UNIVERSITÀ DEGLI STUDI DI TRIESTE

Exome sequencing efficacy and phenotypic expansions involving esophageal atresia/tracheoesophageal fistula plus

This is the peer reviewed (post-print) version of the following article:

Original
Exome sequencing efficacy and phenotypic expansions involving esophageal atresia/tracheoesophageal fistula plus / Sy, M.R., Chauhan, J., Prescott, K., Imam, A., Kraus, A., Beleza, A., Salkeld, L., Hosdurga, S., Parker, M., Vasudevan, P., Islam, L., Goel, H., Bain, N., Park, S., Mohammed, S., Dieterich, K., Coutton, C., Satre, V., Vieville, G., Donaldson, A., et al.. - In: AMERICAN JOURNAL OF MEDICAL GENETICS. PART A. - ISSN 1552-4825. - 188:12(2022), pp. 3492-3504. [10.1002/ajmg.a.62976]

Availability:
This version is available at: 11368/3032878 since: 2022-10-31T14:39:58Z
Publisher:

Published
DOI:10.1002/ajmg.a.62976
Terms of use:

Some rights reserved. More details in the PostPrint rights section below.

License:
Digital Rights Management not defined

Publisher copyright

-.-

(Article begins on next page)

12 October 2026

arTs Università degli Studi di Trieste
Archivio della ricerca – postprint

Exome sequencing efficacy and phenotypic expansions involving esophageal atresia/tracheoesophageal fistula plus

Mary R. Sy[1] | Jaynee Chauhan[2] | Katrina Prescott[2] | Aliza Imam[2] | Alison Kraus[2] | Ana Beleza[3] | Lee Salkeld[4] | Saraswati Hosdurga[5] | Michael Parker[6] | Pradeep Vasudevan[7] | Lily Islam[8] | Himanshu Goel[9,10] | Nicole Bain[11] | Soo-Mi Park[12] | Shehla Mohammed[13] | Klaus Dieterich[14,15] | Charles Coutton[14,16] | Véronique Satre[14,16] | Gaëlle Vieville[14] | Alan Donaldson[17] | Claire Beneteau[18] | Jamal Ghoumid[19] | Kris Van Den Bogaert[20] | Anneleen Boogaerts[20] | Elise Boudry[21] | Clémence Vanlerberghe[19] | Florence Petit[19] | Laura Bernardini[22] | Barbara Torres[22] | Teresa Mattina[23,24] | Diana Carli[25] | Giorgia Mandrile[26] | Michele Pinelli[27,28] | Nicola Brunetti-Pierri[28,29] | Katherine Neas[30] | Rachel Beddow[31] | Pernille M. Tørring[32] | Flavio Faletra[33] | Beatrice Spedicati[34] | Paolo Gasparini[33,34] | Alessandro Mussa[25,35] | Giovanni Battista Ferrero[36] | Anne Lampe[37] | Wayne Lam[38] | Weimin Bi[1,39] | Carlos A. Bacino[1] | Akela Kuwahara[40,41,42] | Jeffrey O. Bush[40,41,42] | Xiaonan Zhao[1,39] | Pamela N. Luna[1] | Chad A. Shaw[1] | Jill A. Rosenfeld[1] | Daryl A. Scott[1,43]

Abstract

Esophageal atresia/tracheoesophageal fistula (EA/TEF) is a life-threatening birth defect that often occurs with other major birth defects (EA/TEF+). Despite advances in genetic testing, a molecular diagnosis can only be made in a minority of EA/TEF+ cases. Here, we analyzed clinical exome sequencing data and data from the DECIPHER database to determine the efficacy of exome sequencing in cases of EA/TEF+ and to identify phenotypic expansions involving EA/TEF. Among 67 individuals with EA/TEF+ referred for clinical exome sequencing, a definitive or probable diagnosis was made in 11 cases for an efficacy rate of 16% (11/67). This efficacy rate is significantly lower than that reported for other major birth defects, suggesting that polygenic, multifactorial, epigenetic, and/or environmental factors may play a particularly important role in EA/TEF pathogenesis. Our cohort included individuals with pathogenic or likely pathogenic variants that affect *TCF4* and its downstream target *NRXN1*, and *FANCA*, *FANCB*, and *FANCC*, which are associated with Fanconi anemia.

These cases, previously published case reports, and comparisons to other EA/TEF genes made using a machine learning algorithm, provide evidence in support of a potential pathogenic role for these genes in the development of EA/TEF.

KEYWORDS
esophageal atresia, exome sequencing, Fanconi anemia, *NRXN1*, *TCF4*, tracheoesophageal fistula

1 | INTRODUCTION

Esophageal atresia (EA) and tracheoesophageal fistula (TEF) are common, life-threatening birth defects seen in ~1:3500–1:4500 live births (Depaepe et al., 1993; Lupo et al., 2017). These defects arise from failure of the proximal foregut to properly separate into distinct respiratory (ventral) and gastrointestinal (dorsal) tubes between 28 days and 37 days postfertilization (Ioannides & Copp, 2009). In the majority of cases, EA and TEF occur together, with isolated EA accounting for ~8% of cases, and isolated H-type TEF with no EA accounting for ~3% of cases (Scott, 1993). EA/TEF is associated with high rates of mortality, which correlate positively with the coexistence of other structural birth defects, and significant morbidity, including the need for multiple hospitalizations and surgeries, feeding intolerance, gastroesophageal reflux, failure to thrive, and recurrent infections (Little et al., 2003; Seo et al., 2010; Sulkowski et al., 2014).

Between 50% and 84% of individuals with EA/TEF have at least one additional congenital anomaly (EA/TEF+) with congenital heart defects (CHD) being particularly common (Cassina et al., 2016; Seo et al., 2010; Sulkowski et al., 2014). A subset of individuals with EA/TEF+ can be described as having VACTERL association, which describes the nonrandom association of vertebral (V), anal (A), cardiac (C), TEF with esophageal atresia (TE), renal (R), and limb (L) anomalies (MIM# 192350). In a subset of cases, a large chromosomal disorder, a microdeletion/microduplication, or a single gene disorder can be identified as the cause (Brosens et al., 2016; Brosens, de Jong, et al., 2014; Felix et al., 2007; Scott, 1993). Common examples include trisomy 18, DiGeorge syndrome (MIM# 188400) caused by recurrent microdeletions of chromosome 22q11.2, and CHARGE syndrome (MIM# 214800) caused by pathogenic variants in *CHD7*. Failure to identify an underlying cause in the majority of EA/TEF cases is likely due to failure to employ optimal genetic testing, limited knowledge about the genes that cause EA/TEF, and/or the role of multifactorial or nongenetic factors in the development of EA/TEF (Brosens, Ploeg, et al., 2014).

Here, we analyze a clinical database of ~17,000 exome sequencing test results to determine the diagnostic efficacy of exome sequencing in individuals with EA/TEF+. We then use data from this clinical cohort and individuals cataloged in the DECIPHER database to identify associations between disease-causing genes and EA/TEF+.

2 | MATERIALS AND METHODS

2.1 | Database analysis and clinical review

We searched coded information from a clinical database of ~17,000 individuals who were referred to Baylor Genetics for exome sequencing to identify individuals with EA/TEF+ based on the phenotypes included in their test indications (Table S1). Individuals for whom a molecular diagnosis was made using array-based copy number variant (CNV) detection assays or other genetic tests were not included in this study. Data for this cohort did not contain information on whether exome sequencing was performed on a proband, duo, or trio basis. Among the 18 individuals in which a molecular finding was reported, inheritance data suggests that no parental samples were submitted for 2 subjects, at least 1 parental sample was submitted for 5 subjects, and both parental samples were submitted for 11 subjects. One individual in the cohort with TEF had a sister with TEF, and the cohort included two brothers, both of whom had TEF. None of these individuals had a molecular finding reported on their exome sequencing report.

We also searched for individuals with EA/TEF who carried sequence variants, or rare, small (<2 Mb, and containing 1–20 protein-coding genes) CNVs cataloged in the DECIPHER database (Firth et al., 2009). Contact was made with each of the submitting centers who then approved the publication of their patient's clinical and molecular data (Tables S2 and S3). Subject S32 is part of the Deciphering Developmental Disorders (DDD) Study. Subjects S35 and S45 were previously published by Mattioli et al. (2017) and Umana et al. (2011), respectively.

Variants reported by Baylor Genetics to be potentially related to the clinical phenotypes listed in the indication for ES testing, and sequence variants reported in DECIPHER, were reanalyzed and classified as pathogenic, likely pathogenic, or variants of uncertain significance based on the American College of Medical Genetics and Genomics (ACMG) standards for variant interpretation using the most current data available (Richards et al., 2015). Each potential diagnosis was then designated as definitive, probable, or provisional based on previously published criteria set forth by Scott et al. (2021). These criteria take into account the ACMG classification of the variant(s), the inheritance pattern within the family, variant configuration (cis vs. trans), the sex of the proband, and the overlap between the phenotypes listed in the indication and phenotypes previously shown to be associated with disorders caused by the affected gene.

2.2 | Calculating diagnostic efficacy

The number of EA/TEF+ cases with a definitive or probable molecular diagnosis was divided by the total number of cases to determine the efficacy rate. This process was then repeated for the subset of EA/TEF+ cases in which CHD was also included in the indication, and the subset of EA/TEF+ cases in which the criteria for VACTERL association were fulfilled (at least two defining phenotypes in addition to EA/TEF) based on the phenotypes listed in the indication.

2.3 | Literature and database searches

Genes affected by putatively deleterious variants that have not been clearly associated with the development of EA/TEF, were designated as EA/TEF candidate genes. We subsequently searched the literature for reports in which each candidate gene, and/or their associated genetic disorder(s), were mentioned in conjunction with EA and/or TEF. We also searched the Mouse Genome Informatics database (MGI; http://www.informatics.jax.org/) to determine if EA and/or TEF were identified in mice bearing deleterious alleles in the mouse homologs of these candidate genes (Blake et al., 2011).

2.4 | Machine learning

We have previously developed a machine learning algorithm that integrates knowledge from genome-scale data sources including Gene Ontology, the MGI, the Protein Interaction Network Analysis platform, the GeneAtlas expression distribution, and transcription factor binding and epigenetic histone modifications data from NIH Roadmap Epigenomics Mapping Consortium, to rank genes based on their similarity to a set of training genes known to cause a phenotype of interest (Callaway et al., 2018; Campbell et al., 2013; Scott et al., 2021). Briefly, the method is a supervised analysis procedure that employs the input training set of known disease genes to construct a pattern in genomic feature space and ranks genes with respect to their similarity to the training set using quantitative similarity metrics. For protein–protein interactions, the method considers the number of paths from the protein encoded by a target gene to the proteins encoded by the training genes using proteome-wide protein interaction network data (Campbell et al., 2014), and then compares this path counts to a genome-wide distribution. For the other knowledge sources, a centroid is created by the mean characteristics of the training set, be they annotations or tissue expression patterns. A multivariate distance metric motivated by Mahalanobis distance computes the similarity of the feature characteristics of a target gene, as annotated in the knowledge source, to this centroid. Each gene is then ranked from highest to lowest according to its similarity to the training set.

To generate EA/TEF-specific pathogenicity scores for all RefSeq genes, we trained this machine learning algorithm with a set of 42 manually curated human genes that are known to cause EA/TEF or whose mouse homologs have been shown to cause EA/TEF. This EA/TEF training gene set included *AAAS*, *AXIN1*, *CHD7*, *CHRD*, *CPLANE2*, *CTNNB1*, *DYNC2H1*, *EFNB2*, *EFTUD2*, *FGFR3*, *FOXF1*, *FUZ*, *GDF3*, *GDF6*, *GLI2*, *GLI3*, *IFT172*, *MKKS*, *MYCN*, *NDN*, *NKX2-1*, *NKX3-2*, *NOG*, *PAX2*, *PCSK5*, *POR*, *RAB25*, *SHH*, *SNRPN*, *SOX17*, *SOX2*, *SOX4*, *TBX1*, *TBX5*, *TCOF1*, *TERC*, *UBE3A*, *UPK3A*, *VANGL1*, *WDR35*, *WHSC1*, and *WNT3* (Blake et al., 2011; Brosens, Ploeg, et al., 2014). The inclusion of genes shown to cause EA/TEF in mice was supported by the strong biological evidence of causality demonstrated in these mouse models. The addition of these genes to the training gene set also resulted in an improvement in cross-validation scores (data not shown).

Cross-validation studies were performed to test the performance of our machine learning procedure (Callaway et al., 2018; Campbell et al., 2013). First, the full set of training genes was randomly broken into four subsets of approximately equal size. The machine learning procedure was then trained on each respective set, and a genome-wide evaluation of all genes was performed including the excluded subset of training genes. The percentiles of the excluded genes were then recorded to assess performance. The procedure was repeated, reciprocally, so that all training genes received cross-validated scores.

To visualize the performance of the procedure, we tabulated the fraction of EA/TEF training set genes with percentile scores exceeding various cutoffs. This allowed us to generate receiver operating characteristic (ROC) style curves where the effectiveness of the procedure corresponds to the area between the curve and a diagonal line which represents the result that would be generated by chance alone (Figure 1a). In this case, the ROC curves generated using data from each knowledge source, and the average of the scores across all knowledge sources, were positive. This provided evidence that our scoring procedure can identify the EA/TEF training genes more efficiently than random chance.

Having validated the algorithm, we then generated EA/TEF-specific pathogenicity scores for all RefSeq genes based on the centile rank of an omnibus score generated using fit data from all knowledge sources as previously described (Callaway et al., 2018; Campbell et al., 2013). By definition, the resulting EA/TEF-specific pathogenicity scores ranged from 0% to 100%, with a median of 50%. In contrast, the EA/TEF training gene set had a range of 72.6%–99.9% with median score of 99% (Figure 1b and Table S4), with *CPLANE2* (72.6%), *TCOF1* (74.2%), *EFTUD2* (80.2%), *SNRPN* (83.8%), and *VANGL1* (84.6%) being outliers.

2.5 | Statistical analyses

Two-tailed Fisher's exact tests were performed using a 2×2 contingency table calculator available through GraphPad QuickCalcs (https://www.graphpad.com/quickcalcs/contingency1/) to compare the diagnostic yields between subcohorts. Box plots were generated using the Alcula.com Statistical Calculator: Box Plot program (http://www.alcula.com/calculators/statistics/box-plot/).

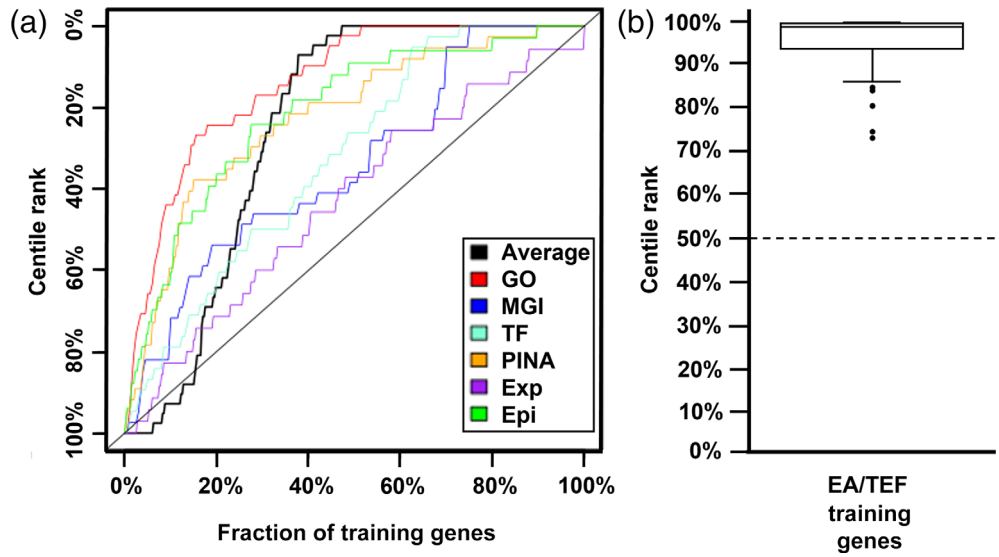

FIGURE 1 Generation and evaluation of EA/TEF-specific pathogenicity scores. (a) Receiver operating characteristic (ROC) style curves were generated in validation studies of our machine learning scoring approach using individual knowledge sources (colored) and the average score from all knowledge sources (black). The positive area underneath each curve indicates that our scoring approach identified training set genes known to cause EA/TEF more efficiently than random chance (diagonal line). (b) After validation, EA/TEF-specific pathogenicity scores were calculated for all RefSeq genes. The EA/TEF training gene set had a range of 72.6%–99.9% with median score of 99% with *CPLANE2* (72.6%), *TCOF1* (74.2%), *EFTUD2* (80.2%), *SNRPN* (83.8%), and *VANGL1* (84.6%) being outliers. This is significantly higher than the 50% median score (dashed line) for all RefSeq genes. EA/TEF, esophageal atresia/tracheoesophageal fistula; Epi, epigenetic histone modifications data from NIH roadmap Epigenomics mapping consortium; Exp, the GeneAtlas expression distribution; GO, gene ontology; MGI, the Mouse Genome Database; PINA, the protein interaction network analysis platform; TF, transcription factor binding data from NIH roadmap Epigenomics mapping consortium.

3 | RESULTS

3.1 | Diagnostic efficacy of clinical exome sequencing

In a clinical database of ~17,000 individuals who were referred for exome sequencing, we identified 67 individuals with EA/TEF+ based on phenotypes included in their test indications. Exome sequencing provided a definitive ($n = 10$; 15%) or probable ($n = 1$; 1.5%) diagnosis in 11 individuals for an efficacy rate of 16.4% (11/67). A provisional diagnosis was made in 7 additional EA/TEF+ case (7/67, 10%). If these provisional cases were included, the efficacy rate for EA/TEF+ would be 26.9% (18/67; Table S1).

In the subset of 39 individuals with EA/TEF+ who had a CHD, a definitive or probable diagnosis was made in eight, for an efficacy rate of 20.5% (8/39). In the subset of 23 individuals with EA/TEF+ who met criteria for VACTERL association, a definitive or probable diagnosis was made in three, for an efficacy rate of 13% (3/23). Although a higher efficacy rate was found for individuals with CHD, and a lower efficacy rate was found for individuals who met criteria for VACTERL association, these differences were not found to be statistically different than the subset of cases that did not have CHD (3/28, 10.7%; $p = 0.3366$) and the subset of cases that did not meet criteria for VACTERL association (8/44, 18.2%; $p = 0.736$), respectively.

3.2 | Genes known to cause EA/TEF

The only gene that was recurrently found to cause EA/TEF in our clinical cohort was *CHD7*. Heterozygous, loss-of-function variants in this gene cause CHARGE syndrome (MIM# 214800). It has been previously reported that ~15%–20% of individuals with CHARGE syndrome have EA/TEF (Lalani et al., 1993). In our cohort, Subjects S3 and S4 carried frameshift variants in *CHD7*, and S5 carried a stop-gain variant (Table S1). Other individuals in our clinical cohort carried pathogenic or likely pathogenic variants in other genes that have been clearly associated EA/TEF. These genes included *EFTUD2* (Subject S6) that causes mandibulofacial dysostosis, Guion-Almeida type (MIM# 610536), *FGFR3* (Subject S9) that causes a variety of skeletal dysplasias, and *MYCN* (Subject S12) that causes Feingold syndrome 1 (MIM# 164280).

3.3 | Candidate genes for EA/TEF

The majority of individuals in our clinical cohort who carried a definitive or probable diagnosis had associated variants in human disease genes that have not been clearly shown to cause EA/TEF. For the remainder of our study, we considered these genes as candidate genes for EA/TEF.

To identify additional candidate genes, we searched the DECIPHER database for individuals with EA/TEF who had sequence

changes or rare, small (<2 Mb and containing 1–20 protein-coding genes) CNVs (Firth et al., 2009; MacDonald et al., 2014). We obtained permission to publish the clinical and molecular data from 41 individuals with EA/TEF who met these criteria; 14 individuals with sequence variants (Subjects S19–S32; Table S2), and 27 individuals with CNVs (Subjects S33–S59; Table S3).

Some of the genes which were altered in individuals with EA/TEF from DECIPHER have been shown to cause EA/TEF including *CHD7* (Subjects S19 and S20) and *EFTUD2* (Subjects S21–S24). The remainder had changes affecting genes not clearly associated with EA/TEF. These genes were considered candidate genes for EA/TEF if they, (1) were affected by single nucleotide variants associated with a definitive or probable diagnosis, (2) were affected by a deletion and were predicted to have high probability of loss-of-function intolerance (pLI > 0.8 in gnomAD), or (3) if they may have been disrupted by a duplication based on the presence of one or more breakpoints within the gene (marked by an † in Table S3) and were predicted to have high loss-of-function intolerance (pLI > 0.8 in gnomAD; Karczewski et al., 2020).

3.4 | Associations between candidate genes and EA/TEF

Since EA/TEF is a relatively common birth defect, it is possible that it arose in some individuals in our cohort independent of the changes detected by exome sequencing or CNV analysis. As a means of determining which candidate genes for EA/TEF were the most likely to be contributing to the development of this disorder, we used an approach previously employed by Scott et al. (2021). Briefly, we determined whether each gene associated with a definitive or probable diagnosis had been previously reported in association with EA/TEF in humans, is known to cause a genetic syndrome previously associated with EA/TEF, and/or had a high similarity to genes known to cause EA/TEF based on a high EA/TEF-specific pathogenicity score (≥85% rank compared with all RefSeq genes) generated using our machine learning procedure. The results of these evaluations are summarized in Tables 1–3.

In Table 1, we list genes for which there is sufficient evidence to suggest an association with EA/TEF. In Tables 2 and 3, we list genes carrying sequence variants, or genes potentially disrupted by CNVs, respectively, for which there is currently insufficient evidence to suggest an association with EA/TEF.

4 | DISCUSSION

Isolated EA/TEF is associated with an empiric sibling recurrence risk of 1%, and an ~2%–4% recurrence risk in the offspring of an affected individual (Shaw-Smith, 2006). These low recurrence risks, and a twin concordance rate of ~2.5%, are consistent with a polygenic/multifactorial inheritance pattern (Robert et al., 1993). The sibling recurrence risk for nonisolated EA/TEF is often described as low in most families, with a minority having a higher risk (~25%–50%) due to a Mendelian disorder. Similarly, in the absence of a genetic diagnosis, the offspring recurrence risk for EA/TEF and/or other VACTERL-associated malformations has been estimated at ~2%–4%, but the offspring recurrence risk for individuals with a genetic diagnosis can be up to 50% (Robert et al., 1993; Scott, 1993). Therefore, identifying an underlying cause for EA/TEF through genetic testing can provide more accurate and individualized recurrence risk estimates.

Other potential benefits of obtaining a molecular diagnosis include improved prognostication and personalized medical management that can optimize the use of medical resources and may lead to the early identification and treatment of associated medical problems (Matias et al., 2019; Niguidula et al., 2018). Despite these potential benefits, exome sequencing is not universally ordered for individuals with EA/TEF+ without a molecular diagnosis. This may be due to uncertainty about the efficacy of exomes sequencing in individuals with EA/TEF+.

4.1 | Efficacy of clinical ES in cases of EA/TEF+

In our clinical cohort of 67 individuals with EA/TEF+, exome sequencing provided a definitive or probable diagnosis in 11 individuals for an efficacy rate of 16.4% (11/67). Given the potential benefits of a molecular diagnosis, this efficacy rate is sufficiently high to suggest that such testing should be considered for all individuals with EA/TEF+ who do not have a molecular diagnosis.

The efficacy rate of clinical exome sequencing in individuals with EA/TEF+ is significantly lower than the efficacy rate reported in individuals with other birth defects (Meng et al., 2017; Retterer et al., 2016) including the 37% (28/76; $p = 0.0082$) rate for individuals with congenital diaphragmatic hernia plus (CDH+) determined using data from the same clinical cohort (Scott et al., 2021). This difference is maintained if provisional diagnoses are included with the efficacy rate for EA/TEF+ being 26.9% (18/67) compared with 46.1% (35/76) for CDH+ ($p = 0.024$; Scott et al., 2021).

To further investigate this pattern, we reviewed chromosomal microarray (CMA) data from Baylor Genetics. We found that pathogenic or likely pathogenic CNVs were reported in 2% (1/51) of CMA cases in which EA/TEF was listed as a phenotype and 10% (22/223) of CMA cases in which CDH was listed as a phenotype. Although this is consistent with the pattern seen in our exome sequencing results, the difference in CMA efficacy rates did not reach statistical significance ($p = 0.09$).

One possible reason for the difference in clinical exome sequencing efficacy rates between EA/TEF+ and CDH+ is that polygenic, multifactorial, or nongenetic factors—environmental, epigenetic, or stochastic factors—may play a greater role in the development of EA/TEF than CDH. Unlike CDH, EA/TEF is a feature of VACTERL association. Several maternal risk factors, such as conception via assisted reproductive technologies, pregestational diabetes, and chronic obstructive lower pulmonary diseases, are positively correlated with the risk of having a child with VACTERL association

TABLE 1 Genes with sufficient evidence to support an association with EA/TEF

Gene	Disorder, MIM number	Subject ID; variant; ACMG interpretation	Number of individuals in our cohort with changes in this gene; level of diagnostic certainty	EA/TEF-specific pathogenicity score (%)	Other cases of EA/TEF reported for this gene/disorder	References
TCF4	Pitt-Hopkins syndrome, MIM# 610954	S17; c.1486+1G>T [NM_001083962.2] p.(?); Pathogenic	1; Definitive	90.7	Yes/Yes	Whalen et al. (2012)
NRXN1	Pitt-Hopkins-like syndrome 2, MIM# 614325; susceptibility to schizophrenia, MIM# 614332; susceptibility to developmental delay, intellectual disability, abnormal behaviors, autism spectrum disorder, and seizures	S31; c.2138C>G, [NM_001135659.3] p.(Ser713*); Likely Pathogenic	1; Probable	90.2	Yes/Yes	Lowther et al. (2017)
FANCA	Fanconi anemia complementation Group A, MIM# 227650	S25; c.[2175_2182del]; [3788_3790del] [NM_000135.4] p.[(Phe726Glufs*65)]; [(Phe1263del)]; pathogenic/pathogenic	1; Definitive	41.8	Yes/Yes	Faivre et al. (2005)
FANCB	Fanconi anemia complementation Group B, MIM# 300514	S41[a]; Deletion; Pathogenic	1; Definitive	40.4	Yes/Yes	Holden et al. (2006) and McCauley et al. (2011)
FANCC	Fanconi anemia complementation Group C, MIM# 227645	S7; c.[1642C>T];[1642C>T] [NM_000136.3] p.[(R548*)]; [(R548*)] Pathogenic/Pathogenic	1; Definitive	51.3	Yes/Yes	Kutler and Auerbach (2004)

Abbreviations: ACMG, American College of Medical Genetics and Genomics; EA/TEF, esophageal atresia/tracheoesophageal fistula.
[a]Previously reported by Umana et al. (2011).

TABLE 2 Genes with definitive or probable diagnoses that do not currently have sufficient evidence to support an association with EA/TEF

Gene	Disorder, MIM number	Subject ID; variant; ACMG interpretation	Level of diagnostic certainty	EA/TEF specific pathogenicity score (%)	Other cases EA/TEF reported for this gene/disorder in humans
NEDD4L	Periventricular nodular heterotopia 7, MIM# 617201	S13; c.814-6T>A [NM_015277.6] p.(?); likely pathogenic	Probable	41.9	No/No
NSD1	Sotos syndrome 1, MIM# 117550	S32; c.5351T>C [NM_022455.5] p.(Ile1784Thr); likely pathogenic	Probable	85.4	No/No
PTPN11	LEOPARD syndrome 1, MIM# 151100; Noonan syndrome 1, MIM# 163950; Metachondromatosis, MIM# 156250	S15; c.922A>G [NM_002834.5] p.(N308D); pathogenic	Definitive	71	No/No
SLC20A2	Basal ganglia calcification, idiopathic, 1, MIM# 213600	S10; c.731-18_738del26 [NM_006749.5] p.(?); pathogenic	Definitive	43.6	No/No
TPO	Thyroid dyshormonogenesis 2A, MIM# 274500	S18; c.[1184_1187dupGCCG];[1472G>A] [NM_000547.6] p.[A397Pfs*76]; [(R491H)]; pathogenic/pathogenic	Definitive	77.4	No/No

Abbreviations: ACMG, American College of Medical Genetics and Genomics; EA/TEF, esophageal atresia/tracheoesophageal fistula.

(Lubinsky, 2018; van de Putte et al., 2020). Epigenetic factors have also been postulated to contribute to the development of VACTERL association (Lubinsky, 2018; Solomon, 2018).

In this study, the efficacy rate of exome sequencing in the subset of individuals with EA/TEF+ who met criteria for VACTERL association (13%; 3/23) was lower than that of the subset of cases that did not meet criteria for VACTERL association (18.2%; 8/44), although the difference was not statistically significant ($p = 0.736$). The efficacy rate observed in VACTERL association cases from our cohort is comparable to the 14% (38/271) positivity rate of individuals referred to a different reference lab (GeneDx) for exome sequencing who had VACTERL association annotated as part of their clinical characteristics (Solomon, 2018). Additionally, 10% (28/271) of individuals in this GeneDx cohort had a reportable variant in a plausible candidate gene, and 34% (91/271) had variants of unknown significance in genes that correlated with the reported phenotypes. Solomon went on to report that the exome sequencing efficacy rate for this cohort was lower than the overall exome sequencing efficacy rate and, more specifically, the exome sequencing efficacy rate of individuals with multiple congenital anomalies (Retterer et al., 2016; Solomon, 2018). Further research into the genetic, epigenetic, and environmental factors that contribute to the development of EA/TEF and VACTERL association is warranted.

4.2 | Phenotypic expansions involving EA/TEF

4.2.1 | *TCF4* and *NRXN1*

Autosomal dominant, pathogenic variants in *TCF4* are associated with Pitt-Hopkins syndrome (MIM# 610954), a neurodevelopmental disorder characterized by global developmental delay, intellectual disability, autism spectrum disorder, distinctive facial features, episodic hyperventilation, and/or breath-holding, seizures, and severe myopia (Amiel et al., 2007; Pitt & Hopkins, 1978; Zweier et al., 2007). Whalen et al. (2012) described identical twins with Pitt-Hopkins syndrome, one of whom had congenital anomalies including EA and a sacral mass, that were not seen in her twin. Subject S17 had EA/TEF, neurodevelopmental phenotypes, dysmorphic features, congenital esotropia, and myopia associated with a de novo c.1486+1G>T, p.(?) [NM_001083962.2] pathogenic variant in *TCF4*. This gave her a definitive diagnosis of Pitt-Hopkins syndrome and suggests that loss of TCF4 function may lead to an increased risk of developing EA/TEF.

TCF4 encodes a basic helix–loop–helix transcription factor that transactivates the *NRXN1β* and *CNTNAP2* promoters in luciferase assays (Forrest et al., 2012). This suggests that *TCF4*, *NRXN1*, and *CNTNAP2* may function in a common pathway during development, and that disruption of this pathway may lead to a common set of phenotypes. Consistent with that hypothesis, autosomal recessive variants in *NRXN1* are associated with Pitt-Hopkins-like syndrome 2 (MIM# 614325), and autosomal dominant *NRXN1* variants are associated with susceptibility to schizophrenia (MIM# 614332), developmental delay, intellectual disability, abnormal behaviors, autism

TABLE 3 Genes with high pLI scores (>0.8) that are deleted or potentially disrupted for which there is currently insufficient evidence to support an association with EA/TEF

Gene (pLI score)	Disorder, MIM no.	Subject ID, CNV type	EA/TEF-specific pathogenicity score (%)	Other cases of EA/TEF reported for this gene/disorder in humans
BRPF1 (pLI = 1)	Intellectual developmental disorder with dysmorphic facies and ptosis, MIM# 617333	S35, Deletion	47.6	No/No
FUBP1 (pLI = 1)	None	S34, Deletion	59.9	No/N/A
GLRA2[a] (pLI = 0.97)	Intellectual developmental disorder, X-linked, syndromic, Pilorge type, MIM# 301076	S45, Deletion	61.5	No/N/A
LARGE1 (pLI = 0.99)	Muscular dystrophy-dystroglycanopathy (congenital with brain and eye anomalies), Type A, 6, MIM# 613154; Muscular dystrophy-dystroglycanopathy (congenital with mental retardation), Type B, 6, MIM# 608840	S44, Deletion	ND	No/No
LINGO2 (pLI = 0.86)	None	S38, Deletion	0.7	No/N/A
MOSPD2[a] (pLI = 0.99)	None	S45, Deletion	63.3	No/N/A
NRDC (pLI = 1)	None	S33, Deletion	ND	No/N/A
USP33 (pLI = 0.82)	None	S34, Deletion	58.5	No/N/A
SVEP1 (pLI = 1)	None	S39, Deletion	91	No/N/A
TMEM108 (pLI = 1)	None	S36, Deletion	84.4	No/N/A
ZFYVE9 (pLI = 0.9)	None	S33, Deletion	89.1	No/N/A
ZNF592 (pLI = 1)	None	S42, Deletion	38.8	No/N/A
ZZZ3 (pLI = 1)	None	S34, Deletion	45.5	No/N/A
DIO2 (pLI = 0.96)	None	S53, Breakpoint of Duplication	40.9	No/N/A
FLNA (pLI = 1)	FLNA-related disorders, MIM# 300321, 314400, 300048, 305620, 300049, 300048, 309350, 311300, 304120, and 300244	S59, Breakpoint of Duplication	74.3	No/No
GMDS (pLI = 0.99)	None	S49, Breakpoint of Duplication	7.3	No/N/A
OTUD7A (pLI = 0.95)	None	S55, Breakpoint of Duplication	11.8	No/N/A
PACS1 (pLI = 1)	Schuurs–Hoeijmakers syndrome, MIM# 615009	S51, Breakpoint of Duplication	79.5	No/No
PDE4D (pLI = 1)	Acrodysostosis 2, with or without hormone resistance, MIM# 614613	S48, Breakpoint of Duplication	73.8	No/No
RORA (pLI = 1)	Intellectual developmental disorder with or without epilepsy or cerebellar ataxia, MIM# 618060	S56, Breakpoint of Duplication	80.9	No/No
SART1 (pLI = 0.88)	None	S51, Breakpoint of Duplication	19.4	No/N/A

Abbreviations: EA/TEF, esophageal atresia/tracheoesophageal fistula; N/A, not applicable; ND, not determined; pLI, probability of loss-of-function intolerance.
[a]Found in a male previously reported by Umana et al. (2011) who carried a deletion of *FANCB*.

spectrum disorder, and seizures (Dabell et al., 2013; Harrison et al., 2011; Kim et al., 2008; Lowther et al., 2017; Schaaf et al., 2012; Zweier et al., 2009). Similarly, autosomal recessive and autosomal dominant variants in *CNTNAP2* are associated with Pitt-Hopkins-like syndrome 1 (MIM# 610042) and autism susceptibility 15 (MIM# 612100), respectively (Alarcon et al., 2008; Zweier et al., 2009).

EA/TEF are not common features seen in *NRXN1*-related and *CNTNAP2*-related disorders. However, TEF has been previously documented in one individual with a deletion overlapping the 5′ end of *NRXN1*, indicating a deletion of one or more of exons 1–4 (Lowther et al., 2017). Subject S31 had TEF, butterfly vertebrae, a dilated vestibule of the inner ear, and a hearing abnormality and carried a likely pathogenic c.2138C>G, p.(Ser713*) [NM_001135659.3] stop-gain variant in *NRXN1*. In contrast, no individuals with EA/TEF harboring putatively deleterious variants in *CNTNAP2* were identified in this or previous studies. However, large (>13 Mb) terminal 7q deletions including *CNTNAP2* have been documented in two patients with EA/TEF (Busa et al., 2016; Speleman et al., 1992) and one patient with esophageal stenosis (Zen et al., 2010).

Taken together these data suggest that deleterious variants in genes associated with Pitt-Hopkins syndrome and related disorders—especially *TCF4* and *NRXN1*—may predispose to the development of EA/TEF. Consistent with this possibility, we note that *TCF4*, *NRXN1*, and *CNTNAP2* had high EA/TEF-specific pathogenicity scores of 90.7%, 90.2%, and 81.1%, respectively.

4.2.2 | *FANCA* , *FANCB*, *FANCC*, and *FANCL*

Fanconi anemia (FA) is a genetically heterogenous disorder that can be caused by pathogenic variants in at least 23 genes and is characterized by a variety of structural birth defects, bone marrow failure, and increased risk for malignancy (Mehta & Ebens, 1993). EA and TEF are seen in ~1.4% and 3.5%, respectively, of individuals with FA (Giampietro et al., 1993). Here, we report individuals with variants in four FA-associated genes: *FANCA*, *FANCB*, *FANCC*, and *FANCL*.

Faivre et al. (2005) described a boy with FA with EA/TEF who carried two pathogenic *FANCA* deletions, one of exons 6–8, and one of exons 11–21. More recently, Feng et al. identified four rare, heterozygous variants in *FANCA* in individuals with EA/TEF (Feng et al., 2018). In our study, Subject S25 is a 2-year-old male with TEF, a ventricular septal defect, aplasia/hypoplasia of the forearm bones, a short thumb, penoscrotal hypospadias, and 2–3 toe syndactyly who carried compound heterozygous, pathogenic c.[2175_2182del];[3788_3790del], p.[(Phe726Glufs*65)];[(Phe1263del)] [NM_000135.4] *FANCA* variants.

Holden et al. (2006) described a three-generation family with X-linked VACTERL-H syndrome caused by a c.1496+5G>A [NM_001018113.3] splice donor variant in *FANCB* that caused skipping of exon 7. This family included a stillborn male fetus with EA/TEF, hydrocephalus associated with an Arnold-Chiari malformation, CHD, unilateral renal agenesis on the right, a dysplastic left kidney, missing thumbs, bilateral absent radii, lumbar spina bifida occulta, and abnormal ears. McCauley et al. (2011) described two boys with X-linked VACTERL-H and EA/TEF. The first had EA, dilation of the lateral and third ventricles with a small fourth ventricle, right renal agenesis, left ureteric and pelvicalyceal dilation, absent thumbs and radii, micropenis, a short neck, and eye and ear anomalies, associated with a maternally inherited deletion of *FANCB* exons 8–10. The second had a TEF, ventriculomegaly, absent thumbs and radii, and carried a c.2150T>G, p.Leu717* stop-gain variant in *FANCB*. In our study, Subject S45 is a previously published male infant with TEF, a ventricular septal defect, renal hypoplasia, anal atresia, aplasia/hypoplasia of the thumb, and hypoplasia of the radius, who carries a chrX:14,543,045–15,074,800 deletion (hg38) that affects *FANCB* (Umana et al., 2011). This deletion also includes *GLRA2*, whose loss is associated with intellectual developmental disorder, X-linked, syndromic, Pilorge type (MIM# 301076), and *MOSPD2*, which has a pLI score of 0.99 in gnomAD but is currently not associated with a genetic disorder in humans (Pilorge et al., 2016).

Cox et al. (1997) reported an Ashkenazi Jewish, nonidentical twin pair with VACTERL-H. The male fetus had TEF, dilated lateral ventricles, right lung lobulation defects, duodenal atresia, intestinal malrotation, an ectopic right kidney, absent right radius and thumb, and small, dysplastic ears. The affected female fetus did not have EA/TEF. Both twins were homozygous for the pathogenic c.456+4A>T [NM_000136.3] variant in *FANCC* that is commonly seen in individuals of Ashkenazi Jewish ancestry (Kutler & Auerbach, 2004). In our study, Subject S7 was a female infant with EA/TEF, a ventricular septal defect, a horseshoe kidney, duodenal atresia, an anteriorly placed anus, a flexion contracture of the left wrist, a short thumb, a radial defect, conductive hearing impairment, atresia of the external auditory canal, absence of the clitoris, vaginal prolapse, clubbing of the fingers, and a sacral dimple, who was homozygous for a pathogenic c.1642C>T, p.(Arg548*) [NM_000136.3] stop variant in *FANCC*.

Vetro et al. (2015) reported a female and a male fetus from the same family that had EA/TEF and cardio/pulmonary, renal, genital, radial, and thumb anomalies. Genetic testing performed on the male fetus revealed a homozygous c.268del, p.(Leu90Phefs*6) [NM_018062.4] frameshift variant in *FANCL*. In our study, Subject S8 is a woman with EA/TEF, abnormal position of inferior vena cava, pyloric stenosis, an inguinal hernia, polyarticular arthritis, irritable bowel syndrome, skeletal abnormalities, and upslanting palpebral fissures, who carries a maternally inherited c.1096_1099dup, p.(Thr367Asnfs*13) [NM_018062.4] variant of unknown significance in the *FANCL* gene. This change occurs in the final exon of the gene and is not expected to trigger nonsense-mediated mRNA decay. A second *FANCL* variant was not identified. Hence, it is unclear whether this variant contributed to the phenotypes seen in Subject S8.

Although there is sufficient evidence to suggest an association between EA/TEF and *FANCA*, *FANCB*, *FANCC*, and possibly *FANCL*, the EA/TEF-specific pathogenicity scores of these genes are relatively low—41.8%, 40.4%, 51.3%, and 38.7% respectively. This suggests that they represent a distinct group of EA/TEF genes whose characteristics differ from those of the other EA/TEF genes in the training set. This difference is likely due, at least in part, to their unique role in DNA damage repair, bone marrow function, and cancer.

4.3 | Other EA/TEF candidate genes

There is currently insufficient evidence to suggest an association between the genes listed in Tables 2 and 3. However, some have high EA/TEF-specific pathogenicity scores (>80%). These genes include *SVEP1* (91%), *TMEM108* (84.4%), and *ZFYVE9* (89.1%) that have not been associated with a specific genetic disorder but have high pLI scores (≥0.9). It is possible that their haploinsufficiency may ultimately be found to cause a genetic syndrome in which EA/TEF is a phenotype.

AFFILIATIONS

[1]Department of Molecular and Human Genetics, Baylor College of Medicine, Houston, Texas, USA
[2]Yorkshire Regional Genetics Service, Leeds Teaching Hospitals NHS Trust, Chapel Allerton Hospital, Leeds, UK
[3]Clinical Genetics Department, University Hospitals Bristol and Weston, Bristol NHS Foundation, Bristol, UK
[4]Whiteladies Medical Group, Bristol, UK
[5]Community Children's Health Partnership, Sirona Health and Care, Bristol, UK
[6]Sheffield Children's NHS Foundation Trust, Sheffield, UK
[7]University Hospitals of Leicester NHS Trust, Leicester, UK
[8]Birmingham Women's and Children's Hospital NHS Foundation Trust, Birmingham, UK
[9]Hunter Genetics, Hunter New England Local Health District, Waratah, New South Wales, Australia
[10]University of Newcastle, Callaghan, New South Wales, Australia
[11]Department of Molecular Medicine, New South Wales Health Pathology, Newcastle, New South Wales, Australia
[12]East Anglian Medical Genetics Service, Cambridge University Hospitals NHS Foundation Trust, Cambridge, UK
[13]Guy's and St Thomas' NHS Foundation Trust, London, UK
[14]Département de Génétique et Procréation, Hôpital Couple Enfant, CHU Grenoble, Grenoble, France
[15]INSERM U1216 Grenoble Institut des Neurosciences, Cellular Myology and Pathology, Grenoble, France
[16]Genetic Epigenetic and Therapies of Infertility Team, Institute for Advanced Biosciences, Inserm U 1209, CNRS UMR 5309, Université Grenoble Alpes, Grenoble, France
[17]Clinical Genetics Department, St Michaels Hospital, Bristol, UK
[18]Nantes Université, CHU de Nantes, UF 9321 de Fœtopathologie et Génétique, Nantes, France
[19]Université de Lille, ULR7364 RADEME, CHU Lille, Clinique de Génétique Guy Fontaine, Lille, France
[20]Center for Human Genetics, University Hospitals Leuven–KU Leuven, Leuven, Belgium
[21]CHU Lille, Institut de Génétique Médicale, Lille, France
[22]Medical Genetics Unit, Fondazione IRCCS Casa Sollievo della Sofferenza, San Giovanni Rotondo, Italy
[23]Department of Biomedical and Biotechnological Sciences, Medical Genetics, University of Catania, Catania, Italy
[24]Scientific Foundation and Clinic G. B. Morgagni, Catania, Italy
[25]Department of Public Health and Pediatrics, University of Torino, Torino, Italy
[26]Medical Genetics Unit, San Luigi University Hospital, University of Torino, Orbassano, Italy
[27]Department of Molecular Medicine and Medical Biotechnology, University of Naples Federico II, Naples, Italy
[28]Telethon Institute of Genetics and Medicine (TIGEM), Pozzuoli, Italy
[29]Department of Translational Medicine, University of Naples Federico II, Naples, Italy
[30]Genetic Health Service NZ, Wellington, New Zealand
[31]Wellington Regional Genetics Laboratory, Wellington, New Zealand
[32]Department of Clinical Genetics, Odense University Hospital, Odense, Denmark
[33]Institute for Maternal and Child Health - IRCCS Burlo Garofolo, Trieste, Italy
[34]Department of Medicine, Surgery and Health Sciences, University of Trieste, Trieste, Italy
[35]Pediatric Clinical Genetics Unit, Regina Margherita Children's Hospital, Torino, Italy
[36]Department of Clinical and Biological Sciences, Università degli Studi di Torino, Torino, Italy
[37]South East Scotland Clinical Genetics Service, Western General Hospital, Edinburgh, UK
[38]Department of Clinical Genetics, Western General Hospital, Edinburgh, UK
[39]Baylor Genetics, Houston, Texas, USA
[40]Department of Cell and Tissue Biology, University of California San Francisco, San Francisco, California, USA
[41]Institute for Human Genetics, University of California San Francisco, San Francisco, California, USA
[42]Eli and Edythe Broad Center of Regeneration Medicine and Stem Cell Research, University of California San Francisco, San Francisco, California, USA
[43]Department of Molecular Physiology and Biophysics, Baylor College of Medicine, Houston, Texas, USA

ACKNOWLEDGMENTS

This study makes use of data generated by the DECIPHER community. A full list of centers who contributed to the generation of the data is available from https://deciphergenomics.org/about/stats and via email from contact@deciphergenomics.org. We note that those who carried out the original analysis and collection of the DECIPHER data bear no responsibility for the further analysis or interpretation of the data. Some individuals described in this article are part of the Deciphering Developmental Disorders (DDD) study. The views expressed in this publication are those of the author(s) and not necessarily those of Wellcome or the Department of Health. The DDD study has UK Research Ethics Committee approval (10/H0305/83, granted by the Cambridge South REC, and GEN/284/12 granted by the Republic of Ireland REC). The research team acknowledges the support of the National Institute for Health Research, through the Comprehensive Clinical Research Network. This study was conducted, in part, within the European Reference Network ITHACA.

FUNDING INFORMATION

This research was funded, in part, by NIH/NICHD grant HD098458 to D.A.S. Funding for the DECIPHER project was provided by Wellcome. The DDD study presents independent research commissioned by the Health Innovation Challenge Fund [grant number HICF-1009-003], a parallel funding partnership between Wellcome and the Department of Health, and the Wellcome Sanger Institute [grant number WT098051].

CONFLICT OF INTEREST

Baylor College of Medicine (BCM) and Miraca Holdings Inc. have formed a joint venture with shared ownership and governance of Baylor Genetics (BG), which performs genetic testing and derives revenue. The Department of Molecular & Human Genetics at Baylor College of Medicine receives revenue from clinical genetic testing completed at BG.

DATA AVAILABILITY STATEMENT

The data generated during this study can be found within the published article and its supplementary files. The clinical exome sequencing variants described in this manuscript have been submitted to ClinVar (https://www.ncbi.nlm.nih.gov/clinvar/).

ETHICS STATEMENT

This work was approved by the institutional review board of Baylor College of Medicine (protocol H-47546) and was conducted in accordance with the ethical standards of this institution's committee on human research and international standards.

ORCID

Himanshu Goel https://orcid.org/0000-0001-6448-6618
Klaus Dieterich https://orcid.org/0000-0002-4362-4062
Laura Bernardini https://orcid.org/0000-0002-3554-2817
Diana Carli https://orcid.org/0000-0001-5690-6504
Michele Pinelli https://orcid.org/0000-0002-5927-1185
Nicola Brunetti-Pierri https://orcid.org/0000-0002-6895-8819
Flavio Faletra https://orcid.org/0000-0003-1483-3612
Daryl A. Scott https://orcid.org/0000-0003-1460-5169

REFERENCES

Alarcon, M., Abrahams, B. S., Stone, J. L., Duvall, J. A., Perederiy, J. V., Bomar, J. M., Sebat, J., Wigler, M., Martin, C. L., Ledbetter, D. H., Nelson, S. F., Cantor, R. M., & Geschwind, D. H. (2008). Linkage, association, and gene-expression analyses identify CNTNAP2 as an autism-susceptibility gene. *American Journal of Human Genetics*, *82*(1), 150–159. https://doi.org/10.1016/j.ajhg.2007.09.005

Amiel, J., Rio, M., de Pontual, L., Redon, R., Malan, V., Boddaert, N., Plouin, P., Carter, N. P., Lyonnet, S., Munnich, A., & Colleaux, L. (2007). Mutations in TCF4, encoding a class I basic helix-loop-helix transcription factor, are responsible for Pitt-Hopkins syndrome, a severe epileptic encephalopathy associated with autonomic dysfunction. *American Journal of Human Genetics*, *80*(5), 988–993. https://doi.org/10.1086/515582

Blake, J. A., Bult, C. J., Kadin, J. A., Richardson, J. E., Eppig, J. T., & The Mouse Genome Database Group. (2011). The Mouse Genome Database (MGD): Premier model organism resource for mammalian genomics and genetics. *Nucleic Acids Research*, *39*(Database issue), D842–D848. https://doi.org/10.1093/nar/gkq1008

Brosens, E., de Jong, E. M., Barakat, T. S., Eussen, B. H., D'Haene, B., De Baere, E., Verdin, H., Poddighe, P. J., Galjaard, R. J., Gribnau, J., Brooks, A. S., Tibboel, D., & de Klein, A. (2014). Structural and numerical changes of chromosome X in patients with esophageal atresia. *European Journal of Human Genetics*, *22*(9), 1077–1084. https://doi.org/10.1038/ejhg.2013.295

Brosens, E., Marsch, F., de Jong, E. M., Zaveri, H. P., Hilger, A. C., Choinitzki, V. G., Holscher, A., Hoffmann, P., Herms, S., Boemers, T. M., Ure, B. M., Lacher, M., Ludwig, M., Eussen, B. H., van der Helm, R. M., Douben, H., Van Opstal, D., Wijnen, R. M., Beverloo, H. B., … de Klein, A. (2016). Copy number variations in 375 patients with oesophageal atresia and/or tracheoesophageal fistula. *European Journal of Human Genetics*, *24*(12), 1715–1723. https://doi.org/10.1038/ejhg.2016.86

Brosens, E., Ploeg, M., van Bever, Y., Koopmans, A. E., IJsselstijn, H., Rottier, R. J., Wijnen, R., Tibboel, D., & de Klein, A. (2014). Clinical and etiological heterogeneity in patients with tracheo-esophageal malformations and associated anomalies. *European Journal of Medical Genetics*, *57*(8), 440–452. https://doi.org/10.1016/j.ejmg.2014.05.009

Busa, T., Panait, N., Chaumoitre, K., Philip, N., & Missirian, C. (2016). Esophageal atresia with tracheoesophageal fistula in a patient with 7q35-36.3 deletion including SHH gene. *European Journal of Medical Genetics*, *59*(10), 546–548. https://doi.org/10.1016/j.ejmg.2016.09.001

Callaway, D. A., Campbell, I. M., Stover, S. R., Hernandez-Garcia, A., Jhangiani, S. N., Punetha, J., Paine, I. S., Posey, J. E., Muzny, D., Lally, K. P., Lupski, J. R., Shaw, C. A., Fernandes, C. J., & Scott, D. A. (2018). Prioritization of candidate genes for congenital diaphragmatic hernia in a critical region on chromosome 4p16 using a machine-learning algorithm. *Journal of Pediatric Genetics*, *7*(4), 164–173. https://doi.org/10.1055/s-0038-1655755

Campbell, I. M., James, R. A., Chen, E. S., & Shaw, C. A. (2014). NetComm: A network analysis tool based on communicability. *Bioinformatics*, *30*(23), 3387–3389. https://doi.org/10.1093/bioinformatics/btu536

Campbell, I. M., Rao, M., Arredondo, S. D., Lalani, S. R., Xia, Z., Kang, S.-H. L., Bi, W., Breman, A. M., Smith, J. L., & Bacino, C. A. (2013). Fusion of large-scale genomic knowledge and frequency data computationally prioritizes variants in epilepsy. *PLoS Genetics*, *9*(9), e1003797. https://doi.org/10.1371/journal.pgen.1003797

Cassina, M., Ruol, M., Pertile, R., Midrio, P., Piffer, S., Vicenzi, V., Saugo, M., Stocco, C. F., Gamba, P., & Clementi, M. (2016). Prevalence, characteristics, and survival of children with esophageal atresia: A 32-year population-based study including 1,417,724 consecutive newborns. *Birth Defects Research. Part A, Clinical and Molecular Teratology*, *106*(7), 542–548. https://doi.org/10.1002/bdra.23493

Cox, P. M., Gibson, R. A., Morgan, N., & Brueton, L. A. (1997). VACTERL with hydrocephalus in twins due to Fanconi anemia (FA): Mutation in the FAC gene. *American Journal of Medical Genetics*, *68*(1), 86–90. https://www.ncbi.nlm.nih.gov/pubmed/8986283

Dabell, M. P., Rosenfeld, J. A., Bader, P., Escobar, L. F., El-Khechen, D., Vallee, S. E., Dinulos, M. B., Curry, C., Fisher, J., Tervo, R., Hannibal, M. C., Siefkas, K., Wyatt, P. R., Hughes, L., Smith, R., Ellingwood, S., Lacassie, Y., Stroud, T., Farrell, S. A., … Shaffer, L. G. (2013). Investigation of NRXN1 deletions: Clinical and molecular characterization. *American Journal of Medical Genetics. Part A*, *161A*(4), 717–731. https://doi.org/10.1002/ajmg.a.35780

Depaepe, A., Dolk, H., & Lechat, M. F. (1993). The epidemiology of tracheo-oesophageal fistula and oesophageal atresia in Europe. EUROCAT working group. *Archives of Disease in Childhood*, *68*(6), 743–748. https://doi.org/10.1136/adc.68.6.743

Faivre, L., Portnoi, M. F., Pals, G., Stoppa-Lyonnet, D., Le Merrer, M., Thauvin-Robinet, C., Huet, F., Mathew, C. G., Joenje, H., Verloes, A., &

Baumann, C. (2005). Should chromosome breakage studies be performed in patients with VACTERL association? *American Journal of Medical Genetics. Part A*, *137*(1), 55–58. https://doi.org/10.1002/ajmg.a.30853

Felix, J. F., Tibboel, D., & de Klein, A. (2007). Chromosomal anomalies in the aetiology of oesophageal atresia and tracheo-oesophageal fistula [review]. *European Journal of Medical Genetics*, *50*(3), 163–175. https://doi.org/10.1016/j.ejmg.2006.12.004

Feng, Y., Chen, R., Da, M., Qian, B., & Mo, X. (2018). Identification of rare heterozygous missense mutations in FANCA in esophageal atresia patients using next-generation sequencing. *Gene*, *661*, 182–188. https://doi.org/10.1016/j.gene.2018.03.097

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

Forrest, M., Chapman, R. M., Doyle, A. M., Tinsley, C. L., Waite, A., & Blake, D. J. (2012). Functional analysis of TCF4 missense mutations that cause Pitt-Hopkins syndrome. *Human Mutation*, *33*(12), 1676–1686. https://doi.org/10.1002/humu.22160

Giampietro, P. F., Adler-Brecher, B., Verlander, P. C., Pavlakis, S. G., Davis, J. G., & Auerbach, A. D. (1993). The need for more accurate and timely diagnosis in Fanconi anemia: A report from the international Fanconi anemia registry. *Pediatrics*, *91*(6), 1116–1120. https://www.ncbi.nlm.nih.gov/pubmed/8502512

Harrison, V., Connell, L., Hayesmoore, J., McParland, J., Pike, M. G., & Blair, E. (2011). Compound heterozygous deletion of NRXN1 causing severe developmental delay with early onset epilepsy in two sisters. *American Journal of Medical Genetics. Part A*, *155A*(11), 2826–2831. https://doi.org/10.1002/ajmg.a.34255

Holden, S. T., Cox, J. J., Kesterton, I., Thomas, N. S., Carr, C., & Woods, C. G. (2006). Fanconi anaemia complementation group B presenting as X linked VACTERL with hydrocephalus syndrome. *Journal of Medical Genetics*, *43*(9), 750–754. https://doi.org/10.1136/jmg.2006.041673

Ioannides, A. S., & Copp, A. J. (2009). Embryology of oesophageal atresia. *Seminars in Pediatric Surgery*, *18*(1), 2–11. https://doi.org/10.1053/j.sempedsurg.2008.10.002

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

Kim, H. G., Kishikawa, S., Higgins, A. W., Seong, I. S., Donovan, D. J., Shen, Y., Lally, E., Weiss, L. A., Najm, J., Kutsche, K., Descartes, M., Holt, L., Braddock, S., Troxell, R., Kaplan, L., Volkmar, F., Klin, A., Tsatsanis, K., Harris, D. J., … Gusella, J. F. (2008). Disruption of neurexin 1 associated with autism spectrum disorder. *American Journal of Human Genetics*, *82*(1), 199–207. https://doi.org/10.1016/j.ajhg.2007.09.011

Kutler, D. I., & Auerbach, A. D. (2004). Fanconi anemia in Ashkenazi Jews. *Familial Cancer*, *3*(3–4), 241–248. https://doi.org/10.1007/s10689-004-9565-8

Lalani, S. R., Hefner, M. A., Belmont, J. W., & Davenport, S. L. H. (1993). CHARGE Syndrome. In M. P. Adam, H. H. Ardinger, R. A. Pagon, S. E. Wallace, L. J. H. Bean, K. Stephens, & A. Amemiya (Eds.), *GeneReviews®*. University of Washington. https://www.ncbi.nlm.nih.gov/pubmed/20301296

Little, D. C., Rescorla, F. J., Grosfeld, J. L., West, K. W., Scherer, L. R., & Engum, S. A. (2003). Long-term analysis of children with esophageal atresia and tracheoesophageal fistula. *Journal of Pediatric Surgery*, *38*(6), 852–856. https://doi.org/10.1016/s0022-3468(03)00110-6

Lowther, C., Speevak, M., Armour, C. M., Goh, E. S., Graham, G. E., Li, C., Zeesman, S., Nowaczyk, M. J., Schultz, L. A., Morra, A., Nicolson, R., Bikangaga, P., Samdup, D., Zaazou, M., Boyd, K., Jung, J. H., Siu, V., Rajguru, M., Goobie, S., … Bassett, A. S. (2017). Molecular characterization of NRXN1 deletions from 19,263 clinical microarray cases identifies exons important for neurodevelopmental disease expression. *Genetics in Medicine*, *19*(1), 53–61. https://doi.org/10.1038/gim.2016.54

Lubinsky, M. (2018). An epigenetic association of malformations, adverse reproductive outcomes, and fetal origins hypothesis related effects. *Journal of Assisted Reproduction and Genetics*, *35*(6), 953–964. https://doi.org/10.1007/s10815-018-1197-2

Lupo, P. J., Isenburg, J. L., Salemi, J. L., Mai, C. T., Liberman, R. F., Canfield, M. A., Copeland, G., Haight, S., Harpavat, S., Hoyt, A. T., Moore, C. A., Nembhard, W. N., Nguyen, H. N., Rutkowski, R. E., Steele, A., Alverson, C. J., Stallings, E. B., Kirby, R. S., & The National Birth Defects Prevention Network. (2017). Population-based birth defects data in the United States, 2010-2014: A focus on gastrointestinal defects. *Birth Defects Research*, *109*(18), 1504–1514. https://doi.org/10.1002/bdr2.1145

MacDonald, J. R., Ziman, R., Yuen, R. K., Feuk, L., & Scherer, S. W. (2014). The database of genomic variants: A curated collection of structural variation in the human genome. *Nucleic Acids Research*, *42*(Database issue), D986–D992. https://doi.org/10.1093/nar/gkt958

Matias, M., Wusik, K., Neilson, D., Zhang, X., Valencia, C. A., & Collins, K. (2019). Comparison of medical management and genetic counseling options pre- and post-whole exome sequencing for patients with positive and negative results. *Journal of Genetic Counseling*, *28*(2), 182–193. https://doi.org/10.1002/jgc4.1054

Mattioli, F., Schaefer, E., Magee, A., Mark, P., Mancini, G. M., Dieterich, K., Von Allmen, G., Alders, M., Coutton, C., van Slegtenhorst, M., Vieville, G., Engelen, M., Cobben, J. M., Juusola, J., Pujol, A., Mandel, J. L., & Piton, A. (2017). Mutations in histone Acetylase modifier BRPF1 cause an autosomal-dominant form of intellectual disability with associated ptosis. *American Journal of Human Genetics*, *100*(1), 105–116. https://doi.org/10.1016/j.ajhg.2016.11.010

McCauley, J., Masand, N., McGowan, R., Rajagopalan, S., Hunter, A., Michaud, J. L., Gibson, K., Robertson, J., Vaz, F., Abbs, S., & Holden, S. T. (2011). X-linked VACTERL with hydrocephalus syndrome: Further delineation of the phenotype caused by FANCB mutations. *American Journal of Medical Genetics. Part A*, *155A*(10), 2370–2380. https://doi.org/10.1002/ajmg.a.33913

Mehta, P. A., & Ebens, C. (1993). Fanconi anemia. In M. P. Adam, G. M. Mirzaa, R. A. Pagon, S. E. Wallace, L. J. H. Bean, K. W. Gripp, & A. Amemiya (Eds.), *GeneReviews®*. University of Washington. https://www.ncbi.nlm.nih.gov/pubmed/20301575

Meng, L., Pammi, M., Saronwala, A., Magoulas, P., Ghazi, A. R., Vetrini, F., Zhang, J., He, W., Dharmadhikari, A. V., Qu, C., Ward, P., Braxton, A., Narayanan, S., Ge, X., Tokita, M. J., Santiago-Sim, T., Dai, H., Chiang, T., Smith, H., … Lalani, S. R. (2017). Use of exome sequencing for infants in intensive care units: Ascertainment of severe single-gene disorders and effect on medical management. *JAMA Pediatrics*, *171*(12), e173438. https://doi.org/10.1001/jamapediatrics.2017.3438

Niguidula, N., Alamillo, C., Shahmirzadi Mowlavi, L., Powis, Z., Cohen, J. S., & Farwell Hagman, K. D. (2018). Clinical whole-exome sequencing results impact medical management. *Molecular Genetics & Genomic Medicine*, *6*(6), 1068–1078. https://doi.org/10.1002/mgg3.484

Pilorge, M., Fassier, C., Le Corronc, H., Potey, A., Bai, J., De Gois, S., Delaby, E., Assouline, B., Guinchat, V., Devillard, F., Delorme, R., Nygren, G., Rastam, M., Meier, J. C., Otani, S., Cheval, H., James, V. M., Topf, M., Dear, T. N., … Betancur, C. (2016). Genetic and functional analyses demonstrate a role for abnormal glycinergic signaling in autism. *Molecular Psychiatry*, *21*(7), 936–945. https://doi.org/10.1038/mp.2015.139

Pitt, D., & Hopkins, I. (1978). A syndrome of mental retardation, wide mouth and intermittent overbreathing. *Australian Paediatric Journal*, *14*(3), 182–184. https://doi.org/10.1111/jpc.1978.14.3.182

Retterer, K., Juusola, J., Cho, M. T., Vitazka, P., Millan, F., Gibellini, F., Vertino-Bell, A., Smaoui, N., Neidich, J., Monaghan, K. G., McKnight, D., Bai, R., Suchy, S., Friedman, B., Tahiliani, J., Pineda-Alvarez, D., Richard, G., Brandt, T., Haverfield, E., … Bale, S. (2016). Clinical application of whole-exome sequencing across clinical indications. *Genetics in Medicine*, *18*(7), 696–704. https://doi.org/10.1038/gim.2015.148

Richards, S., Aziz, N., Bale, S., Bick, D., Das, S., Gastier-Foster, J., Grody, W. W., Hegde, M., Lyon, E., Spector, E., Voelkerding, K., Rehm, H. L., & Committee, A. L. Q. A. (2015). Standards and guidelines for the interpretation of sequence variants: A joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. *Genetics in Medicine*, *17*(5), 405–424. https://doi.org/10.1038/gim.2015.30

Robert, E., Mutchinick, O., Mastroiacovo, P., Knudsen, L. B., Daltveit, A. K., Castilla, E. E., Lancaster, P., Kallen, B., & Cocchi, G. (1993). An international collaborative study of the epidemiology of esophageal atresia or stenosis. *Reproductive Toxicology*, *7*(5), 405–421. http://www.ncbi.nlm.nih.gov/pubmed/8274816

Schaaf, C. P., Boone, P. M., Sampath, S., Williams, C., Bader, P. I., Mueller, J. M., Shchelochkov, O. A., Brown, C. W., Crawford, H. P., Phalen, J. A., Tartaglia, N. R., Evans, P., Campbell, W. M., Tsai, A. C., Parsley, L., Grayson, S. W., Scheuerle, A., Luzzi, C. D., Thomas, S. K., … Cheung, S. W. (2012). Phenotypic spectrum and genotype-phenotype correlations of NRXN1 exon deletions. *European Journal of Human Genetics*, *20*(12), 1240–1247. https://doi.org/10.1038/ejhg.2012.95

Scott, D. A. (1993). Esophageal atresia / tracheoesophageal fistula overview. In M. P. Adam, H. H. Ardinger, R. A. Pagon, S. E. Wallace, L. J. H. Bean, K. Stephens, & A. Amemiya (Eds.), *GeneReviews®*. University of Washington. https://www.ncbi.nlm.nih.gov/pubmed/20301753

Scott, T. M., Campbell, I. M., Hernandez-Garcia, A., Lalani, S. R., Liu, P., Shaw, C. A., Rosenfeld, J. A., & Scott, D. A. (2021). Clinical exome sequencing data reveal high diagnostic yields for congenital diaphragmatic hernia plus (CDH+) and new phenotypic expansions involving CDH. *Journal of Medical Genetics*, *59*(3), 270–278. https://doi.org/10.1136/jmedgenet-2020-107317

Seo, J., Kim, D. Y., Kim, A. R., Kim, D. Y., Kim, S. C., Kim, I. K., Kim, K. S., Yoon, C. H., & Pi, S. Y. (2010). An 18-year experience of tracheoesophageal fistula and esophageal atresia. *Korean Journal of Pediatrics*, *53*(6), 705–710. https://doi.org/10.3345/kjp.2010.53.6.705

Shaw-Smith, C. (2006). Oesophageal atresia, tracheo-oesophageal fistula, and the VACTERL association: Review of genetics and epidemiology. *Journal of Medical Genetics*, *43*(7), 545–554. https://doi.org/10.1136/jmg.2005.038158

Solomon, B. D. (2018). The etiology of VACTERL association: Current knowledge and hypotheses. *American Journal of Medical Genetics. Part C, Seminars in Medical Genetics*, *178*(4), 440–446. https://doi.org/10.1002/ajmg.c.31664

Speleman, F., Van Roy, N., Wiegant, J., Verschraegen-Spae, M. R., Benoit, Y., Govaert, P., Goossens, L., & Leroy, J. G. (1992). Detection of subtle reciprocal translocations by fluorescence in situ hybridization. *Clinical Genetics*, *41*(4), 169–174. https://doi.org/10.1111/j.1399-0004.1992.tb03657.x

Sulkowski, J. P., Cooper, J. N., Lopez, J. J., Jadcherla, Y., Cuenot, A., Mattei, P., Deans, K. J., & Minneci, P. C. (2014). Morbidity and mortality in patients with esophageal atresia. *Surgery*, *156*(2), 483–491. https://doi.org/10.1016/j.surg.2014.03.016

Umana, L. A., Magoulas, P., Bi, W., & Bacino, C. A. (2011). A male newborn with VACTERL association and Fanconi anemia with a FANCB deletion detected by array comparative genomic hybridization (aCGH). *American Journal of Medical Genetics. Part A*, *155A*(12), 3071–3074. https://doi.org/10.1002/ajmg.a.34296

van de Putte, R., van Rooij, I., Haanappel, C. P., Marcelis, C. L. M., Brunner, H. G., Addor, M. C., Cavero-Carbonell, C., Dias, C. M., Draper, E. S., Etxebarriarteun, L., Gatt, M., Khoshnood, B., Kinsner-Ovaskainen, A., Klungsoyr, K., Kurinczuk, J. J., Latos-Bielenska, A., Luyt, K., O'Mahony, M. T., Miller, N., … Roeleveld, N. (2020). Maternal risk factors for the VACTERL association: A EUROCAT case-control study. *Birth Defects Research*, *112*(9), 688–698. https://doi.org/10.1002/bdr2.1686

Vetro, A., Iascone, M., Limongelli, I., Ameziane, N., Gana, S., Della Mina, E., Giussani, U., Ciccone, R., Forlino, A., Pezzoli, L., Rooimans, M. A., van Essen, A. J., Messa, J., Rizzuti, T., Bianchi, P., Dorsman, J., de Winter, J. P., Lalatta, F., & Zuffardi, O. (2015). Loss-of-function FANCL mutations associate with severe Fanconi anemia overlapping the VACTERL association. *Human Mutation*, *36*(5), 562–568. https://doi.org/10.1002/humu.22784

Whalen, S., Heron, D., Gaillon, T., Moldovan, O., Rossi, M., Devillard, F., Giuliano, F., Soares, G., Mathieu-Dramard, M., Afenjar, A., Charles, P., Mignot, C., Burglen, L., Van Maldergem, L., Piard, J., Aftimos, S., Mancini, G., Dias, P., Philip, N., … Giurgea, I. (2012). Novel comprehensive diagnostic strategy in Pitt-Hopkins syndrome: Clinical score and further delineation of the TCF4 mutational spectrum. *Human Mutation*, *33*(1), 64–72. https://doi.org/10.1002/humu.21639

Zen, P. R., Riegel, M., Rosa, R. F., Pinto, L. L., Graziadio, C., Schwartz, I. V., & Paskulin, G. A. (2010). Esophageal stenosis in a child presenting a de novo 7q terminal deletion. *European Journal of Medical Genetics*, *53*(5), 333–336. https://doi.org/10.1016/j.ejmg.2010.06.008

Zweier, C., de Jong, E. K., Zweier, M., Orrico, A., Ousager, L. B., Collins, A. L., Bijlsma, E. K., Oortveld, M. A., Ekici, A. B., Reis, A., Schenck, A., & Rauch, A. (2009). CNTNAP2 and NRXN1 are mutated in autosomal-recessive Pitt-Hopkins-like mental retardation and determine the level of a common synaptic protein in drosophila. *American Journal of Human Genetics*, *85*(5), 655–666. https://doi.org/10.1016/j.ajhg.2009.10.004

Zweier, C., Peippo, M. M., Hoyer, J., Sousa, S., Bottani, A., Clayton-Smith, J., Reardon, W., Saraiva, J., Cabral, A., Gohring, I., Devriendt, K., de Ravel, T., Bijlsma, E. K., Hennekam, R. C., Orrico, A., Cohen, M., Dreweke, A., Reis, A., Nurnberg, P., & Rauch, A. (2007). Haploinsufficiency of TCF4 causes syndromal mental retardation with intermittent hyperventilation (Pitt-Hopkins syndrome). *American Journal of Human Genetics*, *80*(5), 994–1001. https://doi.org/10.1086/515583

SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

How to cite this article: Sy, M. R., Chauhan, J., Prescott, K., Imam, A., Kraus, A., Beleza, A., Salkeld, L., Hosdurga, S., Parker, M., Vasudevan, P., Islam, L., Goel, H., Bain, N., Park, S.-M., Mohammed, S., Dieterich, K., Coutton, C., Satre, V., Vieville, G., … Scott, D. A. (2022). Exome sequencing efficacy and phenotypic expansions involving esophageal atresia/tracheoesophageal fistula plus. *American Journal of Medical Genetics Part A*, *188*A:3492–3504. https://doi.org/10.1002/ajmg.a.62976