary Case Reports for further description of each subject).

To identify additional individuals harboring CNVs at the *ERF* locus, independently of the phenotype, we performed bioinformatic screening of all the 74,008 genomes of participants from families affected with rare disorders available in the 100kGP (main programme v10; RR187). This revealed two additional deletions around *ERF* (Subjects 2 and 3; Table 1, Figures 1a, and S1). The deletion in Subject 2 (264 kb; Figure 1a) had previously been detected by array CGH when it was reported as having arisen de novo; however, closer inspection of the paternal WGS data suggested low levels of mosaicism based on the presence of a few abnormal reads supporting the deletion (Figure S1B). Using the same method as for Subject 1 (Figure S1E), we estimated that approximately 5% of paternal blood cells were mosaic. The deletion in Subject 3 (31.7 kb; Figure 1a) was inherited from his father (Figure S1C), with no indication of mosaicism (Figure S1E). Following informed consent, we obtained DNA samples from each of the family trios and confirmed the previously deduced molecular nature of each deletion by breakpoint-PCR (Table S2) and dideoxy-sequencing (Figure S2). No other causative pathogenic change was identified by 100kGP for any of Subjects 1–3.

In parallel, as part of a clinical genetics investigation, a further de novo deletion including *ERF* was identified by aCGH in Subject 4

(Table 1 and Figure 1a); following informed consent, WGS was carried out using the proband’s DNA to characterize the breakpoints, demonstrating a 265 kb deletion (Figure S1D). There was no evidence of a breakpoint-PCR product in samples from either of the parents of Subject 4, in whom the deletion was quantified as 50%, indicating a de novo origin at conception (Figure S2). Segregation analysis of a rare SNV (chr19:g.42783791G>C, hg19) located within the deleted region established that the deletion arose on the paternal allele (data not shown).

Toward a more comprehensive analysis of genotype–phenotype correlations, additional cases harboring heterozygous deletions around *ERF* that had been identified by aCGH were retrieved from the DECIPHER database (Firth et al., 2009) (Subject 5, ~265 kb; Subject 6, ~51 kb) (Figure 1a), and the respective clinicians/scientists were contacted. However, in Subject 6, an additional confounding chromosomal abnormality was present in the proband (Table 1). Similarly to Subject 1, this rendered it difficult to disentangle the relative contributions of the different chromosome imbalances to the phenotype. Hence, to undertake a detailed genotype–phenotype correlation of deletions surrounding *ERF*, we focused on Subjects 2–5 only. The major clinical features of these four subjects are summarized in Table 1; see Supplementary Case Reports for more detailed information.

Based on the relative size and extent of each deletion, and the degree of associated intellectual disability, we propose that the *ERF*

TABLE 1 Clinical and molecular characterization of subjects harboring ERF deletions

Subject number (ID)	Subject 1 (7125)	Subject 2 (8944)	Subject 3 (8889)	Subject 4 (8939)	Subject 5 (272468)	Subject 6 (381692)
Main phenotype	Syndromic CRS	Syndromic ID	Familial macrocephaly	Syndromic CRS (learning disability of early onset)	Syndromic ID	Syndromic ID
Craniofacial	CRS (S+M+BL), hypertelorism, exorbitism, and macrostomia	narrow face, prominent eyes, mildly high palate, small chin, and low frontal hairline	Macrocephaly and telecanthus (also in father)	CRS (S, not evident on clinical examination)	Microcephaly, long face, and macrostomia	Macrocephaly and mild facial dysmorphism
Intellectual disability	Moderate	Moderate	Not present	Moderate-severe	Moderate	Moderate
Other clinical features	Multiple large freckles	ADHD, Jeavons syndrome	Mild aortic arch hypoplasia	ASD	Short stature and atrial septal defect	
Detection method	GS (not detected on aCGH)	aCGH + GS	GS (not detected on aCGH)	aCGH + GS	aCGH	aCGH
Event	DEL (314 kb)	DEL (264 kb)	DEL (31.7 kb)	DEL (265 kb)	DEL (265 kb)	DEL (51.2 kb)
Coordinates (hg19)	chr19:42456593-42770777	chr19:42488104-42751672	chr19:42731682-42763363	chr19:42537012-42801688	chr19:42492136-42756726 <sup>a</sup>	chr19:42702762-42754032 <sup>a</sup>
Inheritance	De novo mosaic in father (75% blood cells)	De novo mosaic in father (5% blood cells)	Inherited (paternal)	De novo (paternal origin)	Unknown	De novo
Validation (method)	Breakpoint PCR	aCGH [chr19:42532353-42723970]; Breakpoint PCR	Breakpoint PCR	aCGH [chr19:42632509-42756260]; Breakpoint PCR	Independent aCGH	
Additional findings	15q15.3 DEL [chr15:43851119-44048331]x1 pat, 16p13.13p13.11 DUP [chr16:12017784-15551332] x3 mat	11p11.2 DUP [chr11:47,892,568-48,664,526]x3 mat			1q21.1q21.2 (BP3-BP4) de novo DEL [chr1:146641601-147356634]	

Abbreviations: aCGH, array comparative genomic hybridization; ADHD, attention-deficit hyperactivity disorder; ASD, autism spectrum disorder; BL, bilambdoid synostosis; CRS, craniosynostosis; DEL, heterozygous deletion; DUP, heterozygous duplication; GS, genome sequencing; ID, intellectual disability; M, metopic synostosis; S, sagittal synostosis.

<sup>a</sup>Estimated minimum deletion size from aCGH data.



deletions belong to three categories. First, in the case of the smallest deletion (Subject 3, 31.7 kb), which is constitutionally inherited from the father, neither individual has ID. This deletion includes three genes (a small portion of the *ZNF526* 3'-untranslated region (UTR), and whole gene deletion of *GSK3A* and *ERF*), suggesting that possessing a single copy of these genes is not associated with ID.

Second, two of the probands (Subjects 2 and 5, Figure 1b) harbored deletions of apparently similar extent, although only the breakpoints in Subject 2 were confirmed at the sequence level. In addition to deletion of *GSK3A* and *ZNF526*, these deletions include five other genes, *DEDD2*, *POU2F2*, *ZNF574*, *GRIK5*, and *ATP1A3*, extending in a progressively centromeric direction (Figure 1a and Table S1). Only one, *ATP1A3*, is a known disease-associated gene: heterozygous variants have been described in three overlapping neurological disorders, alternating hemiplegia of childhood 2 (OMIM# 614820), rapid-onset dystonia-parkinsonism (dystonia-12; OMIM# 128235), and cerebellar ataxia, areflexia, pes cavus, optic atrophy, and sensorineural hearing loss (CAPOS) syndrome (OMIM# 601338) (Rosewich et al., 2017). Intellectual disability, although reported, is infrequent in these disorders and the causative mutations are typically missense or small in-frame variants (Heinzen et al., 2014; Sweney et al., 2015), with evidence of toxic gain-of-function effects rather than haploinsufficiency (Arystarkhova et al., 2019). Hence, it cannot be assumed that heterozygous deletion of *ATP1A3* would cause moderate ID. Three of the five genes in the extended deletion interval (*ATP1A3*, *GRIK5*, and *POU2F2*) have a pLI score greater than 0.9 (Table S1), indicating evolutionary constraint against loss-of-function alleles (Karczewski et al., 2020). Both Subjects 2 and 5 had a similar degree of moderate ID but were discordant for some other clinical features (notably Jeavons syndrome-type epilepsy in Subject 2). Hence we propose that haploinsufficiency for one or a combination of genes in the *ATP1A3-DEDD2* interval causes moderate ID.

In the third category, the deletion in Subject 4, who has moderate-severe ID and autistic spectrum disorder (ASD), extended more telomeric than any of the other deletions, to encompass the gene *CIC*. Intragenic mutations of *CIC* were previously described in both severe ID and ASD (Guo et al., 2019; Lu et al., 2017), which is likely to explain the more severe ID phenotype in this case.

Although our observations must be regarded as provisional given the small number of cases identified, they represent the beginnings of a map of genotype-phenotype correlations for deletions encompassing *ERF*. Importantly, each deletion appeared unique, with no evidence for a recurrent breakpoint mechanism. In the four cases characterized at the molecular level, most breakpoints occurred in, or in close proximity to, regions rich in repetitive elements, especially *Alu* elements (Figure S3); in three of these, the sequences at the breakpoints show homology of only 2–3 nucleotides (cases 1, 2, and 4; Figure S3), indicating nonhomologous end-joining as the most likely mechanism. In Subject 3, however, nonallelic homologous recombination between two *Alu* elements (*AluY* and *AluSx*) evidently occurred (Figure S3). Of note, the aCGH originally used to identify the deletion in Subject 4 suggested a smaller extent of deletion, not

including *CIC*, in contrast to the larger 265 kb deletion determined by WGS. Moreover, the aCGH in the parents of Subject 2 had suggested that the deletion arose de novo in the child, whereas WGS demonstrated a low level of mosaicism in the father. These two examples illustrate the added value provided by WGS, both for refining molecular diagnoses and for greater precision in recurrence risks.

From a clinical point of view, deletion or functional disruption of the *ERF* gene itself is likely to account for the mild dysmorphic facial features (including variable hypertelorism, exorbitism, and macrostomia) in these individuals (Figure 1b). Importantly, *ERF* haploinsufficiency may predispose to an insidious presentation of craniosynostosis and raised intracranial pressure, without any noticeable change in skull shape (Glass et al., 2019; Twigg et al., 2013). Consequently, we recommend that all children found to harbor *ERF* deletions are referred for three-dimensional computed tomography scanning of the skull. The value of this is demonstrated by Subject 4, who was revealed to have occult sagittal synostosis and pathologically raised ICP. In this individual, sleep apnea associated with enlarged adenoids appeared to be contributing to this symptomatology, and adenotonsillectomy led to the apparent improvement in respiratory function and a burst in newly acquired language skills (Supplementary Case Reports). Clearly, amelioration of potentially reversible causes of learning or behavioral disability is particularly critical when deletion of contiguous genes may in addition be contributing to ID.

## ACKNOWLEDGMENTS

We thank all the families for their participation in this study. We thank Alistair Pagnamenta for initial help with interrogating 100,000 Genomes Project data, Florence Petit for information from DECIPHER, and the MRC-WIMM facility for DNA sequencing. This study was supported by the NIHR Oxford Biomedical Research Centre (AOMW) and NIHR UK Rare Genetic Disease Research Consortium, the MRC through the WIMM Strategic Alliance (G0902418 and MC UU 12025), and the VTCT Foundation (SRFT, AOMW). This study was made possible, in part, through access to the data and findings generated by the 100,000 Genomes Project. The 100,000 Genomes Project is managed by Genomics England Limited (a wholly-owned company of the Department of Health and Social Care). The 100,000 Genomes Project is funded by the NIHR and National Health Service (NHS) England. Wellcome, Cancer Research UK and the MRC have also funded research infrastructure. The 100,000 Genomes Project uses data provided by patients and collected by the NHS as part of their care and support. This study also makes use of data generated by the DECIPHER community. A full list of centers that contributed to the generation of the data is available from <https://decipher.sanger.ac.uk/about/stats> and via email from [decipher@sanger.ac.uk](mailto:decipher@sanger.ac.uk). Funding for the DECIPHER project was provided by Wellcome. The views expressed in this publication are those of the authors and not necessarily those of Wellcome, NIHR, or the Department of Health and Social Care.

## CONFLICT OF INTERESTS

All the authors declare that there are no conflict of interests.

## AUTHOR CONTRIBUTIONS

Fiona Blanco Kelly, Anne Dieux-Coeslier, Rachel Harrison, Diana Johnson, Katherine Lachlan, Jenny E V Morton, Helen Stewart, Pradeep Vasudevan, and Andrew Wilkie undertook patient recruitment and assessment. The Genomics England Research Consortium undertook genome sequencing and Elise Boudry-Labis analyzed array CGH data. Eduardo Calpena undertook most of the bioinformatics and experimental analysis, with input from Simon McGowan and Stephen Twigg. Eduardo Calpena and Andrew Wilkie drafted the manuscript, with the assistance of all other authors. All authors approved the final draft and are accountable for the accuracy of the manuscript.

## DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon request. Information about the identified deletions around *ERF* has been submitted to the ClinVar database (SUB9501313; ClinVar accessions SCV001571604-SCV001571609).

## ORCID

Eduardo Calpena  <http://orcid.org/0000-0001-6399-6528>

Andrew O. M. Wilkie  <https://orcid.org/0000-0002-2972-5481>

## REFERENCES

- Arystarkhova, E., Haq, I. U., Luebbert, T., Mochel, F., Saunders-Pullman, R., Bressman, S. B., Feschenko, P., Salazar, C., Cook, J. F., Demarest, S., Brashear, A., Ozelius, L. J., & Sweadner, K. J. (2019). Factors in the disease severity of ATP1A3 mutations: Impairment, misfolding, and allele competition. *Neurobiology of Disease*, 132, 104577. <https://doi.org/10.1016/j.nbd.2019.104577>
- Chaudhry, A., Sabatini, P., Han, L., Ray, P. N., Forrest, C., & Bowdin, S. (2015). Heterozygous mutations in *ERF* cause syndromic craniosynostosis with multiple suture involvement. *American Journal of Medical Genetics. Part A*, 167A(11), 2544–2547. <https://doi.org/10.1002/ajmg.a.37218>
- Chen, X., Schulz-Trieglaff, O., Shaw, R., Barnes, B., Schlesinger, F., Källberg, M., Cox, A. J., Kruglyak, S., & Saunders, C. T. (2016). Manta: Rapid detection of structural variants and indels for germline and cancer sequencing applications. *Bioinformatics*, 32(8), 1220–1222. <https://doi.org/10.1093/bioinformatics/btv710>
- Farrar, J. E., Vlachos, A., Atsidaftos, E., Carlson-Donohoe, H., Markello, T. C., Arceci, R. J., Ellis, S. R., Lipton, J. M., & Bodine, D. M. (2011). Ribosomal protein gene deletions in Diamond-Blackfan anemia. *Blood*, 118(26), 6943–6951. <https://doi.org/10.1182/blood-2011-08-375170>
- Firth, H. V., Richards, S. M., Bevan, A. P., Clayton, S., Corpas, M., Rajan, D., Van Vooren, S., Moreau, Y., Pettett, R. M., & Carter, N. P. (2009). DECIPHER: Database of chromosomal imbalance and phenotype in humans using Ensembl resources. *American Journal of Human Genetics*, 84(4), 524–533. <https://doi.org/10.1016/j.ajhg.2009.03.010>
- Le Gallic, L., Virgilio, L., Cohen, P., Biteau, B., Mavrothalassitis G. (2004). *ERF* nuclear shuttling, a continuous monitor of Erk activity that links it to cell cycle progression. *Molecular and Cellular Biology*, 24(3), 1206–1218. <https://doi.org/10.1128/mcb.24.3.1206-1218.2004>
- Glass, G. E., O'Hara, J., Canham, N., Cilliers, D., Dunaway, D., Fenwick, A. L., Jeelani, N. O., Johnson, D., Lester, T., Lord, H., Morton, J., Nishikawa, H., Noons, P., Schwiebert, K., Shipster, C., Taylor-Beadling, A., Twigg, S., Vasudevan, P., Wall, S. A., ... Wilson, L. C. (2019). *ERF*-related craniosynostosis: The phenotypic and developmental profile of a new craniosynostosis syndrome. *American Journal of Medical Genetics. Part A*, 179(4), 615–627. <https://doi.org/10.1002/ajmg.a.61073>
- Guo, H., Duyzend, M. H., Coe, B. P., Baker, C., Hoekzema, K., Gerdt, J., Turner, T. N., Zody, M. C., Beighley, J. S., Murali, S. C., Nelson, B. J., University of Washington Center for Mendelian, G., Bamshad, M. J., Nickerson, D. A., Bernier, R. A., & Eichler, E. E. (2019). Genome sequencing identifies multiple deleterious variants in autism patients with more severe phenotypes. *Genetics in Medicine*, 21(7), 1611–1620. <https://doi.org/10.1038/s41436-018-0380-2>
- Heinzen, E. L., Arzimanoglou, A., Brashear, A., Clapcote, S. J., Gurrieri, F., Goldstein, D. B., Jóhannesson, S. H., Mikati, M. A., Neville, B., Nicole, S., Ozelius, L. J., Poulsen, H., Schyns, T., Sweadner, K. J., van den Maagdenberg, A., Vilsen, B., & ATP1A3 Working, G. (2014). Distinct neurological disorders with ATP1A3 mutations. *Lancet Neurology*, 13(5), 503–514. [https://doi.org/10.1016/S1474-4422\(14\)70011-0](https://doi.org/10.1016/S1474-4422(14)70011-0)
- Karczewski, K. J., Francioli, L. C., Tiao, G., Cummings, B. B., Alfoldi, J., Wang, Q., Collins, R. L., Laricchia, K. M., Ganna, A., Birnbaum, D. P., Gauthier, L. D., Brand, H., Solomonson, M., Watts, N. A., Rhodes, D., Singer-Berk, M., England, E. M., Seaby, E. G., Kosmicki, J. A., ... MacArthur, D. G. (2020). The mutational constraint spectrum quantified from variation in 141,456 humans. *Nature*, 581(7809), 434–443. <https://doi.org/10.1038/s41586-020-2308-7>
- Kessi, M., Xiong, J., Wu, L., Yang, L., He, F., Chen, C., Pang, N., Duan, H., Zhang, W., Arafat, A., Yin, F., & Peng, J. (2018). Rare copy number variations and predictors in children with intellectual disability and epilepsy. *Frontiers in Neurology*, 9, 947. <https://doi.org/10.3389/fneur.2018.00947>
- Korberg, I., Nowinski, D., Bondeson, M. L., Melin, M., Kolby, L., & Stattin, E. L. (2020). A progressive and complex clinical course in two family members with *ERF*-related craniosynostosis: A case report. *BMC Medical Genetics*, 21(1), 90. <https://doi.org/10.1186/s12881-020-01015-z>
- von Kriegsheim, A., Baiocchi, D., Birtwistle, M., Sumpton, D., Bienvenut, W., Morrice, N., Yamada, K., Lamond, A., Kalna, G., Orton, R., Gilbert, D., & Kolch, W. (2009). Cell fate decisions are specified by the dynamic ERK interactome. *Nature Cell Biology*, 11(12), 1458–1464. <https://doi.org/10.1038/ncb1994>
- Kuramitsu, M., Sato-Otsubo, A., Morio, T., Takagi, M., Toki, T., Terui, K., Wang, R., Kanno, H., Ohga, S., Ohara, A., Kojima, S., Kitoh, T., Goi, K., Kudo, K., Matsubayashi, T., Mizue, N., Ozeki, M., Masumi, A., Momose, H., ... Hamaguchi, I. (2012). Extensive gene deletions in Japanese patients with Diamond-Blackfan anemia. *Blood*, 119(10), 2376–2384. <https://doi.org/10.1182/blood-2011-07-368662>
- Lavoie, H., Gagnon, J., & Therrien, M. (2020). ERK signalling: A master regulator of cell behaviour, life and fate. *Nature Reviews Molecular Cell Biology*, 21(10), 607–632. <https://doi.org/10.1038/s41580-020-0255-7>
- Lee, E., Le, T., Zhu, Y., Elakis, G., Turner, A., Lo, W., Venselaar, H., Verrenkamp, C. A., Snow, N., Mowat, D., Kirk, E. P., Sachdev, R., Smith, J., Brown, N. J., Wallis, M., Barnett, C., McKenzie, F., Freckmann, M. L., Collins, F., ... Roscioli, T. (2018). A craniosynostosis massively parallel sequencing panel study in 309 Australian and New Zealand patients: Findings and recommendations. *Genetics in Medicine*, 20(9), 1061–1068. <https://doi.org/10.1038/gim.2017.214>
- Lu, H. C., Tan, Q., Rousseaux, M. W., Wang, W., Kim, J. Y., Richman, R., Wan, Y. W., Yeh, S. Y., Patel, J. M., Liu, X., Lin, T., Lee, Y., Fryer, J. D., Han, J., Chahrouh, M., Finnell, R. H., Lei, Y., Zurita-Jimenez, M. E., Ahimaz, P., ... Zoghbi, H. Y. (2017). Disruption of the ATXN1-C1C complex causes a spectrum of neurobehavioral phenotypes in mice and humans. *Nature Genetics*, 49(4), 527–536. <https://doi.org/10.1038/ng.3808>

- Polychronopoulos, S., Verykokakis, M., Yazicioglu, M. N., Sakarellos-Daitsiotis, M., Cobb, M. H., & Mavrothalassitis, G. (2006). The transcriptional ETS2 repressor factor associates with active and inactive Erks through distinct FXF motifs. *Journal of Biological Chemistry*, 281(35), 25601–25611. <https://doi.org/10.1074/jbc.M605185200>
- Provenzano, A., La Barbera, A., Scagnet, M., Pagliuzzi, A., Traficante, G., Pantaleo, M., Tiberi, L., Vergani, D., Kurtas, N. E., Guarducci, S., Bargiacchi, S., Forzano, G., Artuso, R., Palazzo, V., Kura, A., Giordano, F., di Feo, D., Mortilla, M., De Filippi, C., ... Giglio, S. (2021). Chiari 1 malformation and exome sequencing in 51 trios: The emerging role of rare missense variants in chromatin-remodeling genes. *Human Genetics*, 140(4), 625–647. <https://doi.org/10.1007/s00439-020-02231-6>
- Quarello, P., Garelli, E., Brusco, A., Carando, A., Pappi, P., Barberis, M., Coletti, V., Campagnoli, M. F., Dianzani, I., & Ramenghi, U. (2008). Multiplex ligation-dependent probe amplification enhances molecular diagnosis of Diamond-Blackfan anemia due to RPS19 deficiency. *Haematologica*, 93(11), 1748–1750. <https://doi.org/10.3324/haematol.13423>
- Roller, E., Ivakhno, S., Lee, S., Royce, T., & Tanner, S. (2016). Canvas: Versatile and scalable detection of copy number variants. *Bioinformatics*, 32(15), 2375–2377. <https://doi.org/10.1093/bioinformatics/btw163>
- Rosewich, H., Sweney, M. T., DeBrosse, S., Ess, K., Ozelius, L., Andermann, E., Andermann, F., Andrasco, G., Belgrade, A., Brashear, A., Ciccocicola, S., Egan, L., George AL, Jr., Lewelt, A., Magelby, J., Merida, M., Newcomb, T., Platt, V., Poncelin, D., ... Swoboda, K. (2017). Research conference summary from the 2014 International Task Force on ATP1A3-related disorders. *Neurology: Genetics*, 3(2), e139. <https://doi.org/10.1212/NXG.000000000000139>
- Sgouras, D. N., Athanasiou, M. A., Beal, G. J., Jr., Fisher, R. J., Blair, D. G., & Mavrothalassitis, G. J. (1995). ERF: an ETS domain protein with strong transcriptional repressor activity, can suppress ets-associated tumorigenesis and is regulated by phosphorylation during cell cycle and mitogenic stimulation. *EMBO Journal*, 14(19), 4781–4793. Retrieved from <https://www.ncbi.nlm.nih.gov/pubmed/7588608>.
- Sweney, M. T., Newcomb, T. M., & Swoboda, K. J. (2015). The expanding spectrum of neurological phenotypes in children with ATP1A3 mutations, alternating hemiplegia of childhood, rapid-onset dystonia-parkinsonism, CAPOS and beyond. *Pediatric Neurology*, 52(1), 56–64. <https://doi.org/10.1016/j.pediatrneurol.2014.09.015>
- Timberlake, A. T., Furey, C. G., Choi, J., Nelson-Williams, C., Yale Center for Genome, A., Yale Center for Genome, A., Loring, E., Galm, A., Kahle, K. T., Steinbacher, D. M., Larysz, D., Persing, J. A., & Lifton, R. P. (2017). De novo mutations in inhibitors of Wnt, BMP, and Ras/ERK signaling pathways in non-syndromic midline craniosynostosis. *Proceedings of the National Academy of Sciences of the United States of America*, 114(35), E7341–E7347. <https://doi.org/10.1073/pnas.1709255114>
- Twigg, S. R., Vorgia, E., McGowan, S. J., Peraki, I., Fenwick, A. L., Sharma, V. P., Allegra, M., Zaragoulis, A., Sadighi Akha, E., Knight, S. J., Lord, H., Lester, T., Izatt, L., Lampe, A. K., Mohammed, S. N., Stewart, F. J., Verloes, A., Wilson, L. C., Healy, C., ... Wilkie, A. O. (2013). Reduced dosage of ERF causes complex craniosynostosis in humans and mice and links ERK1/2 signaling to regulation of osteogenesis. *Nature Genetics*, 45(3), 308–313. <https://doi.org/10.1038/ng.2539>
- Tønne, E., Due-Tønnessen, B. J., Mero, I. L., Wiig, U. S., Kulseth, M. A., Vigeland, M. D., Sheng, Y., von der Lippe, C., Tveten, K., Meling, T. R., Helseth, E., & Heimdal, K. R. (2020). Benefits of clinical criteria and high-throughput sequencing for diagnosing children with syndromic craniosynostosis. *European Journal of Human Genetics*. <https://doi.org/10.1038/s41431-020-00788-4>
- Yoon, J. G., Hahn, H. M., Choi, S., Kim, S. J., Aum, S., Yu, J. W., Park, E. K., Shim, K. W., Lee, M. G., & Kim, Y. O. (2020). Molecular diagnosis of craniosynostosis using targeted next-generation sequencing. *Neurosurgery*, 87(2), 294–302. <https://doi.org/10.1093/neuros/nyz470>
- Yuan, H., Meng, Z., Liu, L., Deng, X., Hu, X., & Liang, L. (2016). A de novo 1.6Mb microdeletion at 19q13.2 in a boy with Diamond-Blackfan anemia, global developmental delay and multiple congenital anomalies. *Molecular Cytogenetics*, 9, 58. <https://doi.org/10.1186/s13039-016-0268-2>

## SUPPORTING INFORMATION

Additional Supporting Information may be found online in the supporting information tab for this article.

**How to cite this article:** Calpena, E., McGowan, S. J., Blanco Kelly, F., Boudry-Labis, E., Dieux-Coeslier, A., Harrison, R., Johnson, D., Lachlan, K., Morton, J. E. V., Stewart, H., Vasudevan, P., Genomics England Research Consortium, Twigg, S. R. F., & Wilkie, A. O. M. (2021). Dissection of contiguous gene effects for deletions around *ERF* on chromosome 19. *Human Mutation*, 42, 811–817. <https://doi.org/10.1002/humu.24213>