Etiology of Esophageal Atresia and Tracheoesophageal Fistula: “Mind the Gap”

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Abstract Esophageal atresia and tracheoesophageal fistula (EA/TEF) are major congenital malformations affecting 1:3500 live births. Current research efforts are focused on understanding the etiology of these defects. We describe well-known animal models, human syndromes, and associations involving EA/TEF, indicating its etiologically heterogeneous nature. Recent advances in genotyping technology and in knowledge of human genetic variation will improve clinical counseling on etiologic factors. This review provides a clinical summary of environmental and genetic factors involved in EA/TEF.

Keywords Congenital anomaly · Foregut · VACTERL · Feingold syndrome · CHARGE syndrome · AEG syndrome · Genes

Introduction

Esophageal atresia (EA) and tracheoesophageal fistula (TEF) are congenital malformations that occur approximately in 1:3500 live-born infants [1]. Five subtypes are described, based on the location of the atresia and the type of connection between trachea and esophagus [2]. Associated anomalies occur in 50% of patients and include vertebral, anal, cardiovascular, tracheoesophageal, renal, and limb abnormalities (occurring together in the VACTERL association). Better surgical techniques and pre- and postoperative care have improved the prognosis of EA/TEF over the past decades, but patients still have significant short- and long-term morbidity [3]. As with other congenital malformations, EA/TEF occurs at an increased rate in twins, but usually affects only one twin [1]. Once a couple has one child with EA/TEF, the risk of having a second child with this anomaly is increased to 1% [4].

The pathologic mechanism leading to EA/TEF is unknown. The trachea, esophagus, and lungs are foregut-derived structures. During the fourth week of embryonic life, the foregut divides into a ventral respiratory part and a dorsal esophageal part. The underlying mechanism of separation is not known.

EA/TEF is thought to be a multifactorial complex disease, with involvement of genetic and environmental factors. In 6% to 10% of patients a defined genetic syndrome can be diagnosed, leaving 90% of patients of unknown etiology [5]. This paper aims to give an overview of current knowledge and gaps in knowledge on the etiology of EA/TEF. In this review we distinguish between complex genetic syndromes and associations to facilitate targeted genetic follow-up and counseling.

Environmental Factors

Various environmental factors have been suggested as risk factors for the development of tracheoesophageal anomalies,
including maternal exposure to methimazole [6], exogenous sex hormones [7], maternal alcohol and smoking [8], infectious diseases [9], and working in agriculture or horticulture [10]. Previous studies from our group implicate a possible role for maternal in utero exposure to diethylstilbestrol (DES) [11]. Observations in insulin-dependent diabetic mothers suggest that first trimester exposure to maternal diabetes is associated with the development of congenital anomalies, including EA/TEF and VACTERL-associated anomalies [12]. Very recently, studies from the European Surveillance of Congenital Anomalies (EUROCAT) birth registry network found that older mothers are at significantly greater risk of having a child with EA [13].

Administration of the anthracycline antibiotic adriamycin to pregnant rats causes EA/TEF and other major congenital anomalies in the offspring [14]. However, no such association has been reported for humans [15]. A role for vitamin A deficiency in the development of EA/TEF has also been suggested. A vitamin A–deficient diet given to pregnant rats caused severe congenital anomalies in the offspring, including agenesis of the lung and TEF [16]. Ethynitrosourea (ENU), an alkylating and mutagenic agent, induced a recessive mouse mutation with a phenotype that includes abnormal tracheoesophageal septation and VACTERL-associated anomalies [17]. So far, no specific environmental risk factor has consistently been identified.

Genetic Factors

Human Syndromes and Associations Involving EA/TEF

More than 50% of EA/TEF patients have associated anomalies. Certain anomalies, such as cardiovascular defects, renal agenesis, microcephaly, duodenal atresia, limb reduction defects, and polycystic kidney are especially prevalent in patients with EA/TEF [18, 19]. EA/TEF may be present in several syndromes and associations, as described in Table 1.

The department of Pediatric Surgery at the Erasmus MC—Sophia Children’s Hospital admitted more than 300 EA/TEF patients from 1988 to 2009. In 29 patients, a chromosomal abnormality or a single gene disorder was causative to the EA/TEF phenotype. One in every 10 patients had a defined syndrome, which is in line with the literature. Many of the known genetic syndromes were seen in this cohort, including all full trisomies (Down syndrome, Edwards syndrome, Patau syndrome), single gene disorders (eg, CHARGE syndrome, Feingold syndrome, Opitz syndrome, and Fanconi anemia), and some less frequent syndromes (eg, Holt-Oram syndrome and Townes Brocks syndrome). The distribution of syndromes is according to the literature [5, 20].

More than 30% of EA/TEF cases in our cohort (syndromal cases excluded) were defined as VACTERL-associated. EA/TEF is a component of the VACTERL association, which includes vertebral, anal, cardiovascular, tracheoesophageal, renal, and limb anomalies, and is seen in 10% to 30% of EA/TEF patients [1, 19]. The use of this acronym as a clinical entity is still being debated, as is the minimum number of defects that must be present.

Of the VACTERL components, the vertebrae/ribs and the cardiovascular system are most commonly affected in combination with EA/TEF [23]. EA/TEF occasionally occurs in combinations with hemifacial microsomia, cardiac, vertebral, and/or central nervous system anomalies (Goldenhar syndrome), but no genetic basis has been described for this syndrome [21].

VACTERL associated patients are of interest in the search for new genetic factors underlying foregut-related and associated anomalies. However, the combinations of EA/TEF and its associated anomalies resemble and/or overlap phenotypically with defined syndromes, such as CHARGE syndrome, Feingold syndrome, and 22q11 deletion syndrome (discussed below). It is not easy, therefore, to discriminate between the causal syndromes, the more so as the etiology of VACTERL association is still unclear. Specific phenotypes, such as VACTERL-associated anomalies combined with hydrocephalus, did reveal causative mutations in the genes FANCB and PTEN [22, 23]. A recent study found deletions in the FOX gene cluster on chromosome 16q24, and mutations in the FOXF1 gene, in patients with alveolar capillary dysplasia combined with VACTERL-associated anomalies, including EA/TEF [24].

Single Gene Disorders Involving EA/TEF

Feingold Syndrome

Feingold syndrome is caused by germline mutations in, or deletions of, the MYCN gene on chromosome 2p24.1. It is the most frequent cause of familial syndromic gastrointestinal atresias. About 30% to 40% of patients diagnosed with Feingold syndrome have EA/TEF [25]. Marcelis et al. [26] reviewed the clinical features of Feingold syndrome in relation to the genotype; 23 different mutations of the MYCN gene and five deletions encompassing MYCN have been described over the years. The authors suggest that the presence of digital anomalies in combination with microcephaly is enough to justify MYCN analysis [26]. Such analysis should also be considered in patients with EA/TEF in combination with microcephaly.
About 10% of patients with CHARGE syndrome display EA/TEF. This well-defined syndrome—involving coloboma, heart anomalies, choanal atresia, growth and/or mental retardation, genital and ear anomalies—is caused by mutations in the chromodomain helicase DNA-binding (CHD7) gene, present in about 60% of CHARGE patients [27]. This gene is thought to have a role in early embryonic development affecting epigenetic regulation by chromatin.
organization and euchromatic gene expression. Still, its regulatory function and involvement in CHARGE syndrome and foregut development should be clarified in functional studies in humans and animal models.

**AEG Syndrome**

Deletions and mutations of the SOX2 gene are causative for the phenotype of clinical anophthalmia/optic nerve hypoplasia, esophageal atresia, and/or genital anomalies in the AEG syndrome [28]. Studies in chickens and Xenopus implicate a role for Sox2 in the developing foregut. Que et al. [29••,30] demonstrated EA/TEF in Sox2 mutant mice and thus provided evidence that downregulation of Sox2 plays a role in the etiology of EA. The proven relation between murine models and the human phenotype was a breakthrough in the knowledge about syndromic EA/TEF.

**Pallister-Hall Syndrome**

Pallister-Hall syndrome includes bifid epiglottis, hypoplastic hamartoblastoma, postaxial polydactyly, anal atresia, and occasionally laryngeal clefts. Mutations in the GLI3 gene can cause Pallister-Hall syndrome [31]. The foregut-related anomalies, such as laryngeal clefts and lobulation defects of the lungs, form a phenotypic link between human Pallister-Hall patients and the combined knockout mice for Gli2/Gli3. This provides evidence that the Gli-genes and their pathways are important in foregut development [32].

**Opitz G Syndrome**

Opitz G syndrome is characterized by midline abnormalities with mental retardation and agenesis of the corpus callosum. Even though EA/TEF is rare in Opitz G syndrome, the combination of EA/TEF with corpus callosum agenesis warrants testing for mutations in the MID1 gene, causing the X-linked form of Opitz syndrome. Laryngotracheoesophageal defects in general are present in most MID1-mutated males, but EA/TEF is rare [33]. In addition, deletions on chromosome 22q11.2 cause the autosomal-dominant form of Opitz syndrome, which has the same size of the deletion as observed in DiGeorge syndrome [34]. Also, several cases of EA/TEF have been described in patients with DiGeorge syndrome (see below).

**Fanconi Anemia**

Fanconi anemia (FA) is a genetically and phenotypically heterogeneous syndrome characterized by progressive bone marrow failure and early occurrence of acute myeloid leukemia, combined with several congenital malformations, including gastrointestinal malformations. Thirteen genetic subtypes have been described (A, B, C, D1, D2, E, F, G, I, J, L, M, and N), of which FANCA, FANCC, and FANCG are the three most common disease-causing genes [35]. Mutations in the FANCA, FANCB, FANCC, FANCD1 (BRCA2), FANCD2, and FANCG genes have been described in patients with EA/TEF with various combinations of defects, including microcephaly, short stature, pigment changes, and several VACTERL-associated anomalies, including heart, renal, and limb defects [22, 35–39]. EA/TEF is not a common feature of FA, but gastrointestinal malformations, including duodenal atresia, anorectal malformations, and EA/TEF, are seen in 14% of all FA patients [35]. In addition, mutations in the FANCB gene, on chromosome Xp22.31, were causes of X-linked VACTERL with hydrocephalus in patients who also had EA/TEF, lung lobulation defects, and confirmed central nervous system anomalies [22]. The diagnosis of Fanconi anemia calls for early therapeutic interventions and chromosome breakage studies. Faivre et al. [36] advised performance of breakage studies in patients with common VACTERL-associated anomalies, including EA/TEF, in combination with skin pigmentation abnormalities, growth retardation, microcephaly, and/or dysmorphism.

**Chromosomal Abnormalities**

Many well-known chromosomal aberrations are observed in EA/TEF patients, such as Down syndrome (trisomy 21), Edwards syndrome (trisomy 18), and Patau syndrome (trisomy 13) [1]. Deletions on several chromosomal loci, including 22q11 (DiGeorge syndrome), distal 13q, 17q21.3-q24.2, and 16q24.1 are found in some cases [24••, 40–43]. The paper by Felix et al. [42] reviewed the structural chromosomal anomalies reported in 30 patients. Some of the above-mentioned deletions are of interest, because the deleted loci include several candidate genes for EA/TEF, for example, the 17q21.3-q24.2 region including NOG and TBX4. Recently Stankiewicz et al. [24••] described deletions on the 16q24.1 locus, including the forkhead genes FOXF1, FOXC2, and FOXL1. Of these transcription factors, FOXF1 is of particular interest, because heterozygote knockout mice for this protein have EA/TEF.

Better molecular cytogenetic techniques allow screening of large cohorts of patients with congenital anomalies; the copy number variations in microarray studies yield additional deletions and duplications. Such studies in EA/TEF patients are expected to reveal new candidate regions. We confirmed several chromosomal aberrations in patients with EA/TEF: Triple-X syndrome, Xp duplications, 22q11 microduplication syndrome, and a 5q11.2 deletion (unpublished data). Microarray studies will also provide more insight into
the polymorphic regions of the human genome in relation to inherited aberrations in patients with congenital anomalies.

**Murine Models**

Several murine models are described to cause tracheoesophageal anomalies (Table 2) [17, 30, 44]. Genes of developmental pathways are involved, including vitamin A effectors (Raro, Rarβ), effectors of the sonic hedgehog (SHH) pathway (Shh, Gli2, Gli3, Foxf1), other homeobox-containing transcription factors and their regulators (Tbx4, Ttf-1, Pcsk5), and developmental transcriptional regulators (Tbx4, Sox2).

Research into the human homologues of these genes has shed some light on the molecular basis underlying some human cases of EA/TEF. For example, Que et al. [30] found that 70% of null mutant mice for Noggin display EA/TEF. In several human TEF cases there is a deletion of the 17q21.3-24.2 locus, which includes the human orthologue NOG [30, 43]. Mouse haploinsufficient for Foxf1 may show EA/TEF and other foregut-derived anomalies, including lung hypoplasia and lobulation defects [45]. A patient with a heterozygote deletion on chromosome 16q24, including the FOXF1 gene, displayed lung anomalies, EA/TEF and other VACTERL-associated features [24±]. The Gli2 and Gli3 genes are essential for formation of the trachea and the esophagus. Deletions of these genes are associated with congenital defects that resemble the VACTERL association, at least in mice [30, 32]. Szumska et al. [17] describe an ENU-induced recessive mouse mutation in the Pcsk5 gene, a regulator of Hox genes. Such mutations, in a heterozygote form, were also present in two patients with EA/TEF and features of VACTERL association. However, in these cases the mutations were inherited from a phenotypically normal parent. The etiologic role of these mutations remained unclear [17].

In conclusion, EA/TEF can be part of a spectrum of anomalies in specific syndromes with a known cause or it can be part of an association (Fig. 1). Clinicians should be aware of specific combinations of anomalies, and should consider counseling by a clinical geneticist.

**Strategies and Future Prospects**

**Phenotypic Approach**

Patients presenting with esophageal atresia receive treatment by standardized clinical and surgical protocols. Adding standardized genetic protocols for counseling and research would provide a good opportunity to unravel the genetic background of EA and other congenital abnormalities. The clinical protocol for all patients with EA/TEF should include an echocardiogram and vertebral and limb radiographs, complemented with renal ultrasound evaluation if other features of the VACTERL association are present.

**Genotypic Approach**

Counseling by a clinical geneticist is advised, including standard karyotyping and screening for subtelomeric anomalies. Such counseling should consider the genetic information that is available for patients with EA/TEF and other congenital abnormalities. The etiologic role of these genetic factors remains unclear, but they should be considered in the differential diagnosis for patients with EA/TEF and features of the VACTERL association. In conclusion, EA/TEF can be part of a spectrum of anomalies in specific syndromes with a known cause or it can be part of an association (Fig. 1). Clinicians should be aware of specific combinations of anomalies, and should consider counseling by a clinical geneticist.

**Table 2** Overview of genes essential for tracheoesophageal development and their human homologues

| Gene        | Mutant phenotype | Human homologue | Human locus |
|-------------|------------------|-----------------|-------------|
| Shh<sup>-/-</sup> | EA; TEF; lungs form rudimentary sacs | SHH | 7q36 |
| Raro<sup>-/-</sup>; Rarβ<sup>-/-</sup> | TEF; lung hypoplasia or agenesis | RARo; RARβ | 17q21.1; RARβ: 3p24 |
| Gli2<sup>-/-</sup>; Gli3<sup>-/-</sup> | EA; TEF; severe lung phenotype | GLI2; GLI3 | GLI2: 2q14; GLI3: 7p13 |
| Foxf1<sup>-/-</sup> | Lethal before embryonic day 10; extra-embryonic defects | FOXF1 | 16q24.1 |
| Foxf1<sup>-/-</sup> | TEF; rudimentary peripheral lung primordial | TTF1 | 14q13 |
| Sox2<sup>-/-</sup> | EA; TEF; lung branching defects | SOX2 | 3q26.3-q27 |
| Noggin<sup>-/-</sup> | EA; TEF; lung branchng defects; abnormal notochord morphogenesis | NOG | 17q22 |
| Pcsk5 C470R | Abnormal tracheoesophageal septation; hypoplastic lungs | PCSK5 | 9q21.3 |
| Hoxc4<sup>-/-</sup> | Partially or completely blocked esophageal lumen; disruption of esophageal musculature | HOXC4 | 12q13.3 |
| Tbx4 misexpression | TEF | TBX4 | 17q21-q22 |

<sup>a</sup>Ethynitrosourea (ENU)-induced mouse mutation

EA esophageal atresia; TEF tracheoesophageal fistula

*(Adapted from Felix et al. [44], supplemented with findings from recent studies by Szumska et al. [17] and Que et al. [29±, 30])*
aberrations with multiplex ligation-dependent probe amplification. Clinical data should be reviewed and stored in comprehensive databases, together with genetic information, including pedigrees, DNA, tissue, and cell lines. With improvements in microarray technologies, genome-wide screening for copy number variations might replace standard karyotyping in the near future.

Epidemiologic Approach

Strategies that provide more insight into new etiologic factors should be in place to find more evidence for gene-environment interactions, to explore possible molecular mechanisms, and to find new genetic pathways. Environmental factors in families of patients with EA/TEF could be further explored by means of structured and validated questionnaires, adding to the knowledge gained from case-control studies [10]. Such studies require large cohorts, as have been used in population-based registries for surveillance of congenital anomalies, such as EUROCAT, the International Clearinghouse for Birth Defects Surveillance and Research, and the California Birth Defects Monitoring Program.

Experimental Approach

Studies in animal models implicate an essential role for sonic hedgehog signaling and its downstream effectors in the correct embryogenesis of the foregut. Better understanding of normal and abnormal development of the foregut could be achieved by focusing on the overlapping effectors of Shh-signaling, described in patients and animal models, for example, Gli2, Gli3, FOXF1, MID1, Noggin, Bmp4, and TBX1 [30, 46•].

Advances in genotyping technology and in knowledge of human genetic variation have enabled genome-wide association studies to identify susceptibility for common diseases, but also for congenital abnormalities, as proven in neural tube defects [47]. This approach requires large cohorts so as to provide statistical and clinical significance. Direct sequencing of candidate genes in patients and controls will provide an alternative approach that could reveal low-frequency alleles that influence disease susceptibility [48]. In addition, next generation sequencing will soon be an excellent tool in the search for new pathogenic mutations and copy number variations [49]. Also, there is increasing evidence that epigenetic modifications play an important role in developmental defects through DNA methylation and histone modifications [50]. A unifying approach seems to be the answer, therefore: analysis of multiple candidate genes should be done in large groups of well-genotyped individuals using next generation high-throughput genomic technology. This will be of great help in detecting possible gene-gene interactions as well as a possible role for copy number variations and regulatory mutations in patients with EA/TEF.

Conclusions

Many genetic pathways have been implicated in the development of EA/TEF. Patients with distinct phenotypes
may be diagnosed with genetic syndromes such as Feingold syndrome, AEG syndrome, and CHARGE syndrome. There is a substantial gap in our knowledge of how environmental factors combined with genetic factors would disrupt foregut development. We would do well to “mind the gap” by performing clinical studies focusing on phenotyping, combined with targeted molecular genetic studies. In the near future, studies in large cohorts will lead to the discovery of new genes, genetic pathways, and perhaps environmental factors.

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