Dissection of contiguous gene effects for deletions around \textit{ERF} on chromosome 19

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Abstract
Heterozygous intragenic loss-of-function mutations of \textit{ERF}, encoding an ETS transcription factor, were previously reported to cause a novel craniosynostosis syndrome, suggesting that \textit{ERF} is haploinsufficient. We describe six families harboring heterozygous deletions including, or near to, \textit{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 \textit{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...
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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; Laviole 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 (≥333 kb) deletions were reported to have combinations of facial dysmorphism and/or macrocephaly, but “small 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 Canvus 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
detected the chromosome 19 deletion; however, two other im-
balances (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 pro-
gramme v10; RR187). This revealed two additional deletions around
ERF (Subjects 2 and 3; Table1, Figures 1a, and S1). The deletion in
Subject 2 (264 kb; Figure1a) 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 mosai-
cism based on the presence of a few abnormal reads supporting
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)

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 car-
rried out using the proband’s DNA to characterize the breakpoints,
demonstrating a 265 kb deletion (Figure S1D). There was no evi-
dence 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 pa-
ternal 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) (Figure1a), 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 sum-
marized in Table 1; see Supplementary Case Reports for more de-
tailed information.

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

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)
| 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         | 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         | chr19:42456593-42770777 | chr19:42488104-42751672 | chr19:42731682-42763363 | chr19:42537012-42801688 | [chr19:42492136-42756726] | [chr19:42702762-42754032] |
| 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.

*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, POUF2F2, ZNF574, GRIKS, 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) (Rosenwix 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; Sweeney 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, GRIKS, and POUF2F2) 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 macrosomia) 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.

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CONFLICT OF INTERESTS

All the authors declare that there are no conflict of interests.
AUTHOR CONTRIBUTIONS
Fiona Blanco Kelly, Anne Dieux-Coelsier, 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 from the corresponding author upon request. Information about the manuscript.

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., Sabatinil, 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., Atsidasfas, E., Carlson-Donohoe, H., Markello, T. C., Arcetti, 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., Bitezou, 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’Har, J., Canham, N., Cilliars, D., Dunaway, D., Fenwick, A. L., Jeelani, N. O., Johnson, D., Lester, T., Lord, H., Morton, J., Nishikawa, H., Nools, P., Schwibert, K., Shipster, C., Taylor-Beading, 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., Duyzendt, M. H., Cae, B. P., Baker, C., Hoekzema, K., Gerdts, J., Turner, T. N., Zody, M. C., Beighley, J. S., Murali, S. C., Nelson, B. J., University of Washington Center for Mendelian, G., Banishad, 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., Jøhnnessen, S. H., Mikati, M. A., Neville, B., Nicole, S., Ozelius, L. J., Poulsens, 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

Karczewski, K. J., Francioli, L. C., Tiao, G., Cummings, B. B., Alföldi, J., Wang, Q., Collins, R. L., Laricchia, K. M., Ganna, A., Birnbaum, D. P., Gautier, 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/neur.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., Ogah, 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-2011-07-36662

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., Veneselaar, 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., Chahour, M., Finnell, R. H., Lei, Y., Zurita-Jimenez, M. E., Ahimaz, P., ... Zoghbi, H. Y. (2017). Disruption of the ATXN1-CIC network 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-Daitisiotis, 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., Scagni, M., Pagliazzi, A., Traffante, G., Pantaleo, M., Tiberi, L., & Guarducci, S., Bargiacchi, S., Forzano, G., Artuso, R., Palazzo, V., Kura, A., Giordano, F., di Feo, D., Martini, M., De Filippis, C., ... Giglio, S. (2021). Chiar1 mutation 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., Sweeney, M., DeBrosse, S., Ess, K., Ozelius, L., Andermann, E., Andermann, F., Andrasco, G., Belgrade, A., Brashear, A., Ciccodicola, 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.0000000000000139

Sgouras, D. N., Athanasou, M. A., Beal, G. J., Jr., Fisher, R. J., Blair, D. G., & Mavrothalassitis, G. J. (1993). 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/7586068.

Sweeney, 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.1702551114

Twigg, S. R., Vorgia, E., McGowan, S. J., Peraki, I., Fenwick, A. L., Sharma, V. P., Allegra, M., Zaragkoulas, A., Sadigidi 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., Wig, 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 1.6 Mb 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

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