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Clinical and etiological heterogeneity in patients with tracheo-esophageal malformations and associated anomalies

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ABSTRACT

Esophageal Atresia (EA) is a severe developmental defect of the foregut that presents with or without a Tracheo-Esophageal Fistula (TEF). The prevalence of EA/TEF over time and around the world has been relatively stable. EA/TEF is manifested in a broad spectrum of anomalies: in some patients it manifests as an isolated atresia or fistula, but in over half it affects several organ systems. While the associated malformations are often those of the VACTERL spectrum (Vertebral, Anorectal, Cardiac, Tracheo-Esophageal, Renal and Limb), many patients are affected by other malformations, such as micro-cephaly, micrognathia, pyloric stenosis, duodenal atresia, a single umbilical artery, and anomalies of the genitourinary, respiratory and gastrointestinal systems. Though EA/TEF is a genetically heterogeneous condition, recurrent genes and loci are sometimes affected. Tracheo-Esophageal (TE) defects are in fact a variable feature in several known single gene disorders and in patients with specific recurrent Copy Number Variations and structural chromosomal aberrations.

At present, a causal genetic aberration can be identified in 11–12% of patients. In most, EA/TEF is a sporadic finding; the familial recurrence rate is low (1%). As this suggests that epigenetic and environmental factors also contribute to the disease, non-syndromic EA/TEF is generally believed to be a multifactorial condition. Several population-based studies and case reports describe a wide range of associated risks, including age, diabetes, drug use, herbicides, smoking and fetal alcohol exposure. The phenotypical and genetic heterogeneity seen in EA/TEF patients indicates not one underlying cause, but several.

Unraveling the complex multifactorial and heterogeneous etiology of EA/TEF and associated features will require large cohorts of patients. Combined statistical analysis of component findings, genome sequencing, and genome wide association studies will elucidate new causal genetic defects and predisposing loci in the etiology within specific sub-populations. Improved knowledge of environmental risk factors, genetic predisposition and causal genetic syndromes may improve prediction and parental counseling, and prevent co-morbidity.

1. Introduction

Esophageal Atresia (EA) with or without Tracheo-Esophageal Fistula (TEF), is a developmental defect of the foregut characterized by the absence of continuity of the esophagus. EA/TEF (MIM 189960) can be classified in three ways: 1.) the Gross anatomical classification based on the presence and location of atresia and fistula; 2) a classification based on the association with other congenital anomalies (isolated or non-isolated); and 3.) a classification based on the presence of Tracheo-Esophageal (TE) anomalies in a known genetic syndrome (syndromal or non-syndromal). [de Jong et al., 2010a; Genevieve et al., 2007] In the vast majority of patients (78.0–91.8%), the atresia is associated with a TEF, i.e., a distal connection of the esophagus to the trachea. A minority of patients only have an atresia (5.0–13.0%), a

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fistula (2.4–6.5%), an atresia with a proximal connection to the trachea (0.4–5.7%), or an atresia combined with both a proximal and distal fistula (0.1–2.6%). [Bax et al., 2008; Spitz, 2007] In approximately half of patients, TE anomalies are associated with other congenital defects. [de Jong et al., 2008; Shaw-Smith, 2006] Non-syndromal EA/TEF is considered to be a multifactorial disease resulting from a variety of genetic and environmental influences [de Jong et al., 2008; Oddsberg, 2011; Shaw-Smith, 2010].

2. Prevalence, diagnosis and treatment

At ~ 2.5 per 10,000 births, the average prevalence of EA/TEF has been stable over time; regional prevalence ranges roughly between 1 and 4 per 10,000 births, including stillbirths and terminations of pregnancies. [Nassar et al., 2012; Pedersen et al., 2012] Contributing to these regional fluctuations are the quality of registries. While boys are affected more often than girls, with a sex ratio of 3:2 [de Jong et al., 2008; Depaepe et al., 1993], this gender disparity can be confounded by genetic and environmental factors. [Cui et al., 2005; Lisi et al., 2005; Lubinsky, 1997] Although it is preferable to detect EA before birth—in order to schedule delivery at a pediatric surgery and perinatal center, and also to improve parental counseling—EA is usually not detected by ultrasound or MRI [de Jong et al., 2010b]. Prenatal clinical manifestations of EA are polyhydramnios combined with non-visualization of the fetal stomach during ultrasound, at least in the case of an absent TEF. However, these signs are not exclusive to EA; if a TEF is present, amniotic fluid may flow into the fetal stomach resulting in normal fetal stomach filling.

Prenatal detection rates differ substantially between reference centers (10–50%) [Pedersen et al., 2012], and were recently enhanced by combining ultrasound and the so-called amniotic fluid EA index, a promising biochemical approach that measures amniotic fluid alpha-fetoprotein and gamma-glutamyl transpeptidase [Czerkiewicz et al., 2011].

After birth, EA can be suspected if newborns have excessive saliva and/or are in respiratory distress. The diagnosis is confirmed if a nasogastric tube cannot be passed to the stomach. In most cases, tracheo-esophageal defects are repaired using a right sided thoracotomy within 48 h of birth. Ten percent of European centers nowadays use a thoracoscopic procedure [Zani et al., 2013].

3. Early tracheo-esophageal development

Esophagus and respiratory structures develop from one common structure, the foregut. At the end of the third week of development of a human embryo, the endodermal layer folds to form a primitive gut tube. The primitive gut is regionalized and eventually differentiates into specific organs and derivatives of the gut tube, by a time dependent and localized expression and signaling actions of several growth factors (NODAL, FGF4), transcription factors (HEX, SOX2, FOXA2 and CDX2) and molecular pathways. [Faure and de Santa Barbara, 2011] For example, high NODAL levels prime the endodermal layer to an anterior fate, and low levels of NODAL and high levels of FGF4 to a posterior fate. [Zorn and Wells, 2009] The midgut is eventually formed—and the gut tube completed—by the inward-growing foregut, now expressing HEX, SOX2 and FOXA2, and the hindgut, expressing CDX2. [Carlson et al., 2013] Homeobox transcription factors are important for regionalizing the gut tube, and eventually define regional gut identity and specification [Roberts et al., 1995].

In the fourth week of development, the foregut is arranged in a ventral respiratory field marked by high NKX2.1, the absence of SOX2 expression, and a dorsal gastrointestinal tube marked by the reverse NKX2.1/SOX2 expression pattern. In mice, dorsal-ventral patterning of Sox2 and Nkx2.1 is essential for proper foregut morphogenesis. [Morrisey and Hogan, 2010; Que et al., 2007] This developmental process is excellently reviewed by Jacobs et al. (2012) and Morrisey and Hogan (2010). The separation site of the dorsal and ventral foregut is marked by Barx-1, which is expressed in the mesenchyme. [Woo et al., 2011] After this specification, the foregut separates into two sections: a ventral respiratory part with two lung buds, and a dorsal gastrointestinal structure. Key biological processes involved in this separation process are regulated by signals to the epithelium and from the surrounding mesenchyme (Wnt2, Wnt2b, Fgf10 and Bmp4) and notochord (Nog, Shh). [Jacobs et al., 2012] Abnormal foregut morphogenesis can lead to disturbances in dorso-ventral patterning, expression pattern, and the timing of signaling factors in key regulatory networks such as those in Bmp-signaling [Que et al., 2006], Wnt-signaling, RA-signaling [Bayha et al., 2009] and Sonic Hedgehog signaling. [Litingtung et al., 1998] Animal models and affected genes in patients with foregut abnormalities provide clues about a number of important biological processes during foregut separation and morphogenesis, including cell differentiation, proliferation, apoptosis, polarity and cytoskeletal rearrangements and cell-to-cell adhesion [Jacobs et al., 2012].

4. Tracheo-esophageal defects and the VACTERL association

A broad phenotypical spectrum of anomalies is associated with EA. In some cases there is an isolated atresia, but in many more cases, several organ systems are affected. [de Jong et al., 2008; Stoll et al., 2009] Certain malformations are associated with TE anomalies, more often than one would expect by chance; many are of the VACTERL kind (Vertebral, Anorectal, Cardiac, Tracheo-Esophageal, Renal or urinary tract and Limbs malformations). VACTERL association is diagnosed if three or more of the VACTERL component features are present and known genetic syndromes are excluded. [Solomon et al., 2012] As the inclusion criteria for VACTERL-associated malformations differ between institutions, VACTERL incidence ranges between 1 in 10,000 to 40,000 live born infants. [Solomon, 2011; Solomon et al., 2012]

From a developmental perspective, it is difficult to explain this co-occurrence of multiple congenital anomalies. For instance, the VACTERL-associated structures are formed at different points during development, with vertebral organogenesis starting at around day 23, and anorectal development around day 45. [Stevenson and Hunter, 2013] Many theories attempt to explain why the development of various organ systems is impaired: they include environmental exposure before or during organogenesis, epigenetic factors, hemodynamic instability in a monochorionic conception, a malformation sequence after abnormal notochord development that is followed by subsequent vertebral malsegmentation; and disturbances in developmental processes or key regulatory genes and pathways such as Sonic Hedgehog signaling (*SHH*, *GLI2*, *GLI3*). [Pharoah, 2005; Stevenson and Hunter, 2013]

5. Tracheo-esophageal defects and other associated malformations

TE anomalies are often associated with other, non-VACTERL, malformations such as microcephaly, duodenal atresia [Stoll et al., 2009], single umbilical artery, pyloric stenosis [van Beelen et al., 2014], malformations of the genitourinary, respiratory, gastrointestinal and central nervous system; and diaphragmatic hernia, micrognathia and other craniofacial anomalies. [de Jong et al., 2008; Stoll et al., 2009] These malformations are often associated with TE anomalies and one or more of the VACTERL

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