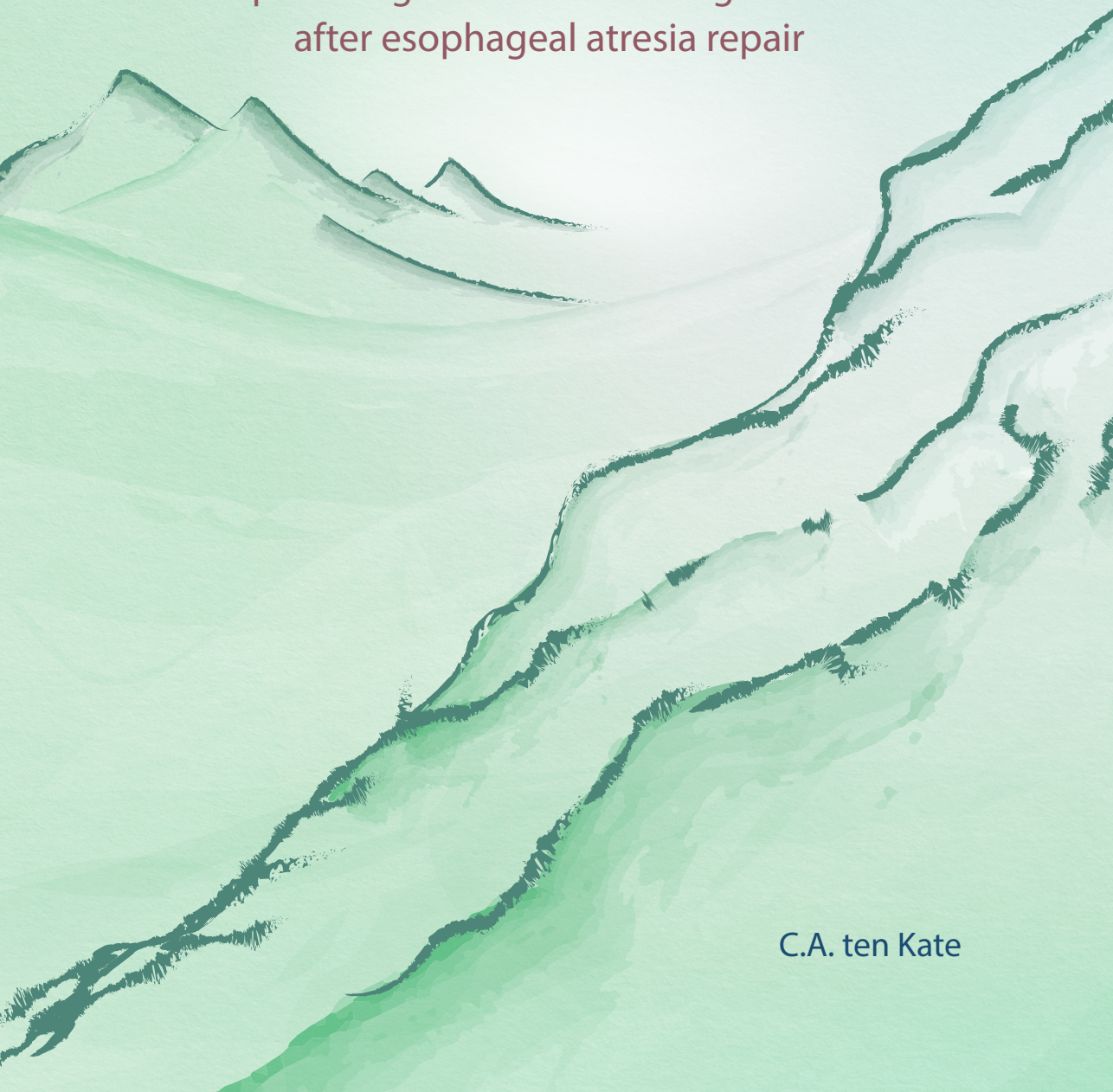
IT **DOESN'T END** WITH CLOSURE

Optimizing health care throughout life
after esophageal atresia repair



C.A. ten Kate

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Optimizing health care throughout life after esophageal atresia repair

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It Doesn't End With Closure

Optimizing health care throughout life after esophageal atresia repair

Het eindigt niet bij sluiting

Optimaliseren van de gezondheidzorg gedurende het leven na correctie van slokdarmatresie

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INTRODUCTION

CHAPTER 1

General Introduction

Esophageal atresia (EA), with or without the presence of a tracheoesophageal fistula (TEF), is a rare congenital malformation that affects 1 in 4000 live-born neonates.[1] The first description of an esophageal anomaly dates from 1670, describing the dissection of a conjoined twin in which one of them had a blind-ending esophagus.[2] In 1929, Vogt et al. first classified different types of EA,[3] and in 1953 Gross et al. proposed the classification that today is still mostly used.[4] This latter classification (see Figure 1) is based on the absence or presence and location of the TEF: EA without a TEF (type A), EA with a proximal TEF (type B), EA with a distal TEF (type C), EA with both a proximal and a distal TEF (type D) and an isolated TEF (type E). The most common form is type C, seen in over 85% of the cases. Types A and B often present as a long gap EA, in which a primary anastomosis of the two esophageal ends is not feasible.[5]

Half of the patients born with EA have associated anomalies or a chromosomal abnormality.[1] In ten percent of the children born with EA, the criteria of a VACTERL association are met. This association is a diagnosis of exclusion in which three or more features of the VACTERL spectrum (vertebral, anorectal, cardiac, tracheoesophageal, renal or limb malformations) are present.[6] Furthermore, EA can be part of genetic syndromes, such as Edward's syndrome (trisomy 18), Down's syndrome (trisomy 21) or CHARGE syndrome (coloboma, heart defects, choanal atresia, retardation, genital and ear abnormalities).[7]

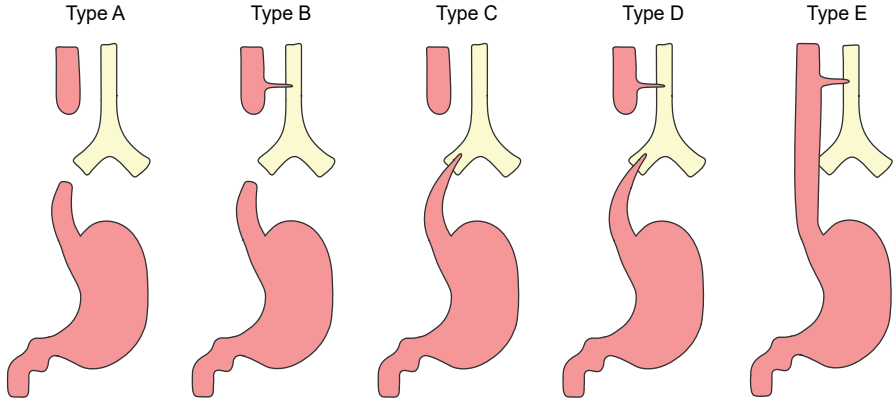

Figure 1. Five types of esophageal atresia, according to the Gross classification.[4]

Foregut morphogenesis and the development of esophageal atresia

The esophagus is derived from the embryonic foregut, as are the respiratory structures. In the third week after conception, the endodermal layer of the embryo folds and forms a primitive gut tube. Time-dependent local expression of several growth factors and molecular pathways

create a foregut, midgut and hindgut, and eventually lead to a separation of the foregut into the esophagus and trachea by the end of the sixth week of pregnancy.[8]

These processes inducing the development of the foregut have been best studied in mouse models. Regional patterns of *Fgf, Wnt, Hedgehog, Bmp* and retinoic acid generate gradients of signaling molecules, and regulate regional identity by activating *Hox* and *ParaHox* transcription factors.[9, 10] In mice, early foregut formation starts with *Foxa2* stimulation of the anterior endoderm.[11] After the formation of the gut tube, signals from the notochord start dorsal-ventral patterning.[12] Dorsal-ventral patterning of the foregut requires an *Fgf4* gradient induced by high *Nkx2.1*/absent *Sox2* in the ventral part, the future trachea, and absent *Nkx2.1*/high *Sox2* in the dorsal future esophagus and stomach.[13] In the ventral foregut, *Wnt2/2b* signaling stimulates the expression of *Nkx2.1*, and *Bmp4* inhibits *Sox2*. In the dorsal foregut, *NOGGIN* regulates the expression of *Sox2*, and *Wnt* signaling inhibits *Nkx2.1*.[8]

In addition, Hedgehog signaling is required for proper intestinal mesenchymal growth, patterning and differentiation.[14, 15] Both *Ihh* and *Shh* are expressed in the epithelium of the primitive gut tube, and differences in expression patterns lead to the development of different gut domains.[15, 16] Endodermal expression of *Shh* signals induces *Bmp4* expression in the mesoderm.[17] *Foxf1/2* is activated via epithelial Hedgehog signaling, stimulating *Bmp4* and reducing non-canonical *Wnt/PCP* signaling in the mesenchyme.[18] Altogether, the combination and precise timing of these dorsal-ventral patterns lead to compartmentalization of the foregut.[19]

Concomitantly, vagal neural crest cells enter the foregut. Enteric neural crest cells migrate in rostro-caudal direction to the end of the gut to form the enteric nervous system, which coordinates movements of the gastrointestinal tract, including the esophageal peristalsis.[20, 21]

Despite the improved understanding of foregut morphogenesis, the exact etiology of EA remains uncertain. For over two decades, it has been a topic of interest of our research group.[22-24] Previous research focused on gene expression patterns,[25] chromosomal anomalies such as copy number variations,[26] and environmental factors such as maternal age or in utero exposure to diethylstilbestrol (DES).[27, 28] Today, EA is considered a multifactorial condition. However, the fact that half of the patients are born with associated anomalies, makes a shared cause suspect. For example, a previous study has suggested involvement of genetic factors in patients with VACTERL association.[29]

Studying patients with multiple rare foregut-derived anomalies could perhaps reveal genetic alterations in genes involved in foregut morphogenesis that are responsible for these defects. The combination of EA and infantile hypertrophic pyloric stenosis (IHPS) is a potential target, because in IHPS the pyloric muscle hypertrophies in the first weeks of life, causing a

narrowing of the pyloric channel.[30] Since the pylorus starts to develop around the fifth week after gestation, there is a clear overlap with the development of the esophagus.[31]

Key question:
Can we propose a causative model for the origin of esophageal atresia in patients with a combination of rare foregut-derived conditions? (**Chapter 2**)

Diagnosis and treatment

Although polyhydramnios in combination with an absent or small stomach bubble on prenatal ultrasound suggests the presence of EA, the diagnosis of EA is mostly made after the child's birth.[32] Typical symptoms are blowing bubbles and respiratory distress caused by a TEF. The diagnosis can be confirmed with a chest X-ray after positioning a nasogastric tube, which will get stuck in the proximal pouch. In all confirmed cases of EA, a clinical geneticist is consulted to evaluate if an underlying genetic syndrome can be diagnosed. However, this consultation often takes place after the surgical repair and does not affect treatment.

The surgical treatment of EA consists of creating an anastomosis of the proximal and distal esophageal pouches, and ligating the TEF if present, usually in the first days of life.[33] In long gap EA, however, the distance between the two pouches is too large to be bridged by a primary anastomosis. This usually concerns EA types A or B.[5] Treatment options include a delayed primary anastomosis (most often used); the traction technique[34]; an anastomosis with a lengthening technique (such as a circular myotomy or a flap esophagoplasty[35]); or esophageal replacement (e.g. jejunal interposition, colon interposition or gastric pull-up).

Anastomotic stricture formation

Despite improved surgical techniques, the most frequent postoperative complication remains the formation of an anastomotic stricture.[36] A recent multicenter study of the Dutch Consortium of Esophageal Atresia (DCEA) reported that 58% percent of the children with EA developed an anastomotic stricture that required at least one endoscopic dilatation. Refractory strictures, requiring five or more dilatations within 28 days interval,[37] were reported in 7% of the total number of children. Risk factors for the development of a refractory stricture were EA type A, anastomotic leakage, and a first dilatation needed within 28 days after surgery.[38]

The European Society for Pediatric Gastroenterology, Hepatology and Nutrition (ESPGHAN) and the North American Society for Pediatric Gastroenterology, Hepatology and Nutrition (NASPGHAN) have stated that the first-line treatment for anastomotic strictures is endoscopic

dilatation under general anesthesia. This procedure involves either balloon dilatation or semi-rigid dilatation (i.e. bougienage), but consensus on the optimal method is lacking.

Key question:
What are the current treatment strategies for endoscopic dilatation procedures in children with esophageal anastomotic strictures worldwide? (**Chapter 4**)

Refractory strictures form a large burden for both the child and parents, as the recurrent dilatations under general anesthesia require the child hospitalization every time. With every dilatation, a new scar is created. A recurrent stricture after dilatation can therefore be considered as a hypertrophic lesion. A potential treatment, in addition to endoscopic dilatation, is the injection of intralesional steroids. For it has been hypothesized that steroids can prevent the regeneration of hypertrophic scar tissue by the inhibition of collagen formation, the enhancement of collagen breakdown and the decreased fibrotic healing.[39, 40]

Although literature on intralesional steroid injections is scarce, reported benefits included a reduction of the number of dilatation procedures,[41] longer intervals between dilatation procedures,[42] enlargement of the luminal diameter,[43] and relief of dysphagia.[44] The treatment is not yet standard of care,[45] because first level evidence is still lacking. Randomized controlled trials on this topic have been performed only in adults with underlying diagnoses other than EA, such as caustic strictures after acid ingestion, peptic strictures, and anastomotic strictures after an esophagectomy with gastric tube reconstruction.

After positive experiences with intralesional steroid injections in a handful of children with EA with recurrent anastomotic strictures, the next step would be to conduct a randomized controlled trial to prove the effectiveness and safety of intralesional steroid injections in addition to endoscopic dilatation in this population on a larger scale.

Key question:
What is the clinical outcome of patients injected with intralesional steroids as a treatment for an anastomotic stricture, in terms of stricture recurrence, postoperative complications and growth? (**Chapter 5**)

Key question:
How effective and safe are intralesional steroid injections combined with endoscopic dilatations to prevent refractory strictures? A study protocol. (**Chapter 6**)

Follow-up and long-term morbidities

With the current treatments strategies, survival rates of are now over 95%.[46-48] The low mortality during childhood has shifted the focus to outcome on the longer term. Long-term morbidities make EA a life-long chronic health condition. Many survivors of EA suffer from esophageal motility problems, gastroesophageal reflux (GER), delayed motor function development, impaired exercise capacity, or airflow obstruction.[49-54] But also in adulthood, many still experience gastrointestinal and respiratory problems, such as dysphagia, GER, coughing, or an impaired exercise capacity.[55-57]

Given the variety of long-term problems, survivors of EA require structured follow-up. In 1999, a longitudinal multidisciplinary follow-up program was initiated at the department of Pediatric Surgery of the Erasmus MC-Sophia Children's Hospital.[58] Standardized follow-up visits with neuropsychological assessments are scheduled until the age of 17 years. Since 2013, the department of Gastroenterology and Hepatology of the Erasmus MC offers prospective screening and surveillance with standardized upper endoscopies with biopsies for adults with EA,[59] every 3 or 5 years depending on a patient's age and histologic results. Standardized screening for lung abnormalities was added by the department of Pulmonology in 2019, for which patients undergo a lung function test and a chest computed tomography scan.

Even though life-long follow-up is recommended in international guidelines,[37] the specific health care needs of adults born with EA are little known. In this age of patient-centered care, the patient's perspective becomes more and more important long-term follow-up and screening programs.

Key question:
What are the medical and psychosocial healthcare needs of adults born with esophageal atresia and their family members, from a patient perspective? (**Chapter 7**)

Barrett's esophagus and esophageal carcinoma

Besides the gastrointestinal and respiratory problems that adults born with EA can encounter, concerns have been raised about the development of Barrett's esophagus (BE) and esophageal carcinoma in this population.[59] BE is a condition in which the squamous mucosa in the distal esophagus is damaged, usually by GER, and replaced by metaplastic columnar mucosa. BE is a premalignant disease, which can progress into esophageal adenocarcinoma in case of neoplastic differentiation.

Compared with the general population, the endoscopic surveillance program in our hospital revealed a 4-times higher prevalence of BE among the first 151 patients, at a much younger age (6.6% vs 1.6%; median age 34 vs. 60 years respectively). In addition, esophageal adenocarcinoma was found in three patients.[59] Surprisingly, also esophageal squamous cell carcinoma predominates in patients with EA at a younger age than the general population.[56]

Currently, surveillance endoscopies are scheduled every 3 or 5 years, depending on the patient's age and histologic results. This interval was chosen based on the American College of Gastroenterology guidelines for BE, in combination with suggestions for screening of adults with EA in literature.[55, 60-62] Nevertheless, the optimal surveillance strategy remains uncertain. Relatively short surveillance intervals may be warranted, but the potential burden of repeated endoscopies for both the patient and the health care system should be taken into account as well.

Key question:
What are the optimal starting age and surveillance interval of endoscopic screening in adults born with esophageal atresia? (**Chapter 8**)

The high prevalence of BE in adults born with EA implies that they have a higher risk to develop BE than do people in the general population. In the general population, male sex, age >50 years, Caucasian race, tobacco smoking, obesity, hiatus hernia and GER are considered as risk factors for BE.[60] Previous research, however, could not find such risk factors in adults born with EA.[59] The question remains whether survivors of EA are more susceptible to develop BE, for example due to a genetic predisposition. From a developmental perspective, a genetic predisposition could make sense, since there is an overlap between genes involved in foregut development[8] and risk loci associated with BE, esophageal adenocarcinoma and esophageal squamous cell carcinoma found in genome wide association studies (for example *TBX5, GDF7, CRTC1, BARX1, FOXP1,* and *FOXF1*).[63-65] Furthermore, there also seems to be an overlap between developmental pathways and pathways that maintain the homeostasis of esophageal epithelium renewal and potentially the development of BE (for example bone morphogenetic protein signaling or Sonic hedgehog signaling).[66]

Key question:
Can we find an increased susceptibility in patients born with esophageal atresia to develop Barrett's esophagus, esophageal adenocarcinoma or esophageal squamous cell carcinoma? (**Chapter 3**)

Psychosocial well-being

Another important aspect of the life course of survivors of EA is psychosocial well-being. One's health status and quality of life are important determinants of the burden of a disease. Health status is understood to reflect a person's well-being in terms of physical, mental and social condition or function. It measures to what extent one is limited in daily life activities by a medical condition.[67] Quality of life is defined as an individual's perception of their position in life in the context of the culture and value systems in which they live, and in relation to their goals, expectations, standards and concerns.[68] Health status and quality of life can be evaluated with patient self-reported and parent proxy-reported outcome measurements, although it is unclear how well these results relate to health and daily functioning. Given the economic burden of long-term follow-up for chronic conditions, patients-reported outcome measurements could be of interest to optimize clinical decision-making.

Nonetheless, one could envision that condition-specific questionnaires may provide better insight in the health status and quality of life of patients with rare diseases, since they provide more sensitive information than generic instruments.[69] For children, the EA-QOL© questionnaire has been developed and validated in Sweden.[70] For adults, a condition-specific questionnaire is currently lacking.

Key question:
How does a child born with esophageal atresia rate his or her health status and quality of life at school age, and how do their parents rate this? (**Chapter 9**)

Key question:
Do patient-reported outcome measures for health status and quality of life relate to clinical outcomes? (**Chapter 10**)

Key question:
How do condition-specific questionnaires rate a patient's health status and quality of life, in relation to generic patient-reported outcome measurements? (**Chapters 11 and 12**)

Aims and outline of this thesis

The studies presented in this thesis were performed with the aim to improve the knowledge on the etiology of EA, short-term complications such as anastomotic stricture formation, and the long-term risk to develop BE or esophageal carcinoma. The research intended to combine

new genetic and clinical insights to enhance the management of these patients, while taking into consideration their quality of life. The ultimate goal is to optimize the health care for all individuals born with esophageal atresia.

In **Chapter 13**, the results of the studies are discussed in a broader perspective, and suggestions for future research are presented. The results of all studies are summarized in English and in Dutch in **Chapter 14**.

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GENETIC PREDISPOSITION

CHAPTER 2

Infantile hypertrophic pyloric stenosis in patients with esophageal atresia

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ABSTRACT

Background

Patients born with esophageal atresia (EA) have a higher incidence of infantile hypertrophic pyloric stenosis (IHPS), suggestive of a relationship. A shared etiology makes sense from a developmental perspective as both affected structures are foregut derived. A genetic component has been described for both conditions as single entities and EA and IHPS are variable components in several monogenetic syndromes. We hypothesized that defects disturbing foregut morphogenesis are responsible for this combination of malformations.

Methods

We investigated the genetic variation of 15 patients with both EA and IHPS with unaffected parents using exome sequencing and SNP array-based genotyping, and compared the results to mouse transcriptome data of the developing foregut.

Results

We did not identify putatively deleterious de novo mutations or recessive variants. However, we detected rare inherited variants in EA or IHPS disease genes or in genes important in foregut morphogenesis, expressed at the proper developmental time-points. Two pathways were significantly enriched ($p < 1 \times 10^{-5}$): proliferation and differentiation of smooth muscle cells and self-renewal of satellite cells.

Conclusions

None of our findings could fully explain the combination of abnormalities on its own, which makes complex inheritance the most plausible genetic explanation, most likely in combination with mechanical and/or environmental factors. As we did not find one defining monogenetic cause for the EA/IHPS phenotype, the impact of the corrective surgery could should be further investigated.

INTRODUCTION

Esophageal atresia (EA), a congenital discontinuity of the esophagus caused by a faulty development of the foregut, can present either as an isolated defect but is often seen in combination with other malformations.[1] EA occurs in about 2.5 cases per 10,000 live births within Europe[2, 3] and over three-quarters of patients present with a tracheoesophageal fistula (TEF).[2, 4] Frequently, the malformations seen in combination with EA are part of the VACTERL (Vertebral, Anorectal, Cardiac, Tracheoesophageal, Renal or urinary tract of Limb malformations) association. VACTERL association is a diagnosis of exclusion in which three or more features of the VACTERL spectrum are present and no known genetic syndrome is identified.[5] Clustering of one or more associated malformations could also be the result of a shared genetic etiology. Recognizing these clusters might be hampered by variable expressivity and/or reduced penetrance.

Another prevalent, but less well-known, associated malformation is Infantile Hypertrophic Pyloric Stenosis (IHPS).[6] In these patients, the pyloric muscle hypertrophies in the first weeks of life, causing a narrowing of the pyloric channel.[7] Seemingly healthy-born infants present at week 3 to 6 of life with projectile postprandial vomiting. This condition requires surgery where the upper layer of the circular smooth muscle of the pylorus will be incised, to release the passage from the stomach to the intestine again. Previously, we have described a 30 times higher prevalence (7.5%) of IHPS in patients with EA compared to the normal population (0.25%).[8] This increased prevalence has been reported in other retrospective studies (3.3-13%) as well.[9, 10] The diagnosis of IHPS is more difficult and often delayed in patients with EA. Relatively common complications after EA repair, such as stenosis of the anastomosis, can protect against reflux and lead to just regurgitation. By the time these patients start vomiting, there is a massive gastroesophageal reflux.

The increased prevalence of IHPS in patients EA suggests a relationship. However, no research has been carried out towards the cause of this increased prevalence. It is unclear if IHPS is the consequence of the surgical repair or the result of a shared genetic etiology. As the esophagus and the pyloric sphincter are both foregut derived structures, we hypothesize that genetic alterations affecting genes important for foregut morphogenesis are the main drivers for the combination of defects seen in these patients. Given the low prevalence of the disorder and the high impact on development, we will concentrate on genes intolerant to heterozygous or recessive variation[11, 12] harboring rare putative deleterious single nucleotide changes or large CNVs.

METHODS

Patient cohort

This study was approved by the Medical Ethical Review Board of Erasmus Medical Center (MEC 193.948/2000/159). We searched the Erasmus University MC-Sophia Children's Hospital EA cohort and the database of the standardized prospective longitudinal follow up program in our hospital for children with congenital anatomical anomalies[13] for patients born between 1970-2017 with a combination of both EA and IHPS in history. Parental informed consent for whole exome sequencing (WES) was obtained for 15 patients.

Detection of genetic variation using exome sequencing

Initially, we included all variants with an minor allele frequency (MAF) below 1% in 1000 Genomes phase 3 version 5, Exome Variant Server 6500 v0.0.30, Genome of the Netherlands,[14] ExAC 0.3 and our in-house cohort (n=906), consisting of individuals captured with the SureSelect Human All Exon 50 Mb Targeted exome enrichment kit v4 (n=279), SureSelect Clinical Research Exome v1 (n=387) and Haloplex Exome target enrichment system (n=240) (Agilent Technologies, Inc., Santa Clara, California). We aimed at finding variants that could be classified as pathogenic or likely pathogenic by the American College of Medical Genetics and Genomics (ACMG) guidelines.[15] All nonsense variants, variants predicted to affect splicing and all heterozygous variants with a Combined Annotation-Dependent Depletion (CADD) score[16] above 20 were selected for individual patient analysis in different downstream tools (see Supplementary Material S1). Prioritized variants were further classified according to the criteria in Supplementary Material S2. Next, we focused on variants with a MAF below 5%, and we selected all protein coding and splicing variants in genes sensitive for recessive variation (Prec <0.9) for evaluation in recessive models. Determination of variant segregation and confirmation of de novo of inherited status of variants was done with Sanger sequencing unless otherwise indicated. Variants were considered ultra-rare[17] when they were absent from the gnomAD (http://gnomad.broadinstitute.org/)[18] dataset. Ultra-rare, X-linked or recessive variants predicted to be deleterious are submitted to the ClinVar database (https://www.ncbi.nlm.nih.gov/clinvar/).

Pathway enrichment analysis of genes affected by rare variants

To investigate if specific pathways are enriched with ultra-rare variants, Gene IDs with variants in canonical splice sites, nonsense variants, protein altering inframe InDels and missense variants were uploaded to Ingenuity pathway Analysis (Qiagen, Venlo, The Netherlands). Additionally, a more stringent set was uploaded with loss of function variants, predicted to be loss of function intolerant (PLI ≥0.9 or Prec ≥0.9) and protein altering variants with a Z-score ≥3.

Expression of candidate genes

Candidate gene expression was determined at relevant developmental time points in mouse. Gene expression of top-ranking genes derived from the individual patient sample prioritizations were determined using datasets (GSE13040, GSE19873, GSE34278, GSE15872, GSE43381) downloaded from the Gene Expression Omnibus (GEO).[19] From these datasets, we used public data on mice on the endoderm, mesoderm and ectoderm at E8.25, foregut at E8.5 and esophagus, stomach, pyloric sphincter and intestine at E11.5-E18.5 (https://www.ncbi.nlm.nih.gov/geo/).[20-24] These datasets were imported into BRB-ArrayTools Version 4.5.0 - Beta_2 (http://linus.nci.nih.gov/BRB-ArrayTools.html), annotated by Bioconductor (www.bioconductor.org), R version 3.2.2 Patched (2015-09-12 r69372) and normalized. We determined differential expression between tissue types and classified upregulated genes being expressed in the tissue under investigation.

Detection of common SNP associated with IHPS

Genome-wide association studies (GWAS) revealed five loci highly associated with IHPS (rs11712066, rs573872, rs29784, rs1933683 and rs6736913), pointing towards *MBNL1*, *NKX2-5*, *BARX1* and *EML4* as candidate genes.[25-27] Unfortunately, rs673913 proved to be resulting in false positive results (e.g. due to sequencing errors or alignment difficulties) in all patients and controls (see Supplementary Material S3). As a result, we did not include rs673913 in our calculation of the polygenic risk score (PGRS). With SNP-array, we genotyped the data of EA patients without IHPS, patients with EA and IHPS, and unaffected controls to determine ancestry as well as proxy SNP prevalence of the four above-mentioned IHPS associated SNPs. The same was done with data of related and unrelated parents of EA patients and parents of EA/IHPS patients. Sanger sequencing was used to confirm the risk allele frequency of these SNPs in our 15 EA/IHPS patients and to validate the chosen proxy SNPs.

Using the odds ratio (OR) of the associated SNPs, we calculated PGRSs: PGRS= $\sum Ln$ *(OR risk allele)*allele count*.[28] For this, we used the OR found in GWAS studies[25, 27] and the OR we calculated from our SNP array data for EA patients versus EA/IHPS patients (see Supplementary Material S4b). A paired t-test, Kruskal-Wallis test and Mann-Whitney test was used to compare the PGRS within each patient en between the different groups. All statistical analyses were performed in SPSS V.24.0 (IBM, Chicago, Illinois, USA), with a significance level of $p<0.05$.

RESULTS

Patient cohort

In total, 27 out of 664 patients (4.1%) born with EA between 1970-2017 developed IHPS. Twenty-one (77.8%) of them were male. A sacral dimple was present in seven patients (25.9%),

anomalies of the vertebrae or ribs in eight patients (29.7%) and genitourinary anomalies in six patients (22.2%) of which two patients (7.4%) had hypospadias. Four patients (14.8%) had three or more anomalies within the VACTERL spectrum.[29] A full phenotypical description of the 27 EA/IHPS patients is given in Table 1. Twenty patients have been described previously.[8]

Detection of genetic variation

Previously, we have described rare Copy Number Variations (CNVs) and their inheritance pattern in patients with EA.[30] Seventeen EA/IHPS patients described in that manuscript are included in this study. None of the six large CNVs identified were de novo, all were inherited from one of the unaffected parents. All CN profiles of main EA and IHPS disease genes[1, 31] were normal. All rare CNVs classified as a variant of unknown significance (VUS), likely deleterious or deleterious are described in Supplementary Material S5.

Exome sequencing resulted in at least 5 Giga-bases of raw sequence data with an average coverage of 70X and 90% of target bases covered over 20X. Quality of the sequence data is listed in Supplementary Material S6.

Mendelian models of inheritance

As none of the parents of the 15 investigated patients were affected, we first considered dominant de novo and recessive modes of inheritance. We could not identify de novo pathogenic variation in known EA and IHPS disease genes.[1, 31] Subsequently, we searched for rare putative damaging variation exome wide and could detect putative deleterious ultra-rare protein coding or splice site variation (n=100). We did not detect any (likely) pathogenic variants in known disease genes. Twenty-five variants turned out to be sequencing artifacts. Furthermore, we could not confirm the segregation of 15 mutations due to lack of parental DNA. We determined the segregation of all remaining ultra-rare variants predicted to be VUS (n=37), or likely deleterious (n=23). However, all putative deleterious variants tested were inherited from one of the unaffected parents.

We inspected the CN profiles from WES-CN and SNP-array for partial overlap with genes affected by heterozygous variant predicted to be deleterious in recessive loss of function intolerant or missense intolerant genes (n=48). We could not detect unmasking of a recessive mutation by a CNV.

We did not detect putative homozygous recessive, compound heterozygous nor X-linked-variants in known disease genes. Given the small sample size of our cohort, we concentrated our analysis on putative recessive inherited variants with a population frequency below 0.05 in genes intolerant to recessive variation (PLIrec >0.9). Furthermore, for putative compound heterozygous inherited variants, we additionally focused on genes that do not often have rare missense variants (missense Z score >2). For putative homozygous and X-linked variants,

Table 1. Phenotype description. EA; esophageal atresia, TEF; tracheoesophageal fistula, IHPS; infantile pyloric stenosis, DES; di-ethylstilbestrol. EA type classification according to Gross classification[76]

Individual	Gender	EA type	Phenotype	Remarks
SKZ_0027	female	C	EA/TEF, IHPS, thin ear helix, seizures	-
SKZ_0096	male	C	EA/TEF, IHPS, syndactyly second-third finger, radial dysplasia, abnormal fibula	VACTERL association
SKZ_0244	male	C	EA/TEF, IHPS, anal atresia, intestinal malrotation, sacral dimple, abnormal os coccygis, abnormal vertebrae L1, thenar hypoplasia, both sides hypoplastic "floating" thumbs, both sides dysplastic radii	VACTERL association, mother is a DES daughter
SKZ_0321	male	C	EA/TEF, IHPS, mild left sided expansion of the pyelocaliceal system , breath holding spells	-
SKZ_0353	female	C	EA/TEF, IHPS, sacral dimple, thin/slender build, diminished hearing, palpebral fissures slant up, hemolytic anemia, short phalanges	Glucose-6-phosphate dehydrogenase deficiency
SKZ_0399	male	C	EA/TEF, IHPS, anal atresia, sacral dimple, 2 umbilical vessels, posteriorly rotated ears, small ears/microtia, flat face, bifid scrotum, small penis/micropenis, small palmar crease, thick fingers, broad thumbs, proximal placement of thumbs, microstomia, thick broad neck, wide nasal bridge, patent ductus arteriosis, 4th toe abnormally placed	VACTERL association
SKZ_0400	male	C	EA/TEF, IHPS, extra ribs, fusion of vertebrae, macrocephaly, bulbar dermoid cyst, auricular tags, short thick/ broad neck	Klippel-Feil syndrome
SKZ_0683	male	C	EA/TEF, IHPS, sacral dimple	-
SKZ_0760	male	C	EA/TEF, IHPS, hemivertebrae, bitemporal narrowing of the head, prominent forehead, hyper mobile/ extensible fingers, narrow thorax/funnel chest, thin lower and upper lip, spasticity, cerebral palsy	-
SKZ_0788	male	C	EA/TEF, IHPS, inguinal hernia, jaundice, deafness	-
SKZ_0790	female	C	EA/TEF, IHPS	-
SKZ_0796	male	C	EA/TEF, IHPS	Vanishing twin
SKZ_0848	male	C	EA/TEF, IHPS, sacral dimple, hypospadias, patent ductus arteriosus	-
SKZ_0887	male	C	EA/TEF, IHPS, abnormal sacrum, fusion of vertebrae, posteriorly rotated ears, small mandible/micrognathia, rocker-bottom feet, sandal gap of toes, open mouth appearance, short neck, jaundice	-
SKZ_1003	male	C	EA/TEF, IHPS, abnormal sacrum, cleft jaw, cleft palate, cleft upper lip, depressed/flat nasal bridge, fused ribs	Methyldopa (aldomet) for hypertension during pregnancy
SKZ_1248	female	C	EA/TEF, IHPS, small large fontanel, deafness, small ears, auricular tags, single palmar crease, small/ hypoplastic deep set ears	-
SKZ_1260	male	C	EA/TEF, IHPS, syndactyly of second-third toe, bifid/fused ribs	-
SKZ_1353	male	C	EA/TEF, IHPS, cleft uvula, epicanthic folds, abnormal dermatoglypic patterns, hyperconvex/clubbed nails, hypoplastic scrotum, hypospadias, bifid scrotum, hydrocele of testis	-
SKZ_1407	female	A	EA, IHPS	-
SKZ_1472	male	C	EA/TEF, IHPS, eczema of hands with hyperhidrosis, blisters and erythema, Xerosis Cutis	Antibiotics for respiratory infection during pregnancy

SKZ_1961	male	C	EA/TEF, IHPS, sacral dimple, mild dysmorphic features, small mouth, pointy ears, long fingers	Maternal hypertension
SKZ_2013	male	A	EA, IHPS, persistent superior vena cava, scoliosis, Horner's syndrome	-
SKZ_2023	male	C	EA/TEF, IHPS, small chin, sacral dimple	-
SKZ_2050	male	C	EA/TEF, IHPS, atrial septum defect	
SKZ_2082	male	C	EA/TEF, IHPS, persistent tracheolaryngeal cleft, anal atresia, atrial septum defect, tracheal-laryngeal anomaly, prostate fistula	VACTERL association
SKZ_2149	male	C	EA/TEF, IHPS	-
SKZ_2171	female	C	EA/TEF, IHPS, spina bifida Th10/11, synostoses vertebrae, hydronephrosis, kyphoscoliosis	Unknown medication for headaches and nerves during pregnancy

X-linked variants, we excluded variants with a similar homozygous variant in GnoMAD and those with a CADD score below 15 (except for variants predicting splicing). Only variants in *COL4A2* (NM_001846:exon22:c.G1438A:p.A480T, NM_001846:exon44:c.G4195A:p.V1399I), *SLC6A2* (NM_001172502:exon1:c.G80A:p.C27Y, NM_001172502:exon2:c.G418A:p.V140I) and *VPS13D* (NM_015378:exon19:c.C4022T:p.S1341L, NM_015378:exon31:c.C7243T:p.H2415Y) were in genes that do not often have rare missense variants. Only the variants in *VPS13D* could both be classified as VUS.

We believe it is difficult to confidently classify the other putative compound heterozygous variants as VUS or higher as neither the gene has a low rate of missense variants, nor it is a missense variation a known disease mechanism (as it is not in a known disease gene). Additionally, we found a homozygous putative splice donor change (*MICAL2*: NM_001346292:exon21:r.spl) and a hemizygous change (*RPGR*:NM_000328:exon14:c.1579_1581del:p.Q527del) we could classify as VUS.

Non-Mendelian models

We found variants in the same gene in multiple patients (see Figure 1). Of these 116 genes (VUS=87, likely deleterious=30), 36 genes were found in ≥3 patients of which six genes were present in more than five patients. We prioritized all rare variants with three in silico tools (see Supplementary Material S1). Fifty-four variants in 34 genes were prioritized by VAAST,[18, 32, 33] which prioritizes based on variant deleteriousness as well as by Phevor and PhenIX which prioritize more on phenotype.[34, 35]

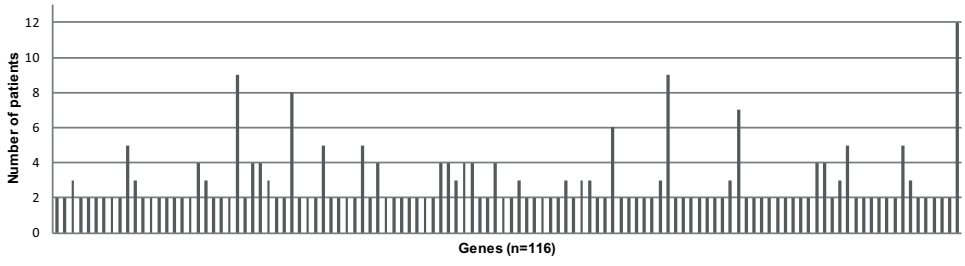

Figure 1. Number of patients with variants per gene. 36 genes were found in ≥3 patients of which six genes were present in more than five patients (*CNTN2*, *DSPP*, *NOTCH4*, *PRRC2A*, *SEC16B*, *ZNF717*). Four (*AMBRA1*, *ATP2A3*, *DSCAM*, *NOTCH1*) out of 116 genes were predicted to be intolerant for missense variants (Z-score ≥ 3).

We evaluated the number of damaging variants in developmental important pathways and known disease genes using 44 ancestry matched controls sequenced on the same platform as our 15 patients. There were no differences between controls and. However, some genes known to be important for foregut morphogenesis or syndromatically associated with EA or IHPS were affected in patients and unaffected in the healthy controls:

TNXB (NM_019105.6:c.4444G>A, p.Val1482Met), *WDR11* (NM_018117.11:c.1138G>T, p.Val380Phe), *PEX3* (NM_003630.2:c.1012A>G, p.Ser338Gly), *TBX3* (NM_016569.3: c.506G>A,p.Arg169Gln), and *GDF6* (NM_001001557.2:c.281C>G, p.Pro94Arg) (see Supplementary Material S7). Furthermore, the number of putative deleterious variants between these two groups did not differ (see Supplementary Material S8). Unfortunately, a burden test comparing the variant profiles of these genes between the patients and their parents was not possible since no WES data of the parents was available.

Pathway enrichment analysis of genes affected by rare variants

First, we evaluated genes with variants in canonical splice sites (n=16), nonsense variants (n=21), protein altering inframe InDels (n=28) and missense variants (n=557). Additionally, a more stringent set was used with loss of function variants, predicted to be loss of function intolerant (PLI ≥0.9, n=4) and protein altering variants with a Z-score ≥3 (n=44). Only when looking at the selected protein altering variants (Z-score ≥3, n=44) or loss of function intolerant (PLI ≥0.9, n=4), two pathways were significantly enriched (p-value <1x10-5): proliferation and differentiation of smooth muscle cells (*INSR*, *ITGB1*, *NOTCH1*, *TCF4*, *PDE4D*, *TERT*, *ANKRD17*, *DICER1*) and self-renewal of satellite cells (*ITGB1*, *NOTCH1*).

Expression of main candidate genes during development

With public micro-array transcriptome data we evaluated which genes were upregulated at a specific time-point in the foregut, esophagus or pyloric sphincter and used the output as an indicator of gene expression (see Supplementary Material S9). Of the genes classified as VUS or likely deleterious in our exome sequencing results, 28 genes were upregulated in both the foregut or esophagus as well as the pyloric sphincter (see Supplementary Material S9). Seven out of 116 genes with putative deleterious variants in more than one patient were differentially expressed in mice foregut: *Adamtsl4* at E8.5, E14.5 and E16.5; *Ankrd26* at E14.5; *Cntn2* at E8.5, E15.5 and E18.5; *Hspg2* at E8.25, E8.5, E14.5 and E18.5; *Kcnn3* at E8.5 and E15.5; *Ldb3* at E8.5, E14.5 and E15.5; *Sec16b* at E8.5, E14.5 and E16.5.

Detection of common SNPs associated with IHPS

We confirmed the selected proxy SNPs found in the SNP array data (see Supplementary Material S4a) using Sanger sequencing of the four loci highly associated with IHPS (rs11712066, rs573872, rs29784 and rs1933683 near genes *MBNL1*, *NKX2-5* and *BARX1*, respectively) in the EA/IHPS patient set. ORs for the four risk loci are shown in Supplementary Material S4b. In total, 28 EA patients (53.6% male), 16 EA/IHPS patients (93.8% male), 80 EA parents (46.3% male, n=66 related), 24 EA/IHPS parents (50.0% male) and 1297 controls (47.8% male) were compared. We did not find a significantly higher incidence of any risk allele for EA/IHPS patients compared to EA patients. Based on the ORs from the literature, we calculated a median PGRS of 0.56 for EA patients and 0.70 for EA/IHPS patients. When using the OR from the SNP array data, we found a median PGRS of 0.39 for EA patients and 0.58 for

EA/IHPS patients. When comparing all groups together, there was no significant difference in PGRS (see Supplementary Material S4c). When comparing the groups separately, there was a nearly significant difference for the PGRS for EA patients compared to EA/IHPS patients (p=0.08, see Supplementary Material S4d). We did not detect rare putatively deleterious variants in *MBNL1*, *NKX2-5* and *BARX1* in the patient exome sequencing data.

DISCUSSION

We hypothesized that the increased prevalence of IHPS in patients with EA compared to the prevalence of IHPS in the normal population was driven by genetic alterations affecting genes important for foregut morphogenesis. The combination of EA and IHPS makes sense from a developmental perspective as the esophagus and the pyloric sphincter are both foregut derived structures. Organ specification during embryonic development is under tight spatiotemporal control of specific growth factors, transcription factors and signaling cascades.[21, 36] Disturbances in these pathways could impact proper development. The esophagus, as well as the stomach, starts developing from the fourth week after conception onwards. The stomach turns around its anterior-posterior axis during embryonic development.[37] The developing pylorus can be visualized with immunostaining at week six after gestation and differentiates during fetal life.[38]

Environmental[39-44] and genetic contributions[1, 5, 31] have been described for both EA and IHPS as single entities, or in combination with other anatomical malformations. For example, it has been suggested that in utero exposure to diethylstilbestrol (DES) is associated with the development of EA.[45] Moreover, both malformations are variable features in often phenotypically overlapping genetic syndromes (see Table 2), which indicates a genetic background for EA and IHPS. More evidence for a genetic contribution can be deduced from twin studies and animal models.[46] The concordance rates in monozygotic twins compared to dizygotic twins is higher for EA[47] and IHPS[48] as single entities. Also, the recurrence risk is elevated for siblings and offspring of affected individuals with EA in combination with other associated anomalies.[49-52] In contrast, the recurrence risk for isolated EA is low[53] and moderate for IHPS.[48, 54] Different than for EA, there has been reported a male predominance for IHPS (4:1).[55] There have been risk loci associated to IHPS.[25-27, 56, 57] To date, no risk loci have been described for EA.

Absence of rare highly penetrant pathogenic changes

As mentioned, EA and IHPS can be part of specific genetic syndromes (see Table 2). None of the fifteen patients had a pathogenic alteration in one of those known disease genes. This is in line with previous studies in which limited causal changes could be detected in patients with EA and associated anomalies.[30, 58, 59]

Table 2. Genetic syndromes and mutated genes with tracheoesophageal and pyloric anomalies as variable features. This table is modified from two reviews on esophageal atresia[1] and infantile hypertrophic pyloric stenosis[31]. AD = autosomal dominant, AR = autosomal recessive, U = unknown, NA = not applicable, XL = X-linked, EA = esophageal atresia, TEF = tracheoesophageal fistula, IHPS = infantile hypertrophic pyloric stenosis. [A] In literature IHPS is associated with other genes responsible for this syndrome. [B] No overlap in EA and IHPS phenotype for this syndrome, the gene mutated in this syndrome can be responsible for different syndromes in which either EA or IHPS are variable features. [C] More genes associated to possible several subtypes of this syndrome.

Syndrome	Esophageal or pyloric anomaly	Inheritance	Loci	Gene(s)	OMIM	Ref
Esophageal atresia or stenosis						
Epidermolysis bullosa, junctional, with pyloric stenosis or atresia [C]	Esophageal and pyloric atresia or stenosis	AR	2q31.1 17q25.1	*ITGA4* *ITGA6*	226730 226730	77, 78
Ehlers-Danlos syndrome [C]	EA and IHPS	AD	2q32.2	*COL3A1*	130050	79
Trisomy 13	EA/TEF and IHPS	AD	13	multiple	NA	80, 81
Trisomy 18	EA/TEF and IHPS	AD	18	multiple	NA	1, 82
Trisomy 21	EA/TEF and IHPS	AD	21	multiple	190685	1, 82
Fryns syndrome	EA/TEF and IHPS	U	unknown	unknown	229850	1, 83
Fetal alcohol syndrome	EA/TEF and IHPS	NA	NA	NA	NA	84 1, 85, 86
Motility anomalies of the esophagus						
Epidermolysis bullosa dystrophia [C]	Esophageal strictures and stenosis	AR, AD AR	3p21.31 11q22.2	*COL7A1* *MMP1*	131750 226600	
Cornelia de Lange syndrome [B,C]	Esophageal stenosis and dysmotility and IHPS	AD	5p13.2	*NIPBL*	122470	87-89
Apert syndrome	Esophageal stenosis and IHPS	AD	10q26.13	*FGFR2*	101200	90, 91
Congenital generalized lipodystrophy	Esophageal dysmotility and IHPS	AR	17q21.2	*PTRF*	613327	92, 93
Opitz-Kaveggia syndrome	Nutcracker esophagus and IHPS	XL	Xq13	*MED12*	305450	94, 95
Noonan syndrome [C]	Esophageal dysmotility and IHPS	AD	12q24.13	*PTPN11*	163950	96, 97
Visceral neuropathy	Dilated non-peristaltic esophagus and IHPS	U	unknown	unknown	243180	98, 99
Costello syndrome	Loss of elastic fibers in esophagus, IHPS	AD	11p15.5	*HRAS*	218040	100, 101 102, 103
Other associations						
Chronic idiopathic intestinal pseudo obstruction [B, C]	Gastro-intestinal dysmotility and IHPS	XL	Xq28	*FLNA*	300048	101, 104
Fronto-metaphyseal dysplasia [B]	EA/TEF	XL	Xq28	*FLNA*	305620	105
X-linked periventricular heterotopia [B]	IHPS	XL	Xq28	*FLNA*	300049	106
FG syndrome [B, C]	Esophageal dysmotility and IHPS	XL	Xq28	*FLNA*	300321	31, 107
CHARGE syndrome [B, C]	EA/TEF	AD	8q12.1-q12.2	*CHD7*	214800	1
Hypogonadotropic hypogonadism with or without anosmia [B, C]	IHPS [A]	AD	8q12.1-q12.2	*CHD7*	612370	108, 109

Subsequently, we determined the segregation of heterozygous ultra-rare alterations in genes intolerant to variation and recessive variation in genes intolerant to recessive variation.[11, 12] We did not identify ultra-rare de novo dominant, recessive or X-linked deleterious protein coding alterations in these genes. Although we could confirm a compound heterozygous variant in *FAM46A* in one patient and an X-linked variant in SH3KBP1 in another patient, *FAM46A* and *SH3KBP1* were not differentially expressed at the time points important for foregut morphogenesis. Given the male predominance, it is surprising that no X-linked alterations were identified. Additionally, it is unlikely that a dominant – inherited high penetrant – change is a likely cause of EA and IHPS as the parents of these patients are unaffected. It could be that a rare variant burden exists. However, we have not detected it, likely due to limited sample size. Focusing on known candidate genes did also not reveal enrichment (see Supplementary Material S7b).

Coding sequences of genes crucial in esophageal and pyloric sphincter formation are affected

Subsequently, we focused on genes involved in foregut development by combining the results of literature research[22, 60-67] with data of previous expression studies[20-24] (see Figure 2). Given their described importance in normal development, variations in multiple of these genes might explain the higher incidence of IHPS in patients with EA. Five of these genes (*TNXB*, *WDR11*, *PEX3*, *TBX3* and *GDF6*) were affected in patients and unaffected in healthy controls. These variants might not be sufficient to result in disease but are predicted to impact the protein and might contribute together with other unknown factors to disease development.

Seven genes (*ADAMTSL4*, *ANKRD26*, *CNTN2*, *HSPG2*, *KCNN3*, *LDB3*, *SEC16B*) with variants in more than one patient were differentially expressed in the developing foregut, esophagus or pyloric sphincter in mice between E8.25 and E16.5. Most of these variants had a population frequency above the prevalence of EA. If these variants are highly penetrant, they would not be the likely cause. To study reduced penetrance, drastically increased sample sizes are needed for an analysis going beyond known intolerant genes.

Haplotypes associated with IHPS development could have an impact in some patients

Additionally, we investigated the IHPS associated risk haplotypes rs11712066, rs573872, rs29784 and rs1933683[25-27] in EA/IHPS patients, as well as EA patients, EA parents, EA/IHPS parents and healthy controls. Although we could not identify a significantly higher single risk allele frequency for EA/IHPS patients, we found a slightly higher PGRS for EA/IHPS patients compared to EA patients (p=0.08). Further research is needed on a larger scale to confirm the impact of this haplotype.

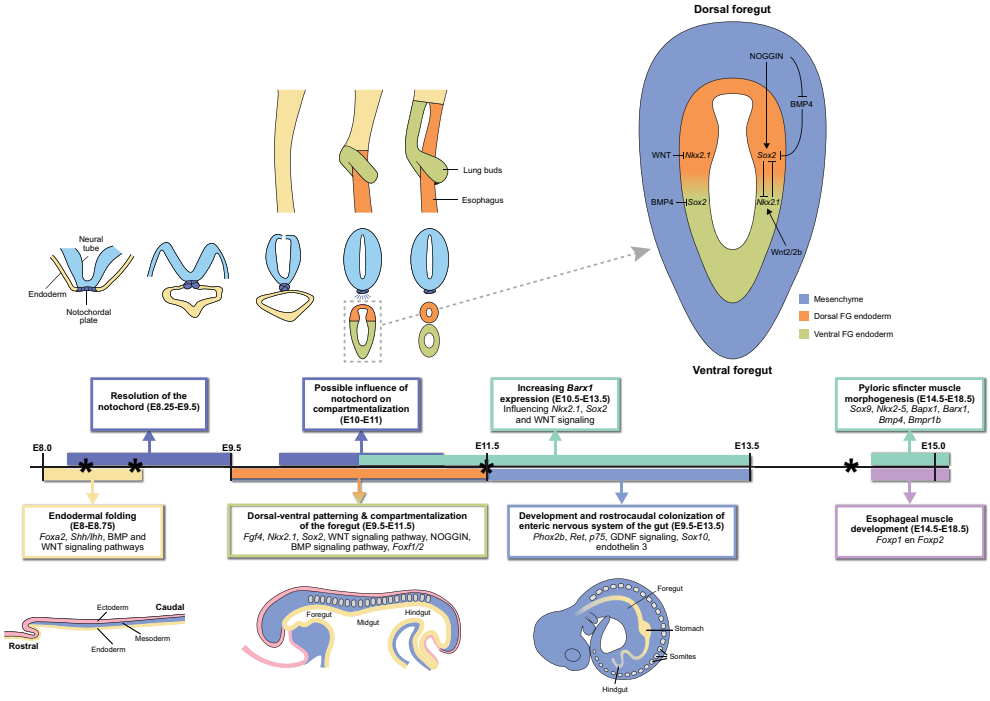

Figure 2. Timeline of models and genes known to be important for foregut development in mice[60, 110, 111] [Top] Visualization of lung bud formation and the genes known to be of importance during tracheoesophageal separation. [Bottom] Timeline of esophageal and pyloric sphincter development. In mice, early foregut formation starts with *Foxa2* stimulation of the anterior endoderm at E8.0.[60] The endodermal sheet folds and forms a tube at E8.75.[22] Next, signals from the notochord start dorsal-ventral patterning around E9.0, with high *Nkx2.1*/absent *Sox2* in the ventral future trachea and absent *Nkx2.1*/high *Sox2* in the dorsal future esophagus and stomach.[61] These dorsal-ventral patterns lead to compartmentalization of the foregut. Between E9.5 and E11.5 the foregut separates in the primordial esophagus and stomach, and in the primordial trachea. Primordial lung buds become apparent at E9.5.[22] The separation site is marked by mesenchymal expression of *Barx1*.[62] The esophagus is completely separated from the trachea at E11.5. Pyloric sphincter formation is mostly studied in chick and mouse models. This formation starts with the thickening of the circular smooth muscle layer between the antrum and the duodenum around E14.5 and the primordial pyloric sphincter is complete around E18.5.[63, 64] In addition to its functioning in foregut separation, the *Barx1* homeobox gene is also vital for stomach differentiation and stomach smooth muscle development. It inhibits *Wnt* signaling 62 and modulates the expression of *Bapx1*, another important factor required for pyloric sphincter morphogenesis.[65-67] * = time points used in expression analysis.

Possible contribution of non-genetic factors

Furthermore, previous studies have suggested the contribution of non-genetic factors as an explanation for the combined occurrence of EA and IHPS. The most common thought is that mechanical and/or environmental factors disturb the developmental field. Environmental risk factors like pesticides, smoking, herbicides and periconceptional alcohol or multivitamin use[39-44] have been suggested for both EA and IHPS. Impaired gastric contractility and esophageal

relaxation were observed in Adriamycin and doxorubicin induced EA in mice.[68, 69] To which extent these factors influence the fetal development, depends on the specific risk factors and their timing.

IHPS might be an acquired condition related to surgery or treatment of EA

Last, IHPS could also be the result of the atresia itself, potentially as a result of the surgical procedure or the postoperative treatment. Previous studies have suggested vagal nerve lesions, a gastrostomy and transpyloric feeding tubes as possible causes for an increased incidence of IHPS after correction of EA.[70] IHPS has been suggested to be a neuromuscular disorder with the involvement of smooth muscle cells, interstitial cells of Cajal and the enteric nervous system. The hypertrophy could be the result of discoordinated movements of the pyloric sphincter and the contractions of the stomach,[71] perhaps as the result of absent nitric oxide synthase activity.[72] Mechanistically, this association between EA and IHPS seems plausible. However, it does not explain why IHPS is not fully penetrant in patients with EA. Further research on the cause and other specific clinical risk factors for patients with EA should be considered, e.g. the late start of enteral feeding or the long-term tube feeding.

Models for EA/IHPS disease etiology

Starting off, we hypothesized that genetic defects, disturbing foregut morphogenesis, would be responsible for the combination of EA and IHPS. A monogenetic syndromic model is unlikely to explain the increased incidence of IHPS in these patients, since we have not detected a central causative gene. The phenotypical spectrum of our EA/IHPS cohort is very heterogeneous and could be the result of impacts on multiple genes, each gene unique to each individual patient. Therefore, it remains possible that IHPS is a rare and less well-known feature of the syndromic phenotype of EA.

We propose two different multifactorial models in which the combination of CNVs, deleterious protein alterations,[1, 73] severe changes in the developmental field during the organogenesis[74, 75] and/or environmental inducing epigenetic changes[44] together modulates the phenotypical spectrum seen in these patients.

The first is a burden model (see Figure 3A). Genetic, epigenetic, environmental and mechanical factors form a burden of risk factors, which balances with protective mechanisms. In this model, the point of balance is not shifted by a mutation in a central gene. Although each person has certain risk factors, in most individuals this will not lead to affected organ systems. There is an intermediate range between normal and affected in which individuals can have the genetic burden but lack an abnormal phenotype (reduced penetrance) or their symptoms differ in severity (variable expressivity). The latter would fit the results in this study. Mechanical or environmental factors could make the difference in shifting the balance.

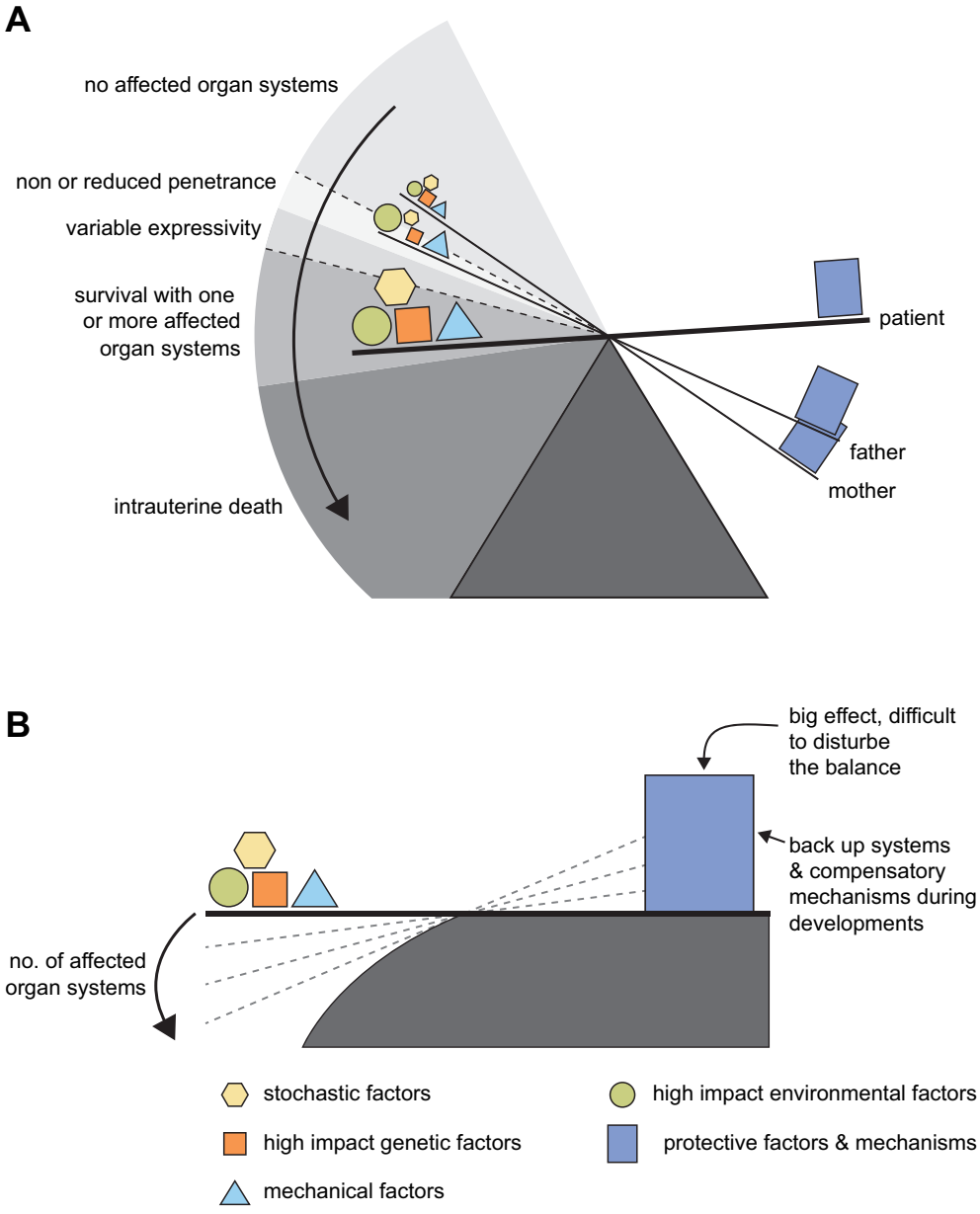

Figure 3. Two models for EA/IHPS etiology. A = burden model, B = slippery slope model. The combination of multiple high impact factors (genetic, environmental, mechanical and/or stochastic) together can modulate the phenotypical spectrum. These risk factors are in balance with protective factors like backup systems and compensatory mechanisms.

The second is a slippery slope model (see Figure 3B) in which the burden of low impact genetic variants and environmental disturbances alone does not impact the balance, until it crosses a certain threshold. The protective mechanisms (e.g. compensatory mechanisms) during development are very strong, making it really difficult to shift the balance. Most fetuses will not develop any malformations despite the combined genetic and environmental burden. Once the threshold is reached, the balance is immediately greatly disrupted and often multiple organ systems are affected. This model also fits with the phenotypical results in this study since four patients had three or more anomalies within the VACTERL spectrum. In this model there is a high tolerance for low impact genetic variation and only high impact variation (aneuploidies, exposure to toxic substances, pathogenic changes in developmental crucial genes) will shift the balance.

CONCLUSIONS

To conclude, the presence of genetic variation in genes involved in foregut development and/or EA or IHPS disease genes might contribute to disease development. We found putative deleterious variation in genes expressed in both the developing esophagus as in the developing pyloric sphincter.

We propose two multifactorial models in which the combination of multiple high impact genetic, mechanical and environmental factors together can shift the balance from normal to abnormal development. A burden model with reduced penetrance or variable expressivity is most likely as genetic factors seem to contribute. Future research should investigate the incidence of IHPS in larger cohorts of patients with EA to further explore this hypothesis. To investigate the role of treatment or surgery, clinical factors related to the surgical correction of EA – for example vagal nerve lesions after surgery, the late start of oral feeding or transpyloric feeding tubes – should be systematically registered.

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S1. Variant prioritization using different in silico tools

Variant burden test and prioritization using Opal

We used the Variant Annotation, Analysis & Search Tool (VAAST)[1-3] cohort analysis embedded in Opal 4.29.5 (Fabric Genomics, Oakland, CA, USA) to rank the variants in the individual patients. Secondly, we performed a burden test on the full exomes using Exome Variant Server 6500 v0.0.30 and 1000 Genomes phase 3 version 5 as a control cohort. We used a 1% allele frequency cut-off for recessive (hemizygous and homozygous) variants and 0.1% cut-off for heterozygous variants. Compound heterozygosity was not considered in this analysis as we did not know the phase of the haplotypes. Only putative protein changing (nonsense, missense, initiator codon variants, in-frame indels, splice sites and splice regions) variants were taken into account. Since we were only interested in putative deleterious variants we used an Omicia score of 0.79 as a threshold as this cut-off has a false positive rate of 5%. Omicia is an algorithm included in the Opal software that combines $SIFT_4$, PolyPhen[5], MutationTaster[6] and PhyloP[7] to predict deleteriousness of variants.

For the VAAST burden test we used a minimum significance of 0.05 and a gene had to have at least two distinct variants in the case set. These genes were used as a gene panel in the individual patient analysis. Individual variants were prioritized before individual inspection as follows. First, all recessive (X-linked and putative homozygous and compound heterozygous), putative rare (MAF ≤0.001%) and damaging de novo variants were selected. Secondly, the top 10 of variants ranked by the VAAST 1.1 prioritization algorithm and subsequently the top 10 variants re-ranked by the Phevor algorithm[8] were included. We used the Human Phenotype Ontology (HPO)[8] terms esophageal atresia and pyloric stenosis as phenotype terms in the algorithm. Finally, variants passing the pre-filtering criteria in genes from the burden test were included.

Variant prioritization using bioinformatic genotype-phenotype correlation tools

Three modules were used: PhenIX[9] (http://compbio.charite.de/PhenIX/), the Exomiser[10] (http://www.sanger.ac.uk/resources/software/exomiser/submit/) and the HPO prioritization incorporated within the Cartagenia software. Settings were as followed. Using PhenIX the full patient phenotype in HPO terms was used, the exome target region filter is on and allele frequency filter of 0.1%, pathogenicity filter was on and mode of inheritance unknown. Genes were prioritized using PhenIX which compares patient phenotypes against human phenotypes only. As a cut-of we used a gene relevance score of 0.8 in combination with a variant score of 0.8, or a total score of 0.9. When using the Exomiser tool we used similar settings: full patient phenotype in HPO terms, exome target region filter is off, allele frequency filter 0.1%, pathogenicity filter on. We did not remove dbSNP variants nor used an inheritance model. Genes are now prioritized using hiPhive, which compares phenotypes against all species. As a cut-off we used a phenotype score of 0.8 in combination with a variant score of 0.8, or an Exomiser score of 0.9.

Supplementary Table S1. Results of variant prioritization.

Patient	Gen	Effect	HGVS cDNA-level nomenclature	mis_z	pLI	Rank VAAST	Rank Phevor full	Rank PhenIX
SE14-262	*CYBRD1*	nonsynonymous	NM_024843.3:c.44C>T	1.05894	0.351788246	4	4	-
SE14-262	*CYBRD1*	nonsynonymous	NM_024843.3:c.677G>A	1.05894	0.351788246	4	4	-
SE14-262	*DMBX1*	frameshift	NM_147192.2:c.468delC	1.140106	0.915995647	6	4	-
SE14-262	*ITGB1*	stopgain	NM_002211.3:c.2252G>A	3.46808	0.914111641	1	1	-
SE14-262	*NBAS*	nonsynonymous	NM_015909.3:c.5467A>T	-3.0608	1.89431E-25	3	2	-
SE14-262	*NBAS*	nonsynonymous	NM_015909.3:c.5465A>C	-3.0608	1.89431E-25	3	2	-
SE14-262	*PNMA3*	nonsynonymous	NM_013364.5:c.631G>T	0.91102	0.005337808	2	3	-
SE14-262	*RRAGD*	nonsynonymous	NM_021244.4:c.1020C>G	2.37809	0.812902091	1	1	-
SE14-263	*DMGDH*	nonsynonymous	NM_013391.3:c.2309G>A	-0.2114	8.90328E-17	2	2	-
SE14-263	*DMGDH*	.	NM_013391.3:c.1684-6A>G	-0.2114	8.90328E-17	2	2	-
SE14-263	*JAKMIP1*	.	NM_001099433.1:c.835-3_835-2insT	3.41787	0.993166784	1	1	-
SE14-264	*ATP6V0A1*	nonsynonymous	NM_001130020.1:c.857G>A	4.2013	0.997249735	2	2	-
SE14-264	*ATP6V0A1*	nonsynonymous	NM_001130020.1:c.1361G>A	4.2013	0.997249735	2	2	-
SE14-264	*DST*	nonsynonymous	NM_001144769.2:c.10877C>T	0.12119	1	1	1	5
SE14-264	*DST*	stopgain	NM_001144769.2:c.5636C>G	0.12119	1	1	1	5
SE14-264	*ENPEP*	.	NM_001977.3:c.787-3T>C	-0.6208	3.57621E-19	5	4	-
SE14-264	*ENPEP*	nonsynonymous	NM_001977.3:c.2226G>T	-0.6208	3.57621E-19	5	4	-
SE14-264	*RBM28*	nonsynonymous	NM_018077.2:c.2273A>G	-0.2601	2.04684E-09	4	3	-
SE14-264	*RBM28*	nonsynonymous	NM_018077.2:c.188A>G	-0.2601	2.04684E-09	4	3	-
SE14-264	*TTC40*	frameshift	NM_001200049.2:c.4178_4184delAGGGAAA	-0.7027	4.17912E-25	3	6	-
SE14-264	*TTC40*	nonsynonymous	NM_001200049.2:c.1675G>A	-0.7027	4.17912E-25	3	6	-
SE14-264	*USH1C*	stopgain	NM_153676.3:c.778G>T	-1.3496	2.72436E-12	2	5	-
SE14-265	*HAO2*	nonsynonymous	NM_001005783.1:c.137G>A	-1.4801	4.47633E-08	4	4	-
SE14-265	*HAO2*	nonsynonymous	NM_001005783.1:c.239T>A	-1.4801	4.47633E-08	4	4	-
SE14-265	*NR0B1*	nonsynonymous	NM_000475.4:c.600C>G	1.94223	0.791995495	3	2	-
SE14-265	*PJA1*	nonsynonymous	NM_145119.3:c.1721T>C	0.97753	0.894042635	2	3	18
SE14-265	*TRAPPC11*	.	NM_021942.5:c.734+1G>T	0.143586	0.000208772	4	2	-
SE14-265	*ZNF716*	frameshift	NM_001159279.1:c.443_444delGT	-2.3904	2.56318E-08	1	1	-
SE14-265	*ZNF716*	stopgain	NM_001159279.1:c.444T>A	-2.3904	2.56318E-08	1	1	-
SE14-265	*ZNF716*	nonsynonymous	NM_001159279.1:c.445T>A	-2.3904	2.56318E-08	1	1	-
SE14-266	*EIF2S3*	.	NM_001415.3:c.133+8T>C	3.81316	0.916323958	4	5	-

SE14-266	*NAGK*	nonsynonymous	NM_017567.4:c.568G>A	-0.814	8.57691E-09	2	2	-
SE14-266	*UPB1*	.	NM_016327.2:c.792-8T>C	-0.3329	1.85087E-07	5	3	-
SE14-266	*UPB1*	.	NM_016327.2:c.792-7C>T	-0.3329	1.85087E-07	5	3	-
SE14-267	*HELZ2*	nonsynonymous	NM_001037335.2:c.3353T>G	0.24759	1.25629E-09	2	3	-
SE14-267	*HELZ2*	nonsynonymous	NM_001037335.2:c.3328T>C	0.24759	1.25629E-09	2	3	-
SE14-267	*PHYH*	nonsynonymous	NM_001037537.1:c.379G>T	-0.1062	1.80976E-05	4	2	4
SE14-267	*PHYH*	nonsynonymous	NM_001037537.1:c.56C>T	-0.1062	1.80976E-05	4	2	-
SE14-267	*PPT1*	stopgain	NM_000310.3:c.451C>T	-0.2555	3.75207E-05	1	3	-
SE14-267	*SH3KBP1*	nonsynonymous	NM_031892.2:c.1061C>T	1.86749	0.998643128	1	1	-
SE14-268	*DNAH3*	.	NM_017539.1:c.10826+5G>A	-1.7439	9.63058E-51	1	2	-
SE14-268	*DNAH3*	nonsynonymous	NM_017539.1:c.1744G>A	-1.7439	9.63058E-51	1	2	-
SE14-269	*ASTN2*	nonsynonymous	NM_014010.4:c.2791C>T	1.76093	0.989172284	3	3	-
SE14-269	*ASTN2*	nonsynonymous	NM_014010.4:c.769T>A	1.76093	0.989172284	3	3	-
SE14-269	*CNTN3*	.	NM_020872.1:c.947-7A>G	0.67362	0.014141786	1	2	-
SE14-269	*CNTN3*	nonsynonymous	NM_020872.1:c.481G>A	0.67362	0.014141786	1	2	-
SE14-269	*GPR98*	nonsynonymous	NM_032119.3:c.1718G>T	-3.9078	8.11098E-24	2	1	-
SE14-269	*GPR98*	nonsynonymous	NM_032119.3:c.9440G>A	-3.9078	8.11098E-24	2	1	-
SE14-270	*ARHGAP8*	nonsynonymous	NM_001017526.1:c.205G>A	#N/A	#N/A	4	4	-
SE14-270	*KANK2*	nonsynonymous	NM_001136191.2:c.1840C>T	0.81821	0.981511999	2	2	-
SE14-270	*KANK2*	nonsynonymous	NM_001136191.2:c.601C>A	0.81821	0.981511999	2	2	-
SE14-270	*MDN1*	inframe	NM_014611.2:c.14355_14357delGGA	-0.2874	0.999999999	5	5	-
SE14-270	*MDN1*	nonsynonymous	NM_014611.2:c.2153C>T	-0.2874	0.999999999	5	5	-
SE14-270	*MID2*	nonsynonymous	NM_012216.3:c.1757T>C	1.62644	0.985076414	1	1	14

S2. Filter and prioritization criteria for the exome sequencing results

Supplementary Table S2. Filter and prioritization criteria. MAF = minor allele frequency, CADD = Combined Annotation-Dependent Depletion, PLI = probability of likely intolerance. # Determine inheritance pattern or confirm de novo status. Classification is as followed. If inheritance pattern is confirmed the variant is classified as a class 3 variant. Additionally, if the gene can be associated to the phenotype the variant will be classified as class 4. If a previous association is present for the gene, the variant will be classified as a class 5 variant. All other variants will be classified as class 1 (MAF above 1%) or class 2 (MAF below 1%).

Model	Pattern	MAF	Missense	Nonsense	Putative splicing	Class
All	All	>1%	CADD ≤20 and/or Z-score ≤3	PLIrec ≤0.9	PLIrec ≤0.9	1
Recessive	X-linked#	≤0.001%	CADD ≥20 and/or Z-score ≥3	PLIrec ≥0.9	PLIrec ≥0.9	3-5
	Homozygous#	≤1%	all	PLIrec ≥0.9	PLIrec ≥0.9	3-5
	Compound heterozygous#	≤0.001%	all	PLIrec ≥0.9	PLIrec ≥0.9	3-5
	Rare variants	>0.001%	CADD ≤20	PLI <0.9	PLI <0.9	2
	Putative de novo#	≤0.001%	CADD ≥20 and Z-score ≤3	PLI <0.9 and CADD ≥20	-	3
Dominant	Putative de novo#	≤0.001%	CADD≥ 20 and Z-score ≥3	PLI ≥0.9 and CADD ≥20	PLI ≥0.9	4-5
Both	Burden	See text				3

S3. Rs673913 is present as a mostly homozygous variant in EA/IHPS patients, EA patients and controls

Supplementary Table S3. Variants of Rs673913.

Sample	Genotype	Allele depth	Depth of coverage
EA_01	homozygous	0.24	24
EA_02	homozygous	0.14	14
EA_03	homozygous	0.51	51
EA_04	homozygous	0.76	76
EA_05	homozygous	0.80	8
EA_06	homozygous	0.54	54
EA_07	homozygous	0.70	70
EA_08	homozygous	0.14	14
EA_09	homozygous	0.11	11
EA_10	homozygous	0.17	17
EA_11	homozygous	0.17	17
EA_12	homozygous	0.24	24
EA_13	homozygous	0.27	27
EA_14	homozygous	0.19	19
EA_15	homozygous	0.19	19
EA_16	homozygous	0.29	29
EA_17	homozygous	0.24	24
SE14-263	heterozygous	9.12	21
SE14-264	homozygous	0.29	29
SE14-265	homozygous	0.20	20
SE14-266	homozygous	0.22	22
SE14-267	homozygous	0.16	16
SE14-268	homozygous	0.19	19
SE14-269	homozygous	0.31	31
SE14-270	homozygous	0.22	22
SE14-271	homozygous	0.32	32
SE14-272	homozygous	0.17	17
SE14-273	homozygous	0.20	20
SE14-274	homozygous	0.18	18
SE14-275	homozygous	0.26	26
SE14-276	homozygous	0.20	20
SE14-262	homozygous	0.23	23
1_F	homozygous	0.54	54
2_M	homozygous	0.80	80
3_F	homozygous	0.99	99
4_M	homozygous	0.100	100
5_M	homozygous	0.87	87
6_F	homozygous	0.86	86
7_F	homozygous	0.116	116
8_M	homozygous	0.69	69
9_M	homozygous	0.99	99
10_F	homozygous	0.115	115
11_M	homozygous	0.83	83
12_F	homozygous	0.80	80
13_M	homozygous	0.97	97
14_F	homozygous	0.72	72

15_M	homozygous	0.89	89
16_M	homozygous	0.96	96
17_F	homozygous	0.82	82
18_F	homozygous	0.74	74
19_M	homozygous	0.93	93
20_F	homozygous	0.65	65
21_M	homozygous	0.136	136
22_F	homozygous	0.77	77
23_M	homozygous	0.88	88
24_F	homozygous	0.117	117
25_M	homozygous	0.66	66
26_F	homozygous	0.78	78
27_M	homozygous	0.59	59
28_F	homozygous	0.97	97
29_M	homozygous	0.84	84
30_F	homozygous	0.93	93
31_M	homozygous	0.95	95
32_F	homozygous	0.97	97
33_M	homozygous	0.100	100
34_F	homozygous	0.94	94
35_M	homozygous	1.113	113
36_M	homozygous	0.99	99
37_F	homozygous	0.95	95
38_M	homozygous	0.93	93
39_F	heterozygous	47.47	94
40_M	homozygous	0.81	81
41_F	homozygous	0.52	52
42_M	homozygous	0.112	112
43_F	homozygous	0.80	80
44_M	homozygous	0.77	77

S4. Detection of common SNPs associated with IHPS

Supplementary Table S4a. Selection of proxy SNPs for common SNPs for IHPS.

Common SNP	Risk/non risk SNP array	Risk/non risk Sanger	Score	Proxy SNP	Exclusion markers	Risk/non risk proxy SNP	Risk/non risk proxy SNP array	D'	R'
rs1933683	G/C	G/C	3.29 (1.27-8.56)	rs10992984	-	G/A	G/A	0.8143	0.6572
rs1933683	G/C	G/C	3.29 (1.27-8.56)	rs10821279	-	T/C	T/C	0.9733	0.9389
rs1933683	G/C	G/C	3.29 (1.27-8.56)		rs12684081	G/A	G/A	1	0.0581
rs1933683	G/C	G/C	3.29 (1.27-8.56)	-	rs10821272	C/T	C/T	1	0.0173
rs1933683	G/C	G/C	3.29 (1.27-8.56)	-	rs11793324	C/T	C/T	1	0.0173
rs1933683	G/C	G/C	3.29 (1.27-8.56)	-	rs7872123	T/C	C/T	1	0.2088
rs1933683	G/C	G/C	3.29 (1.27-8.56)	-	rs10821278	C/A	C/A	1	0.0205
rs11712066	C/T	G/A	2.08 (0.61-7.10)	rs1494014	-	T/C	A/G	0.982	0.9528
rs11712066	C/T	G/A	2.08 (0.61-7.10)	rs16863993	-	G/A	G/A	0.9707	0.5206
rs11712066	C/T	G/A	2.08 (0.61-7.10)	-	rs502775	C/T	C/T	1	0.0902
rs11712066	C/T	G/A	2.08 (0.61-7.10)	-	rs556925	A/G	A/G	1	0.0504
rs11712066	C/T	G/A	2.08 (0.61-7.10)	-	rs1993937	C/A	G/T	1	0.0546
rs11712066	C/T	G/A	2.08 (0.61-7.10)	-	rs6807750	G/A	G/A	1	0.0679
rs11712066	C/T	G/A	2.08 (0.61-7.10)	-	rs325786	T/C	A/C	1	0.0504
rs11712066	C/T	G/A	2.08 (0.61-7.10)	-	rs325781	C/A	C/A	1	0.0504
rs573872	G/T	G/T	0.66 (0.23-1.89)	rs510173	-	T/C	T/C	1	1
rs573872	G/T	G/T	0.66 (0.23-1.89)	-	rs579468	G/A	G/A	1	0.1426
rs573872	G/T	G/T	0.66 (0.23-1.89)	-	rs12490796	G/T	G/T	1	0.0888
rs573872	G/T	G/T	0.66 (0.23-1.89)	-	rs514105	C/T	C/T	1	0.4782
rs29784	T/C	T/C	1.55 (0.73-3.31)	rs39796	-	C/T	G/A	1	1
rs29784	T/C	T/C	1.55 (0.73-3.31)	-	rs8543	T/G	T/G	0.9833	0.1329

Supplementary Table S4b. Odds ratios used for the calculation of the polygenic risk scores. OR calculated from the SNP array data for EA patients vs. EA/IHPS patients. Bold is the common SNP associated with IHPS, *Italy* is the proxy SNP. OR = odds ratio, CI = confidence interval, EA = esophageal atresia, IHPS = infantile hypertrophic pyloric stenosis

		rs11712066 *rs1494014*	**rs29784** *rs39796*	**rs573872** *rs510173*		**rs1933683** *rs1933683*
Literature						
OR (95% CI)	n=3402[11]	1.61 (1.44-1.79)	1.42 (1.30-1.55)	1.41 (1.28-1.56)	n=4951[12]	1.34 (1.24-1.43)
SNP array data						
OR (95% CI)	n=44	0.84 (0.28-2.51)	1.47 (0.61-3.53)	1.19 (0.31-4.55)	n=40	2.88 (0.74-11.10)
Chi-square test (p-value)		0.76	0.39	0.80		0.12

Supplementary Table S4c. Overview of PGRS for all groups. PGRS = polygenic risk score, N = number of patients, OR = odds ratio, IQR = interquartile range, EA = esophageal atresia, IHPS = infantile hypertrophic pyloric stenosis

Group	**N**	**PGRS based on ORs from literature**			**PGRS based on ORs from SNP array data**		
		Median (range)	**IQR**	**Kruskal-Wallis test**	**Median (range)**	**IQR**	**Kruskal-Wallis test**
EA patients	28	0.56 (0-1.18)	0.47	p=0.48	0.39 (-0.17-1.44)	0.73	p=0.47
EA/IHPS patients	16	0.70 (0-1.47)	0.74		0.58 (-0.17-1.83)	1.17	
EA parents	80	0.74 (0-2.00)	0.69		0.39 (-0.33-3.06)	0.77	
EA/IHPS parents	24	0.64 (0-1.18)	0.47		0.47 (-0.17-1.83)	0.80	
Controls	1297	0.69 (0-2.34)	0.69		0.39 (-0.33-3.06)	0.60	

Supplementary Table S4d. Comparison of PGRS for all groups. Results of Mann-Whitney test to compare the PGRS of all group with each other. PGRS = polygenic risk score, OR = odds ratio, EA = esophageal atresia, IHPS = infantile hypertrophic pyloric stenosis.

Group	**PGRS based on ORs from literature** *p-value*	**PGRS based on ORs from SNP array data** *p-value*
EA patients vs. EA/IHPS patients	0.41	0.08
EA patients vs. EA parents	0.17	0.21
EA patients vs. EA/IHPS parents	0.99	0.28
EA patients vs. controls	0.19	0.20
EA/IHPS patients vs. EA parents	0.89	0.32
EA/IHPS patients vs. EA/IHPS parents	0.42	0.42
EA/IHPS patients vs. controls	0.98	0.19
EA parents vs. EA/IHPS parents	0.19	0.84
EA parents vs. controls	0.64	0.79
EA/IHPS parents vs. controls	0.21	0.69

S5. Rare copy number variations classified as variance of unknown significance, likely deleterious or deleterious in patients with esophageal atresia and pyloric stenosis

Methods

Micro-array analysis was performed using the single-nucleotide polymorphism (SNP) CytoSNP-850Kv0 BeadChip (Illumina Inc., San Diego) using standard protocols and the GenomeStudio genotyping module (v1.9.4, www.illumnia.com). Visualization of Copy Number Variations (CNVs), Runs of Homozygosity (ROH) and comparisons to in-house control cohorts as well as published cohorts of affected and control individuals was done using Biodiscovery Nexus CN7.5. (Biodiscovery Inc., Hawthorne, CA, USA) and described previously.[13] None of the six large CNVs identified were *de novo*, all were inherited from one of the unaffected parents. Patient SKZ_400 had a paternal inherited rare gain of chromosomal region 11q15. Patient SKZ_0887 had maternal inherited putative deleterious gains on Xq26.1 and Xp22.33. Patient SKZ_1003 had a maternal inherited loss of chromosomal region 17q11 and patient SKZ_1248 maternal inherited rare gains in chromosomal regions 4q35 and 5p15.1. Additional exon-level CN-profiling using the normalized coverage profiles[14] of the exome sequencing data confirmed the presence of the CNV seen with SNP-array. All CN profiles of main EA and IHPS disease genes[15, 16] were normal. There were no overlapping rare CNVs in this patient cohort.

Supplementary Table S5. Copy number variations. Results are adapted from Brosens et al.[13]

Patient	Remarks
SKZ_0027	-
SKZ_0096	-
SKZ_0244	-
SKZ_0321	-
SKZ_0353	No chromosome breakage susceptibility, normal karyotype
SKZ_0399	Normal karyotype, arr [hg19] (1–22)×2,(X,Y)×1
SKZ_0400	arr[hg19] 11q15(163,146,681–183,022,312)×3 pat
SKZ_0683	arr [hg19] (1–22)×2,(X,Y)×1
SKZ_0760	Normal karyotype, arr [hg19] (1–22)×2,(X,Y)×1
SKZ_0788	Normal karyotype
SKZ_0790	-
SKZ_0796	Vanishing twin, arr [hg19] (1–22)×2,(X,Y)×1
SKZ_0848	Normal karyotype, arr [hg19] (1–22)×2,(X,Y)×1
SKZ_0887	arr[hg19] 2q37.1(163,146,681–183,022,312)×1 pat, Xq26.1(163,146,681–183,022,312)×3 mat, Xp22.33 (163,146,681–183,022,312)×3 mat
SKZ_1003	arr[hg19] 17q11(163,146,681–183,022,312)×1 mat
SKZ_1248	arr[hg19] 5p15.1(163,146,681–183,022,312)×3 mat, 4q35.2(163,146,681–183,022,312)×3 mat
SKZ_1260	arr [hg19] (1–22)×2,(X,Y)×1
SKZ_1353	arr [hg19] (1–22)×2,(X,Y)×1
SKZ_1472	-
SKZ_1961	Normal karyotype, two PCSK5 mutations (MIP)
SKZ_2013	-
SKZ_2023	arr [hg19] (1–22)×2,(X,Y)×1
SKZ_2050	arr [hg19] (1–22)×2,(X,Y)×1

SKZ_2082	arr [hg19] (1–22)×2,(X,Y)×1
SKZ_2149	-
SKZ_1407	-
SKZ_2171	-

S6. Quality of the sequence data

Patient	Total reads	Aligning reads	pct_align	Base coverage			Coverage over n	
				Mean	Mode	Median	n=20	n=50
SKZ_0399	42854772	42314856	0.987401263	56.64	25	45	83.8	44.7
SKZ_0400	46613188	45591952	0.978091265	62.3	29	49	86	49.4
SKZ_0683	43291154	42514371	0.982056773	57.8	26	46	84.6	46
SKZ_0760	45632214	44929427	0.984598884	59.24	27	47	85	46.9
SKZ_0796	43588700	42774240	0.981314882	59.04	28	46	84.6	46.5
SKZ_0848	43507430	42919681	0.986490836	58.07	26	46	84.9	46.2
SKZ_0887	43801008	42911745	0.979697659	57.7	26	45	84	45.2
SKZ_1003	55873420	55158395	0.98720277	73.58	34	58	89.4	58.2
SKZ_1248	42930782	42129043	0.981324845	56.37	27	45	84	44.4
SKZ_1260	45625830	43399055	0.95119486	60.56	29	48	85.3	48
SKZ_1353	53021520	52336597	0.98708217	67.91	32	54	87.4	54
SKZ_1961	48874528	48201036	0.986219979	65.32	30	51	87.1	51.8
SKZ_2023	43479534	42885941	0.986347761	57.89	28	46	84.8	45.9
SKZ_2050	44593276	44012085	0.986966847	59.02	27	46	84.9	46.6
SKZ_2082	43361998	41942437	0.967262556	56.78	28	45	84.1	44.7

S7. Summary of overlapping top candidate genes

Supplementary Table 7a. Variant allele count per gene. LB = likely benign, VUS = variant of unknown significance, LD = likely deleterious. See Supplementary Table S7b for the complete results, adapted from Peeters et al. and Brosens et al.[15, 16]

	EA/IHPS patients (n=15)				Healthy controls (n=44)			
	Rare (MAF ≤0.001%)		Ultra-rare (MAF 0%)		Rare (MAF ≤0.001%)		Ultra-rare (MAF 0%)	
	LB	VUS/LD	LB	VUS/LD	LB	VUS/LD	LB	VUS/LD
Important in normal foregut development (Figure 2)	1	1	0	0	3	5	1	2
Genes associated with genetic syndromes involving both EA and IHPS as variable features (Table 2)	1	0	0	0	2	4	1	1
Genes associated with IHPS[16]	0	0	0	0	0	2	0	0
Genes involved in neuromuscular and connective tissue syndromes associated with IHPS[16]	1	1	1	0	1	2	1	0
Genes involved in syndromes and signaling disturbances associated with IHPS[16]	0	3	0	0	1	6	1	1
Genes involved in ciliopathies and disturbances of gene regulation associated with IHPS[16]	0	1	0	0	4	2	1	0
Genes involved in lymphatic abnormalities and syndromes of environmental and unknown origin associated with IHPS[16]	0	0	0	0	1	0	0	0
Genes involved in genetic syndromes and abnormalities	1	1	1	1	6	6	0	1

Supplementary Table S7b. Overlap top candidate genes. Variant allele count per gene. LB = likely benign, VUS = variant of unknown significance, LD = likely deleterious. Adapted from Peeters et al. and Brosens et al.[15, 16]

		EA/IHPS patients (n=15)				Healthy controls (n=44)			
		Rare (MAF ≤0.001%)		Ultra-rare (MAF 0%)		Rare (MAF ≤0.001%)		Ultra-rare (MAF 0%)	
		LB	VUS/LD	LB	VUS/LD	LB	VUS/LD	LB	VUS/LD
Important in normal foregut development (Figure 2)	*FOXA2*	0	0	0	0	0	0	0	0
	FOXP2	0	0	0	0	0	0	0	0
	GLI1	0	0	0	0	1	0	1	0
	GLI2	0	0	0	0	1	0	0	0
	GLI3	1	0	0	0	1	1	0	0
	SHH	0	0	0	0	0	0	0	0
	FGF4	0	0	0	0	0	1	0	1
	NKX2.1	0	1	0	0	0	1	0	0
	SOX2	0	0	0	0	0	1	0	1
	NOGGIN	0	0	0	0	0	0	0	0
	BAPX1	0	0	0	0	0	0	0	0
	BARX1	0	0	0	0	0	0	0	0
	PHOX2B	0	0	0	0	0	0	0	0
	RET	0	0	0	0	0	0	0	0
	p75	0	0	0	0	0	0	0	0
	GDNF	0	0	0	0	0	1	0	0
	SOX10	0	0	0	0	0	0	0	0
Genes associated with genetic syndromes involving both EA and IHPS as variable features (Table 2)	*ITGA4*	0	0	0	0	0	0	0	0
	ITGA6	0	0	0	0	2	0	1	0
	COL3A1	0	0	0	0	0	1	0	0
	COL7A1	1	0	0	0	0	1	0	0
	MMP1	0	0	0	0	0	1	0	1
	NIPBL	0	0	0	0	0	0	0	0
	FGFR2	0	0	0	0	0	0	0	0
	PTRF	0	0	0	0	0	0	0	0
	MED12	0	0	0	0	0	0	0	0
	PTPN11	0	0	0	0	0	1	0	0
	HRAS	0	0	0	0	0	0	0	0
	FLNA	0	0	0	0	0	0	0	0
	CHD7	0	0	0	0	0	0	0	0
Genes associated with IHPS[16]	*NOS1*	0	0	0	0	0	0	0	0
	MYH11	0	0	0	0	0	1	0	0
	GRIN2A	0	0	0	0	0	0	0	0
	TRPC5	0	0	0	0	0	1	0	0
	TRPC6	0	0	0	0	0	0	0	0
	SLC7A5	0	0	0	0	0	0	0	0
	GLP-2	0	0	0	0	0	0	0	0
	MLN	0	0	0	0	0	0	0	0
	NPY	0	0	0	0	0	0	0	0

Genes involved in neuromuscular and connective tissue syndromes associated with IHPS[16]	*PTRF*	0	0	0	0	0	0	0	0
	MTM1	0	0	0	0	0	0	0	0
	SCN4A	0	0	0	0	0	1	0	0
	NPHS1	0	0	0	0	0	0	0	0
	WT1	0	0	0	0	1	0	1	0
	TNXB	1	0	0	0	0	0	0	0
	COL3A1	0	0	0	0	0	1	0	0
	KAL1	0	0	0	0	0	0	0	0
	PROK2	0	0	0	0	0	0	0	0
	CHD7	0	0	0	0	0	0	0	0
	FGF8	0	0	0	0	0	0	0	0
	WDR11	0	1	0	0	0	0	0	0
	FGFR1	0	0	0	0	0	0	0	0
	FGFR2	0	0	0	0	0	0	0	0
	ZDHHC9	0	0	0	0	0	0	0	0
Genes involved in syndromes and signaling disturbances associated with IHPS[16]	*PYCR1*	0	0	0	0	0	1	0	1
	PAH	0	0	0	0	0	0	0	0
	DHCR7	0	0	0	0	0	0	0	0
	STS	0	0	0	0	1	0	1	0
	PEX1	0	0	0	0	0	1	0	0
	PEX3	0	1	0	0	0	0	0	0
	PEX5	0	1	0	0	0	1	0	0
	PEX6	0	0	0	0	0	0	0	0
	PEX10	0	0	0	0	0	0	0	0
	PEX12	0	0	0	0	0	1	0	0
	PEX13	0	0	0	0	0	0	0	0
	PEX14	0	0	0	0	0	0	0	0
	PEX16	0	0	0	0	0	1	0	0
	PEX19	0	0	0	0	0	0	0	0
	PEX26	0	0	0	0	0	0	0	0
	FLNA	0	0	0	0	0	0	0	0
	CFC1	0	0	0	0	0	0	0	0
	MED12	0	0	0	0	0	0	0	0
	FGS3	0	0	0	0	0	0	0	0
	FGS4	0	0	0	0	0	0	0	0
	FGS5	0	0	0	0	0	0	0	0
	FAM123B	0	0	0	0	0	0	0	0
	TBX3	0	1	0	0	0	0	0	0
	GJA1	0	0	0	0	0	0	0	0
	PKD1	0	0	0	0	0	1	0	0
	PKD2	0	0	0	0	0	0	0	0

Genes involved in ciliopathies and disturbances of gene regulation associated with IHPS[16]	*KAL1*	0	0	0	0	0	0	0	0
	FGFR1	0	0	0	0	0	0	0	0
	PROKR2	0	0	0	0	0	0	0	0
	PROK2	0	0	0	0	0	0	0	0
	CHD7	0	0	0	0	0	0	0	0
	FGF8	0	0	0	0	0	0	0	0
	WDR11	0	1	0	0	0	0	0	0
	PKHD1	0	0	0	0	4	0	1	0
	NIPBL	0	0	0	0	0	0	0	0
	SMC1A	0	0	0	0	0	0	0	0
	HDAC4	0	0	0	0	0	0	0	0
	MAA	0	0	0	0	0	0	0	0
	BCOR	0	0	0	0	0	0	0	0
	RECQL4	0	0	0	0	0	0	0	0
	GPC3	0	0	0	0	0	0	0	0
	HNF1B	0	0	0	0	0	0	0	0
	HRAS	0	0	0	0	0	0	0	0
	PTPN11	0	0	0	0	0	1	0	0
	KRAS	0	0	0	0	0	0	0	0
	SOS1	0	0	0	0	0	1	0	0
	RAF1	0	0	0	0	0	0	0	0
	NRAS	0	0	0	0	0	0	0	0
Genes involved in lymphatic abnormalities and syndromes of environmental and unknown origin associated with IHPS[16]	*CCBE1*	0	0	0	0	1	0	0	0
	SHFL1	0	0	0	0	0	0	0	0

Genes involved in genetic syndromes and abnormalities affecting trachea-esophageal development[15]	*SOX2*	0	0	0	0	0	1	0	0
	MYCN	0	0	0	0	0	0	0	0
	CHD7	0	0	0	0	0	0	0	0
	SEMA3E	0	0	0	0	0	0	0	0
	FANCA	0	0	0	0	2	0	0	0
	FANCB	0	0	0	0	0	0	0	0
	FANCC	0	0	0	0	0	0	0	0
	FANCD1	0	0	0	0	0	0	0	0
	FANCG	0	0	0	0	0	0	0	0
	BAPX1	0	0	0	0	0	0	0	0
	TCOF1	0	0	0	0	1	0	0	0
	MID1	0	0	0	0	0	1	0	0
	UBE3A	0	0	0	0	0	1	0	1
	NDN	0	0	0	0	0	0	0	0
	SNRPN	0	0	0	0	0	0	0	0
	TBX1	0	0	0	0	0	0	0	0
	PAX2	0	0	0	0	0	0	0	0
	HNF1B	0	0	0	0	0	0	0	0
	DSTYK	0	0	0	0	0	1	0	0
	UPK3A	0	0	0	0	2	0	0	0
	ROBO2	0	0	0	0	0	0	0	0
	TRAP1	0	0	0	0	0	1	0	0
	FOXF1	0	0	0	0	0	0	0	0
	GDF6	0	1	0	0	0	0	0	0
	GDF3	0	0	0	0	0	0	0	0
	MEOX2	0	0	0	0	0	0	0	0
	GLI3	1	0	0	0	1	1	0	0
	EFTUD2	0	0	0	0	0	0	0	0
	RBM8A	0	0	0	0	0	0	0	0

S8. Comparison with control cohort: number of variants

Supplementary Table S8. Comparison with control cohort. # = number of variants divided by the number of patients. A Chi^2 test showed no significant differences between the patients and the healthy controls (all p>0.05)

	Ultra-rare variants (MAF 0%)				Rare variants (MAF ≤0.001%)			
	EA/IHPS patients (n=15)		Healthy controls (n=44)		EA/IHPS patients (n=15)		Healthy controls (n=44)	
		#		#		#		#
Putative deleterious	296	*19.73*	725	*16.48*	912	*60.8*	2667	*60.6*
LOF intolerant	28	*1.87*	85	*1.93*	81	*5.4*	272	*6.2*
De novo variants	0	-	Unknown	-	0	-	Unknown	-
Recessive	291	*19.4*	715	*16.25*	898	*59.9*	2631	*59.80*
Compound heterozygous	3	*0.2*	3	*0.06*	8	*0.53*	6	*0.14*
X-linked	11	*0.73*	23	*0.52*	28	*1.87*	95	*2.16*

S9. Results of gene expression analysis

Supplementary Table S9. Results of gene expression analysis. Differential expression of these genes was studied in public gene expression datasets. Bold printed genes were classified as VUS or likely deleterious. These datasets are described in: [17-21].

Upregulated at	Genes	GEO ID	Ref
E8.25 endoderm	***AGRN***, ***CLCNKB***, ***COL16A1***, *COL9A2*, *CPT2*, *DMBT1*, ***HSPG2***, *LAMC2*, ***NKX2-3***, ***PCSK9***, *PTPRF*, *PTPRU*, ***RET***	GSE13040	[17]
E8.25 mesoderm	*FMOD*, ***HSPG2***, ***MAN1C1***, ***NKX2-3***, ***NUP133***, *TAF12*, *TAL1*, *VANGL1*	GSE13040	[17]
E8.25 ectoderm	***AGRN***, *COL9A2*, *CPT2*, *DMBT1*, ***NUP133***, ***PCSK9***, *PTPRF*, *TAF12*	GSE13040	[17]
E8.5 foregut	*A3GALT2*, ***ADAMTSL4***, ***ANKRD29***, *ANXA12*, ***APBB1IP***, ***ARHGAP29***, ***ARHGEF10L***, *ASAP3*, *ASCL2*, *ATAD3C*, *C1orf56*, ***CAMTA1***, ***CC2D1B***, *CCDC18*, *CDH23*, ***CDHR5***, ***CHD5***, ***CLCN6***, ***CNTN2***, ***COL11A1***, *CPT2*, ***CROCC***, *CRYZ*, *CTBS*, *CTNNBIP1*, ***CTNND1***, ***CUL2***, *DMBT1*, ***DNAJC11***, *ECHDC2*, *EFCAB7*, *EMC1*, ***ENTPD1***, ***EPHA2***, ***EXT2***, ***EXTL1***, *FAM13C*, ***FAM20B***, *FMO5*, ***GPATCH3***, ***HIVEP3***, ***HMCN1***, ***HMGCS2***, *HOOK1*, ***HSPG2***, ***ITGB3BP***, ***ITIH5***, ***KCNN3***, ***KCNQ4***, ***KIAA1217***, *LAMC2*, ***LDB3***, *LRRC8B*, ***MAN1C1***, ***MAP3K6***, *MARVELD1*, *MICAL2*, ***MLLT10***, ***MTHFR***, *MXRA8*, ***MYOF***, *NEURL1*, ***NKX2-3***, ***NUP133***, ***OBSCN***, ***OPN4***, *OTOG*, ***OTUD7B***, ***PADI1***, ***PADI3***, ***PARS2***, ***PCSK9***, *PDE4DIP*, ***PKN2***, *PLXDC2*, ***PPM1J***, ***PRDM16***, ***PRPF38B***, ***PRRC2C***, ***PTCH2***, *PTPRF*, *PTPRU*, ***PUM1***, ***RET***, *RFX5*, *SASS6*, ***SEC16B***, ***SERINC2***, *SFXN2*, *SLC5A9*, ***SRSF4***, ***SYDE2***, *TAF12*, *TAL1*, ***TMEM82***, *TRIM34*, ***TRIM6***, *TRIT1*, ***VPS13D***, *VWA5B1*, ***ZBTB7B***	GSE19873	[18]
E11.5 esophagus	***AGRN***, *ANKRD27*, ***ANXA11***, ***COL11A1***, ***COL16A1***, ***CROCC***, ***DNAJC11***, ***ENTPD1***, *FMOD*, ***HMCN1***, ***HSPG2***, *LAMC2*, *LRRC56*, ***OTUD7B***, *PGGHG*, ***PKN2***, *PTPRU*, ***RYR2***, ***SERINC2***, *SLC5A9*, ***SRSF4***, ***SYDE2***, *VANGL1*	GSE13040, GSE34278	[17, 19]
E14.5 pyloric sphincter	***ADAMTSL4***, ***AGRN***, ***ANKRD26***, ***ARHGAP29***, *BSCL2*, ***CAMTA1***, *CCDC18*, ***CDHR5***, ***CEP85***, ***COL11A1***, *COL9A2*, *CPT2*, *CRYZ*, *CTBS*, *DMBT1*, *ECHDC2*, *EFCAB7*, *FAM13C*, *FMO5*, *FMOD*, ***HAO2***, ***HIVEP3***, ***HMCN1***, ***HMGCS2***, ***HSPG2***, ***HYI***, ***ITGB3BP***, *LAMC2*, ***LDB3***, ***MAP7D1***, ***MYOF***, *NEURL1*, ***NKX2-3***, ***NUP133***, ***PCSK9***, ***PKN2***, *PLXDC2*, ***PRDM16***, ***RET***, ***SEC16B***, ***SERINC2***, ***TMEM82***, *TRIM34*, ***ZBTB7B***	GSE15872	[20]
E15.5 esophagus	***AGRN***, ***ANKRD28***, *ART5*, ***CNTN2***, *CPT2*, *DMBT1*, *EMC1*, *FMO5*, ***HMGCS2***, ***LDB3***, *LRRC56*, ***OTUD7B***, *PGGHG*, *PTPRU*, ***RYR2***, ***SRSF4***, *ST7L*, ***SYDE2***, *VANGL1*	GSE34278	[19]
E16.5 pyloric sphincter	***ADAMTSL4***, ***AGRN***, ***ARHGAP29***, *BSCL2*, *CALY*, ***CNTN2***, ***COL11A1***, *COL9A2*, ***DNAJC11***, *FAM13C*, *FMO5*, ***HMCN1***, ***HMGCS2***, *HOOK1*, ***MAP7D1***, ***MIA3***, *MICAL2*, ***MYOF***, *NEURL1*, ***NUP133***, ***PKN2***, *PTPRU*, ***PUM1***, ***RET***, ***SEC16B***, ***SERINC2***, ***VPS13D***, ***ZBTB7B***	GSE15872	[20]
E18.5 esophagus	***AGRN***, ***ARHGAP29***, *BSCL2*, *CALY*, ***CDHR5***, ***CROCC***, *CTNNBIP1*, *ECHDC2*, *EFCAB7*, *EMC1*, ***EPHA2***, *FAM13C*, *FMO5*, *FMOD*, ***HMCN1***, ***HMGCS2***, ***HSPG2***, ***ITIH5***, *LAMC2*, ***MAN1C1***, ***MIA3***, *MXRA8*, ***OTUD7B***, ***PADI1***, *PDE4DIP*, *PLXDC2*, ***PRDM16***, ***PRPF38B***, ***PRRC2C***, ***RET***, ***RYR2***, *SASS6*, ***SERINC2***, *SLC5A9*, *ST7L*, ***SYDE2***, *TRIM34*, *TRIT1*, *TSTD1*, *VANGL1*	GSE43381	[21]

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CHAPTER 3

Intrinsic cellular susceptibility to Barrett's esophagus in adults born with esophageal atresia

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ABSTRACT

The prevalence of Barrett's esophagus (BE) in adults born with esophageal atresia (EA) is four times higher than in the general population and presents at a younger age (34 vs. 60 years). This is (partly) a consequence of chronic gastroesophageal reflux. Given the overlap between genes and pathways involved in foregut and BE development, we hypothesized that EA patients have an intrinsic predisposition to develop BE. Transcriptomes of esophageal biopsies of EA patients with BE (n=19, EA/BE); EA patients without BE (n=44, EA only) and BE patients without EA (n=10, BE only) were compared by RNA expression profiling. Subsequently, we simulated a reflux episode by exposing fibroblasts of 3 EA patients and 3 controls to acidic conditions. Transcriptome responses were compared to the differential expressed transcripts in the biopsies. Predisposing single nucleotide polymorphisms, associated with BE, were slightly increased in EA/BE versus BE-only patients. RNA expression profiling and pathway enrichment analysis revealed differences in retinoic acid metabolism and downstream signaling pathways and inflammatory, stress response and oncological processes. There was a similar effect on retinoic acid signaling and immune response in EA patients upon acid exposure. These results indicate that epithelial tissue homeostasis in EA patients is more prone to acidic disturbances.

Simple Summary:

We investigated the increased prevalence of Barrett's esophagus in adults with esophageal atresia. A higher polygenic risk score and disturbances in inflammatory, stress response and oncological pathways upon acid exposure suggest a genetic susceptibility and increased induction of inflammatory processes. Although further research is required to explore this hypothesis, this could be a first-step into selecting patients that are more at risk to develop Barrett's esophagus and/or esophageal carcinoma. Currently, an endoscopic screening and surveillance program is in practice in our institution for patients born with esophageal atresia, to early detect (pre)malignant lesions. Since recurrent endoscopies can be a burden for the patient, selecting patients by for example genetic susceptibility would allow to only include those at risk in future practice.

INTRODUCTION

Esophageal atresia (EA) is a congenital foregut malformation, of which improved survival rates have resulted in a growing adult population.[1] This raises new challenges in patient care as more emphasis is placed on long-term morbidities than short-term mortality. Respiratory and gastrointestinal symptoms require long-term follow-up.[2] Many adults born with EA (EA adults) suffer from chronic gastroesophageal reflux (GER), which is often underreported by patients due to an altered perception of discomfort.[3] GER can lead to reflux esophagitis, a nonspecific inflammation of the esophagus. Furthermore, the mucosal damage resulting from GER induces the replacement of esophageal squamous epithelium by gastric columnar epithelium containing goblet cells. This precursor lesion, intestinal metaplasia (IM) also known as Barrett's esophagus (BE), can develop via dysplasia into esophageal adenocarcinoma (EAC).[4] Basal cells at the squamous-columnar junction are the origin of the BE cell population.[5] BE tissue has crypts composed of various combinations of goblet cells, mucinous cells, endocrine cells, enterocytes and Paneth cells.[6] The prevalence of BE in EA adults is 4-5 times higher than in the general population (6.6% vs. 1.6%), and presents at a much younger median age (34 vs. 60 years).[3] In the Erasmus MC-Sophia Children's hospital cohort, EAC has been reported in three EA patients, and – surprisingly – also esophageal squamous cell carcinoma (ESCC) is seen more frequently in patients with EA at a younger age compared with the general population.[3]

Disturbances in developmental signaling pathways are often associated with metaplasia and cancer transformation. The overlap of these pathways, disease genes and risk loci for foregut morphogenesis and BE development are suggestive of a shared etiology. During embryonic development the foregut separates into the future trachea and esophagus under the influence of spatiotemporal regulated transcriptional programs. These are regulated by gradients of morphogens that lay the blueprint for their interacting cells to develop into the various esophageal cell types and structures. Six intertwined pathways are crucial in this process: TGFB-BMP, Notch, FGF, WNT, Hedgehog and retinoic acid (RA) signaling.[7] TGFB-BMP signaling,[8] SHH signaling[9] as well as RA signaling[10] are dysregulated in BE. Additionally, genome-wide association studies (GWAS) de-scribe risk loci for the development of BE, EAC and ESCC near genes involved in these foregut developmental genes and pathways. These include *TBX5*, *GDF7*, *CRTC1*, *BARX1*, *FOXP1* and *FOXF1*.[11]

Given the increased incidence of BE in EA adults, endoscopic surveillance is recommended.[12] Surveillance leads to early detection of BE or esophageal carcinoma, but could also create an unnecessary burden of repeated endoscopies for those not at risk as well as substantial added health care costs. Identifying patients at risk for developing BE could be a first step towards a tailor-made surveillance strategy. In this study, we hypothesize that patients born with EA have an increased (genetic) susceptibility for BE development. We aim to identify

this predisposition by comparing risk loci burden and transcriptomes of patients with EA who have developed BE with EA patients without BE, and patients with BE without an EA history. We show that in both groups BE is histopathologically similar. However, the effect of acid reflux seems different with intrinsic cellular differences in inflammatory and stress response pathways, RA metabolism and signaling.

MATERIALS AND METHODS

Study population

Our institutional review board approved this case-control study (MEC-2018-1500). In our surveillance program, patients undergo upper endoscopies with histologic evaluation of biopsies taken according to a standardized protocol.[3] Biopsies and blood used in this study were retrieved from the Biobank Esophageal Atresia (MEC-2015-645) and the Biobank Barrett (MEC-2010-094). Mucosal esophageal biopsies were taken from two sites: (1) unaffected esophageal squamous cell epithelium (SQ), in EA patients taken above the original anastomosis; and (2) the GEJ or – if present – from Barrett's mucosa. Sample extraction protocol and storage are described in Supplementary Methods SM1. Additionally, we genotyped six EA/BE patients from a Finnish cohort study (447/E7/2005),[13] as well as 730 ancestry matched (broadly European) unaffected controls. For the in vitro experiments we used human fibroblasts from EA patients and healthy controls. EA fibroblast lines were taken during routine diagnostic procedures. Control fibroblast lines are anonymized lines that taken previously during unrelated routine diagnostic procedures and stored for research purposes. We compared three groups of patients: patients with EA who have developed BE (EA/BE), patients with EA without BE (EA only), and patients with BE without EA in history (BE only) BE-only patients were matched for age and gender with EA/BE patients. See Figure 1 for study set-up.

Histopathological evaluation

Hematoxylin and eosin-stained histological slides were retrieved from the archives of all patients of whom biopsies had been collected for RNA sequencing. All slides were blinded reassessed by a BE expert pathologist, according to a review-based checklist.[6] Potential differences were scored between the three groups.

SNP Genotyping and calculation of predisposing SNPs, associated with BE

DNA extraction and quantification was done according standard procedures (see Supplementary Methods SM2). Processing of the SNP array genotyping chips (Infinium Global Screening Array v1.0 or v3.0 Illumina, Inc., San Diego, CA, USA) was done according to the manufacturer's standard protocol. Output was generated using Illumina Genome studio v2.0 (Illumina, San Diego, CA, USA). Predisposition loci (and corresponding lead or proxy SNPs)

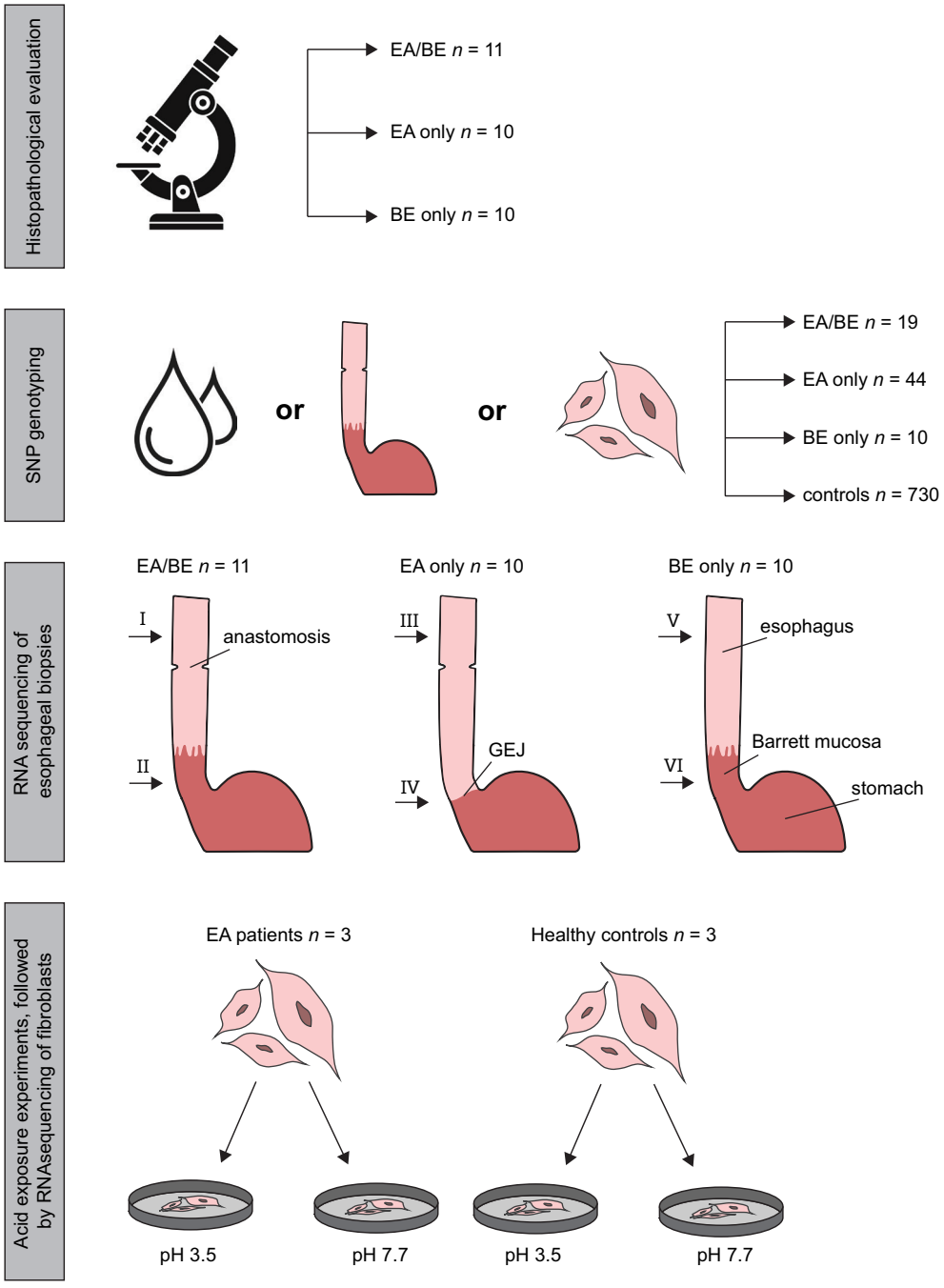

Figure 1. Schematic overview of the study set-up and number of patients included in each part. We compared three groups of patients: patients with esophageal atresia (EA) who have developed Barrett's esophagus (BE, EA/BE), patients with EA without BE (EA only), and patients with BE without EA in history (BE only). BE only patients were matched for age and gender with EA/BE patients. Roman numerals I to VI indicate the subgroups, based on the location of the biopsies. GEJ = gastroesophageal junction.

associated with BE, EAC and/or ESCC were derived from literature (see Supplementary Table S2.1). We used genotype data from EA/BE patients (n=19), EA only patients (n=44), BE only patients (n=10) and controls (n=730) to see if previously BE associated SNPs were more prevalent in EA/BE patients (see Supplementary Methods SM3). We used the allele counts and published ORs of the associated SNPs to calculate a polygenic risk score (PGRS) using an additive model: PGRS= $\sum Ln(OR\ risk\ allele) \times allele\ count$. Since we do not know if these ORs are precise enough to calculate the risk for the combination of EA and BE, we used the ORs of the associated SNPs calculated from our study population in a second calculation. Using a Kruskal-Wallis test and Mann-Whitney tests, we compared the PGRS between the different groups. All statistical analyses were performed in SPSS V.25.0 (IBM, Chicago, IL, USA), with a significance level of $p<0.05$.

RNA sequencing, differential gene expression and pathway enrichment analysis

RNA extraction and quantification was done according standard procedures (see Supplementary Methods SM2). Genome-wide individual gene expression levels – including raw counts – are available in Supplementary Material S4 and S9. Differential expression was calculated between (sub)groups (see Supplementary Methods SM4). Genes with a maximum group mean >2, a fold change ≥1.5 and a false discovery rate (FDR) p-value <0.05 were considered significantly differentially expressed. All differentially expressed genes per subgroup analysis were uploaded into the Ingenuity Pathway Analysis (IPA) software (Qiagen, Venlo, The Netherlands). Core analysis was performed for each (sub)group. A p-value of <0.05 and a Z-score of ≥2 were considered significant. Our ethics committee does not allow sharing of individual patient or control genotype information in the public domain, including sequencing reads.

Acid exposure experiments

In absence of available epithelial cells for in-vitro studies we used fibroblasts. Activated fibroblasts generate extracellular matrix components and regulate inflammation.[14] There are several lines of evidence supporting a role for fibroblasts in BE proliferation and cancer.[15, 16] To simulate a one-time acid reflux episode on RNA level, human fibroblasts from EA patients (n=3) and healthy controls (n=3) were exposed to pH adjusted cell culture medium conditions (SM5). Hydrochloric acid was added to culture medium until the desired pH level was reached. Subsequently, cells were washed with phosphate buffered saline (PBS) and given standard medium. After 24 hours, survival was measured with the TC20™ Automated Cell Counter (Bio-Rad Laboratories B.V., Veenendaal, The Netherlands). Cell morphology was evaluated with the Olympus IX70-S8F Inverted Fluorescence Microscope (Olympus Corporation, Tokyo, Japan). RNA was isolated and sequenced as described in Supplementary Methods SM2 en SM4. Expression levels were compared with the RNA sequencing results of the esophageal biopsies.

Study approval

The Medical Ethics Committee of the Erasmus Medical Center Rotterdam approved this study (MEC-2015-645, MEC-2010-094, MEC-2012-387). All authors had access to the study data and reviewed and approved the final manuscript.

RESULTS

Study population

Patient characteristics are depicted in Supplementary Table S1.1 and S1.2. Histopathological assessment (see Supplementary Table S1.3) of the biopsies is summarized in Supplementary Table S1.4. Columnar epithelium was present in all groups, except for two EA only patients (see Supplementary Table S1.5). Since EA only patients were selected as not having metaplasia in the distal esophagus at endoscopy, this means that most biopsies could contain part of the cardia as well. Neutrophil granulocytes were absent in the majority of EA only patients, while a varying degree of nonspecific inflammatory cell infiltrate was present in most of them. Focusing on the characteristics of BE, IM with the presence of goblet cells was similarly present in EA/BE patients and BE only patients. The amount of IM was larger in BE only patients. No dysplasia was found in any of the samples.

SNP (Single Nucleotide Polymorphism) genotyping

Given the limited sample size of our study population, we used ORs selected from literature to calculate the contribution of predisposing associated SNPs (polygenetic risk score, PGRS). Supplementary Table S2.1 depicts an overview of the included SNPs and ORs. Using these ORs, we found a median PGRS of 3.24 (range 1.39-4.68) for EA/BE patients, of 2.98 (1.19-4.74) for EA only patients and of 2.63 (1.85-3.53) for BE only patients. There were no statistical significant differences between these groups (see Figure 2A, panel a, all $p > 0.05$). When using our own data, we did find significant differences in PGRS between these groups (see Figure 2A, panel b). A higher risk allele frequency was found for EA/BE patients versus BE only patients for rs3784262 near *ALDH1A2* ($p=0.017$), and a lower risk allele frequency of rs3072 near *GDF7* ($p=0.009$) (see Figure 2B). See also Supplementary Material S3.

RNA sequencing of esophageal biopsy specimens

An average of 88,378,214 reads per sample were generated (62,471,354-165,874,334). Of these reads, 98% (94.9-98.4) aligned to the human reference genome. A total of 9752 transcripts had a mean expression of ≥2 RPKM and were considered expressed. See Supplementary Material S4 for the quality report. PCA of the gene expression data confirmed clustering of the samples into the three groups (Supplementary Material S5). PCA and quality control procedures included the exclusion of two outliers (BBE-017 and BBE-079).

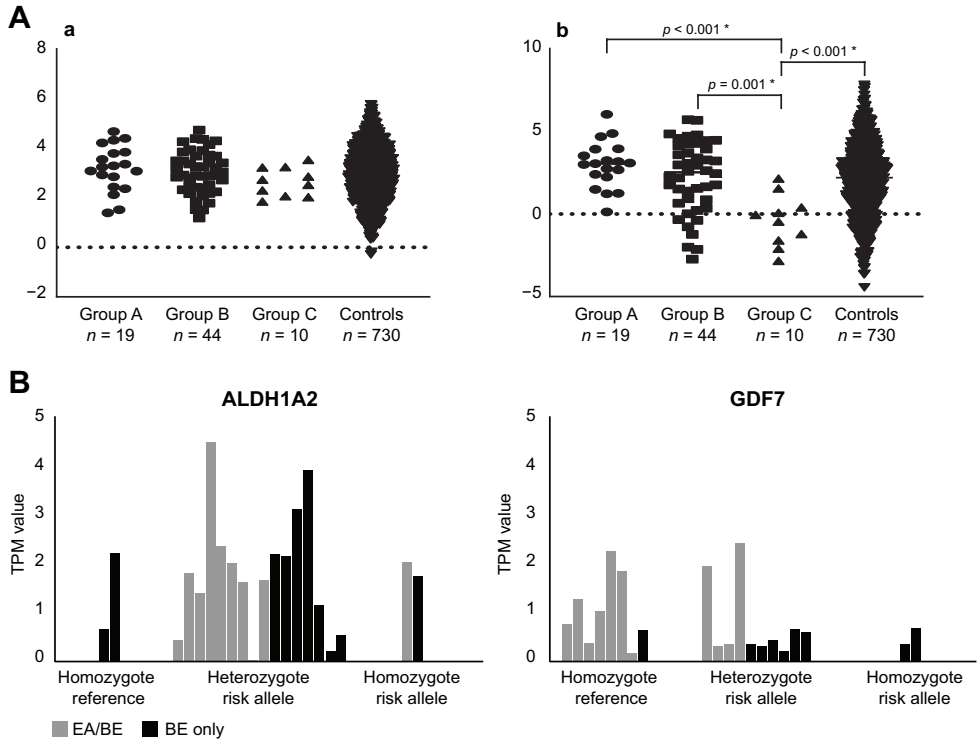

Figure 2. (A) Polygenic risk scores (PGRS) per patient. Group A = patients with esophageal atresia (EA) and Barrett's esophagus (BE), group B = patients with EA without BE, group C = patients with BE without EA in history. Panel a (left) are PGRS based on odds ratios (ORs) selected from the literature. No statistical significant differences between the groups were observed. Panel b (right): PGRS based on ORs calculated from our study population. We found a median PGRS of 3.05 (range 0.14-6.04) for EA/BE patients, of 2.52 (−2.73-5.72) for EA only patients and of −0.24 (−2.83-2.15) for BE only patients. A Kruskal-Wallis test revealed a significant difference in PGRS based on ORs calculated from our study population between the four groups (p=0.001). T-statistics indicated a difference between BE only patients versus EA/BE patients (p<0.001), EA only patients (p=0.001) and controls (p<0.001). Asterisk (*) indicates significance p<0.05. (B) Gene expression levels for *ALDH1A2* and *GDF7* per patient, sorted based on the genotype of the patients. A higher risk allele frequency was found for EA/BE patients versus BE only patients for rs3784262 near *ALDH1A2* (p=0.017) and a putative protective allele for rs3072 near *GDF7* (p=0.009). Looking at gene expression levels, *GDF7* has slightly elevated TPM values for patients homozygote for the reference allele. No significant differences could be detected for these two associated SNPs. TPM = transcripts per million, EA = esophageal atresia, BE = Barrett's esophagus. Complete results can be found in Supplementary Material S3.

Differential expression and pathway enrichment analysis of esophageal biopsy specimens

Seven known BE disease genes[11] were differentially expressed between EA only patients and EA/BE or BE only patients (see Figure 3 and Supplementary Table S6.1). Enriched pathways between EA/BE patients and BE only patients were involved in RA signaling, stress response and inflammatory pathways, and oncological processes (see Figure 4 and Supplementary Table S6.2).

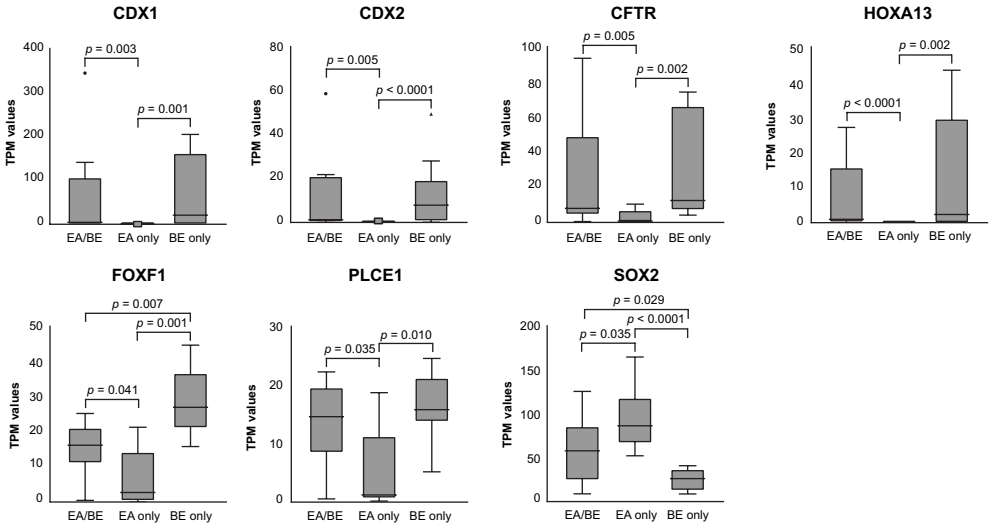

Figure 3. Gene expression levels per group for selected disease genes, involved in foregut morphogenesis and/or associated with Barrett's esophagus in literature, presented as median (interquartile range) with minimum and maximum values. We compared biopsies of the gastroesophageal junction between three groups of patients: patients with esophageal atresia (EA) who have developed Barrett's esophagus (BE) (EA/BE, n=11), patients with EA without BE (EA only, n=10), and patients with BE without EA in history (BE only, n=10). TPM = transcripts per million, EA = esophageal atresia, BE = Barrett's esophagus.

Acid exposure experiments

To study the effect of GER on RNA level, we simulated a reflux episode in in vitro experiments (see Figure 1). First, we optimized the acid exposure experiment (see Supplementary Methods). Next, we exposed fibroblasts from three EA patients and three healthy controls for 30 minutes to medium with pH 3.5 or to normal medium (control). Cells exposed to pH 3.5 showed cell rounding and irregular cell membranes (see Supplementary Figure S7.1). After acid exposure, there was a clear difference between upregulated and downregulated genes, both in patients and controls (see Supplementary Figure S7.2). Ten pathways were enriched with differentially expressed genes between patients and controls (see Supplementary Table S7.3), that contained 244 differentially expressed genes. Subtracting the genes that were also differentially expressed without acid exposure, 81 genes of interest remained (see Supplementary Figure S7.4). Pathway analysis of these 81 genes confirmed enrichment of pathways mostly involved in inflammatory processes (see Supplementary Table S7.5). Finally, we compared the results of the pathway analysis of the biopsies with those of the fibroblasts after acid exposure. Of the enriched pathways between GEJ samples of EA/BE patients and BE only patients, 20 pathways were also enriched between fibroblasts of EA patients and controls after acid exposure (see Table 1). In total, seven genes within these pathways were differentially expressed in both the GEJ samples and the acid-exposed fibroblasts (see Supplementary Figure S7.6).

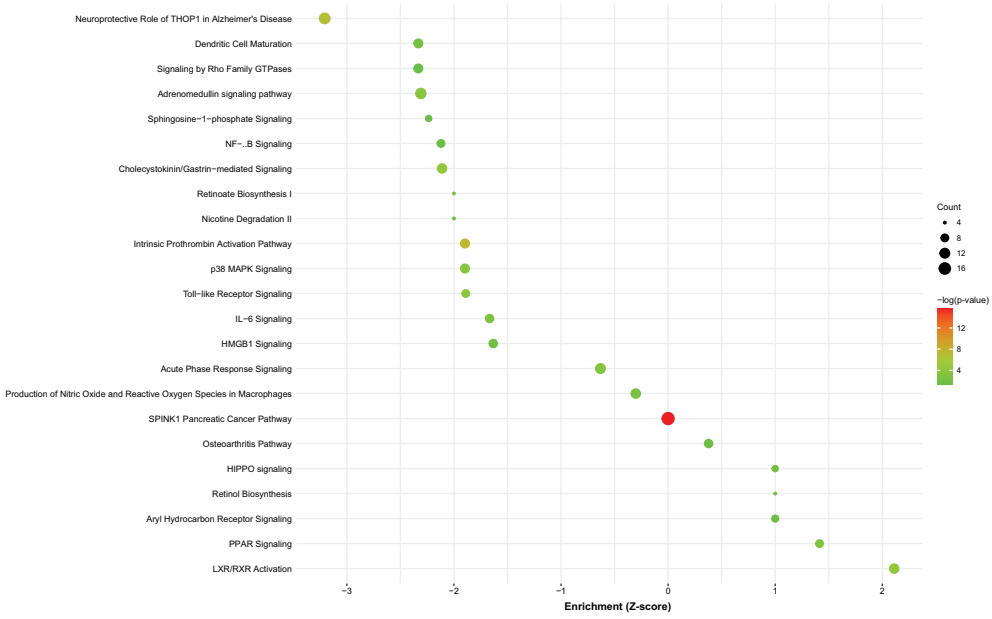

Figure 4. Bubble plot of canonical pathways, significantly enriched by differentially expressed genes, between gastroesophageal junction (GEJ) samples of group A (esophageal atresia (EA) with Barrett's esophagus (BE)) and GEJ samples of group C (BE only). The color and size of the dots represent the range of the p-value and the number of molecules mapped to the indicated pathways. Settings: p-value <0.05 (=−log(p-value) >1.3), z-score <−2 or >2. SPINK1 Pancreatic Cancer Pathway is also the only significantly upregulated pathway, when comparing group A (EA/BE) with group C (BE only). Plotted by http://www.bioinformatics.com.cn, a free online platform for data analysis and visualization.

DISCUSSION

In this first translational case-control study in adults born with EA, we compared EA patients who developed BE (EA/BE) to EA patients who did not develop BE (EA only) and BE patients without a history of EA (BE only). Previous studies described an increased prevalence of BE in EA adults – and at a much younger age – compared with the general population.[3] Over the years, several risk loci associated with BE and/or esophageal carcinoma have been published, of which many near genes involved in foregut development (see Supplementary Material S2).[11] This overlap made us hypothesize that EA patients have an increased (genetic) susceptibility to develop BE.

BE characteristics of EA/BE patients and BE only patients

There is a twenty-year difference in the age at which biopsies were taken between EA/BE patients and BE only patients. We confirmed the lack of morphological differences between these two groups. Although endoscopic esophagitis was absent in the majority of the BE only patients, neutrophil granulocytes were present in these patients. The typical characteristics

Table 1. Overlap between canonical pathways, significantly enriched by differentially expressed genes, in the esophageal biopsy specimens versus the acid-exposed and non-exposed fibroblasts. II = gastroesophageal junction (GEJ) samples from group A (esophageal atresia (EA) and Barrett's esophagus (BE)), VI = GEJ samples from group C (BE only). n = total number of canonical pathways significantly enriched by differentially expressed genes. N/A = not applicable, Z-score could not be calculated. Grey box indicates that a pathway was not present in the results of that pathway analysis.

	Esophageal biopsy specimens		**Fibroblasts from acid exposure experiment**					
	II vs. VI (n=353)		**EA patients vs. controls (acid-exposed) (n=258)**		**EA patients vs. controls (non-exposed) (n=314)**		**Acid-exposed vs. non-exposed (all samples) (n=578)**	
Canonical Pathways	*–log(p-value)*	*Z-Score*	*–log(p-value)*	*Z-Score*	*–log(p-value)*	*Z-Score*	*–log(p-value)*	*Z-Score*
Agranulocyte Adhesion and Diapedesis	1.69	N/A	-	-	1.52	N/A	-	-
Altered T Cell and B Cell Signaling in Rheumatoid Arthritis	3.22	N/A	2.57	N/A	2.05	N/A	-	-
Atherosclerosis Signaling	4.93	N/A	2.04	N/A	2.23	N/A	-	-
Cholecystokinin/Gastrin-mediated Signaling	4.38	2.111	2.35	0	1.39	N/A	-	-
Communication between Innate and Adaptive Immune Cells	2.39	N/A	2.47	N/A	-	-	-	-
Dendritic Cell Maturation	2.27	2.333	4.600	−0.707	2.19	−1.633	-	-
Extrinsic Prothrombin Activation Pathway	1.36	N/A	2.34	N/A	-	-	-	-
Glucocorticoid Receptor Signaling	4.51	N/A	1.53	N/A	2.03	N/A	-	-
Graft-versus-Host Disease Signaling	4.23	N/A	3.600	N/A	-	-	-	-
HMGB1 Signaling	2.09	1.633	2.37	N/A	-	-	-	-
IL-6 Signaling	2.89	1.667	1.33	N/A	-	-	5.02	2.117
Intrinsic Prothrombin Activation Pathway	7.92	1.897	2.61	N/A	-	-	-	-
LXR/RXR Activation	4.31	−2.111	2.12	−1	-	-	-	-
MSP-RON Signaling Pathway	4.58	N/A	1.44	N/A	1.81	N/A	-	-
Osteoarthritis Pathway	1.44	−0.378	-	-	6.98	−1.265	-	-
PPAR Signaling	2.81	−1.414	2.33	0	-	-	2.57	−2.524
Production of Nitric Oxide and Reactive Oxygen Species in Macrophages	2.68	0.302	1.48	N/A	-	-	-	-
Retinol Biosynthesis	1.95	−1	1.49	N/A	-	-	-	-
Role of Macrophages, Fibroblasts and Endothelial Cells in Rheumatoid Arthritis	2.59	N/A	3.710	N/A	3.87	N/A	-	-
Role of Osteoblasts, Osteoclasts and Chondrocytes in Rheumatoid Arthritis	3.10	N/A	3.280	N/A	4.53	N/A	-	-
Sphingosine-1-phosphate Signaling	1.44	−2.236	1.40	N/A	2.38	−1	-	-

of BE (columnar metaplasia with presence of goblet cells) were equally present, although the larger amount of IM in BE only patients is indicative of a more advanced stage. Paneth cells were present in some patients of both groups, a variety more often reported in BE.[6]

The contribution of BE associated SNPs in EA/BE patients

The overlap of genes involved in foregut development and risk loci for BE insinuates a genetic predisposition for EA patients to develop BE. For example, *FOXF1*, which is expressed in the developing foregut,[17] *BARX1*, which is expressed at the tracheoesophageal separation site and inhibits Wnt signaling,[18] and FOXP1, which regulates esophageal muscle development,[19] have all been associated with BE in previous GWAS studies.[11] *FOXP1* has also been implicated as a tumor suppressor gene in several tissues including the gastrointestinal tract.[20] The ORs of these risk loci were often small and the GWAS studies included large sets of BE patients in order to detect these predispositions.

Regardless, there seems to be an elevated risk for EA patients. EA/BE patients have a higher median PGRS compared with BE only patients (3.24 vs. 2.63, p=0.069), which was confirmed and reached significance when using ORs calculated from our study population (p<0.001, see Supplementary Material S3 and Figure 2A). Despite the small cohorts, the higher PGRS in EA/BE patients is suggestive for an increased predisposition, and a possible contribution for the earlier age of onset of BE in these patients. Such a relationship (higher PGRS and earlier disease onset) has been demonstrated previously in patients with atrial fibrillation.[21] However, differences in PGRS are not likely to be sufficient on their own to exclude EA patients from (pre)malignant screening protocols. Ideally, a screening algorithm would contain multiple risk factors of which the PGRS could be one. Further research would be required to confirm the impact of risk loci for BE and their potential benefit in surveillance strategies for EA patients.

Two predisposing associated SNPs proved enriched when comparing EA/BE patients with BE only patients: rs3784262 near *ALDH1A2* (OR 3.94, p=0.017) and rs3072 near *GDF7* (OR 0.22, p=0.009). ALDH1A2 (also known as RALDH2) is an enzyme that catalyzes the transformation of retinaldehyde into RA, a key morphogen in foregut development.[22] Lack of RA signaling results in increased TGFB-BMP signaling and hampers lung bud induction.[23] In contrast, BE is characterized by a higher expression of this enzyme, resulting in higher levels of RA.[24] *GDF7* is also a component of the TGFB-BMP signaling pathway. TGFB-BMP signaling is essential in esophageal formation by inhibiting *SOX2* in the ventral foregut[25] but also contributes to the differentiation of columnar epithelium and BE development by interacting with *CDX1* and *CDX2*.[26] Interestingly, the associated SNP *GDF7* seems a protective locus in EA/BE patients (OR 0.22, p=0.009). The trends shown by these results are illustrative but more research is needed. Though EA/BE patients could have an increased genetic risk, the current sample sizes do not allow to draw firm conclusions.

EA/BE patients have comparable gene expression of BE disease genes as BE only patients

An earlier age of BE onset in EA patients could mean that epithelial homeostasis in these patients is more prone to disturbances. To investigate this, we sequenced RNA extracted from esophageal biopsies of three groups (EA/BE, EA only and BE only). We evaluated the expression of BE disease genes but found no difference in expression between EA/BE patients and BE only patients. In both groups, these genes were upregulated compared to EA only patients, indicating that the BE found in EA/BE patients is similar to the BE in BE only patients (see Figure 3).

EA/BE patients have an increased inflammatory response

Since the expression of disease genes could not explain the earlier age of onset, we explored the complete transcriptome and corresponding differentially expressed genes and pathways. Many of the enriched pathways in EA/BE patients compared with BE only patients, hinted at upregulated inflammatory (e.g., IL-6 signaling) and stress response pathways, downregulated oncological processes and dysregulated RA signaling (see Supplementary Table S6.2). Inflammatory cells produce carcinogenic compounds that can initiate DNA damage. The secretion of growth factors and cytokines increase proliferation and transition to tumor cells.[27] SPINK1 expression itself has the potential to be a BE biomarker as it lacks expression in unaffected esophageal tissue.

Human studies and in vitro experiments have shown that exposure of esophageal tissue to low pH and/or bile acids may induce cell proliferation and reduce cell apoptosis through an increased expression of cyclo-oxygenase-2 (COX-2), prostaglandin E2 (PGE2), mitogen-activated protein kinase (MAPK) and NF-κB pathways.[28-31] In our data, p38 MAPK signaling and NF-κB signaling are upregulated in EA/BE patients compared with BE only patients. Given their proliferative and anti-apoptotic role, these pathways could be valuable for BE staging. Quante and coworkers showed that transgenic mice, overexpressing human IL-1β, presented with chronic inflammation, BE and esophageal dysplasia. Oral exposure to bile acids led to elevated IL-6 levels, accelerating BE development and progression into EAC, and implicating an IL-1β-IL-6 signaling cascade.[32] Clinical management of BE is focused around chemical inhibition of acid exposure and decrease of inflammation. Inhibition of gastric acid secretion with proton pump inhibitors (PPIs) reduces the transition to dysplasia in BE patients[33] and a combination of non-steroidal anti-inflammatory drugs (NSAIDs) and statins may reduce neoplastic progression.[34] Recently, it has been shown that the combination of high-doses esomeprazole and aspirin reduces high-grade dysplasia and EAC in BE patients.[35] Given the potentially altered response to acid in EA patients, the effectiveness of PPIs and NSAIDs in this population warrant further investigation.

Furthermore, stress response pathways are upregulated. Cholecystokinin/gastrin-mediated signaling is an activator of actin stress fiber formation and intertwined with stress response pathways as p38 MAPK Signaling, Sphingosine-1-phosphate signaling and signaling by Rho Family GTPases. These processes may lead to the conversion of squamous epithelium to columnar metaplasia. Another study showed that low pH and/or bile acids can induce oxidative stress, which causes DNA damage.[36] In combination with reduced apoptosis this can lead to dysplasia. When this is followed by neo-plastic progression BE can develop into EAC.

Dysregulation of RA metabolism and signaling

RA is increased in BE and works – like bile acids – through the RXR receptors to transform squamous epithelium to columnar epithelium.[10] LXR/RXR activation, involved in RA mediated gene activation, is downregulated in EA/BE patients compared with BE only patients. Retinol biosynthesis is also downregulated, whilst its downstream processes in all trans RA synthesis (Retinoate Biosynthesis I) are upregulated. Peroxisome proliferator-activated receptors (PPARs) are transcription factors activated by RA, generally upregulated in BE,[37] but downregulated in EA/BE patients. Like discussed above, the downregulation of these pathways could indicate that BE only patients are at a more advanced stage than EA/BE patients. Given the clinical differences (age and length of BE) between these patients, this does make sense.

Downregulation of the Hippo/YAP pathway

Downregulation of oncological pathways in EA/BE patients could be indicative of either a decreased progression rate to dysplasia or a less advanced state of progression compared with BE only patients. The Hippo/YAP pathway is important in cell proliferation, survival, and differentiation. Yes-association protein (YAP) expression is associated with dysplasia and adenocarcinoma.[38] Hippo signaling is involved in cell contact inhibition[39] as is Aryl Hydrocarbon Receptor Signaling.[40] Hippo activation (and YAP inactivation) is necessary for programmed cell death after detachment from the extracellular matrix.[41] Therefore, downregulation of this pathway could (in theory) decrease anoikis and increase the risk of tumor cell metastasis.

EA patients seem to be more sensitive to acid reflux exposure

EA patients are earlier in life and more frequently exposed to GER. Chronic GER could be a consequence of the surgical repair: the lower esophageal sphincter is often retracted above the diaphragm, resulting in the loss of the natural reflux barrier function of the GEJ.[42] Other factors contributing to GER are impaired motility, delayed bolus clearance and delayed gastric emptying.[43] There seems to be a direct relationship of these symptoms with EA, as Adriamycin induced EA rats have impaired esophageal relaxation and a decreased number of ganglia and nerve fibers in the esophageal myenteric plexus.[44] The prevalence of mucosal damage is related to the level of pH exposure and to the composition of the acid reflux.[45] Animal studies have shown that acid fluids can activate pepsin, which inflicts injury and leads to mucosal damage.[46]

We speculated that GER could result in an upregulation of inflammatory pathways. Additionally, EA patients could have a predisposition that makes them more sensitive to acid reflux than the general population. To explore these hypotheses, we performed in vitro experiments to simulate a one-time reflux episode in fibroblasts of EA patients and healthy controls. The enriched pathways of the GEJ biopsies of EA/BE patients showed an overlap with the enriched pathways of the fibroblasts of EA patients after acid exposure – but not with those of healthy controls. These overlapping pathways were again mostly involved inflammatory or oncological processes. For example, LXR/RXR Activation, PPAR Signaling and Retinol Biosynthesis were also enriched in fibroblasts of EA patients after acid exposure, hinting at intrinsic disturbances of RA signaling in EA patients under the influence of GER.

We do not know of the three patients used in the in vitro experiment will develop BE in time as the fibroblasts are derived of patients currently aged 29, 30 and 39 years old. It is, however, interesting that we could detect a similar predisposition in just 3 EA patients, and as a general response (in fibroblasts) to acid.

Strengths and limitations

The main strength of this study is the broad investigative approach by combining histology, genotype, transcriptome and in vitro results. Some limitations should be addressed. First, due to the relative low incidence of EA and corresponding small sample sizes, we mostly observed trends and more EA/BE patients are needed to draw more robust conclusions. At this point, the difference in gene expression between EA/BE patients and EA only patients is negligible. This could be due to the fact that most biopsies could contain part of the cardia. However, the power would increase substantially if we would know which EA patients have not developed BE throughout their life, as the current EA only population is a mixture of patients who have not yet and will never develop BE. Second, EA is a heterogeneous disease. Our study population included both patients with isolated EA and patients with syndromes or multiple anomalies. This phenotypic heterogeneity might also be the results of a genetic heterogeneity. Thirdly, BE can present as a heterogeneous metaplastic mosaic, consisting of multiple individual crypts that arose from independent clones,[47] which have distinct ploidies, copy number variations (CNV) and point mutations.[48] Heterogeneity in these crypts pose a risk of sampling error. Even within long segment BE, IM can be focally distributed.[49] Recent progress in genetic analysis of BE stem cells and EAC indicates that there are patient-specific driver genes affected in both the precursor lesion[50] and subsequent cancer of the esophagus.[51] Perhaps the heterogeneous background of de novo mutations[52] and de novo CNVs[53] in EA contributes to this patient-centered susceptibility. This could have created larger variances in gene expression per evaluated group. Subsequent experiments using single-cell sequencing of definite IM could reveal differences between patients that cannot be detected in whole biopsy specimens. Lastly, morphological differences were absent. However, segment length

differences could be related to a difference in disease stage[54] and impact gene networks are prone to disturbances.

CONCLUSIONS

Altered regulation of p38 MAPK, NF-κB and RA signaling could have implications for (or be related to) the dysplastic progression. If Hippo/YAP signaling remains down-regulated upon progression to cancer, the metastasis risk could be higher in EA patients due to reduced anoikis. An increased PGRS and upregulation of inflammatory pathways hint at a multifactorial contribution underlying the earlier age of onset of BE in EA patients. We did not evaluate mechanical factors such as loss of the natural reflux barrier due to the surgical repair and clinical factors such as impaired esophageal motility. These factors increase the level of acid exposure and likely add to the effect of risk loci and primed inflammatory pathways.

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S1. Study population and histopathological evaluation

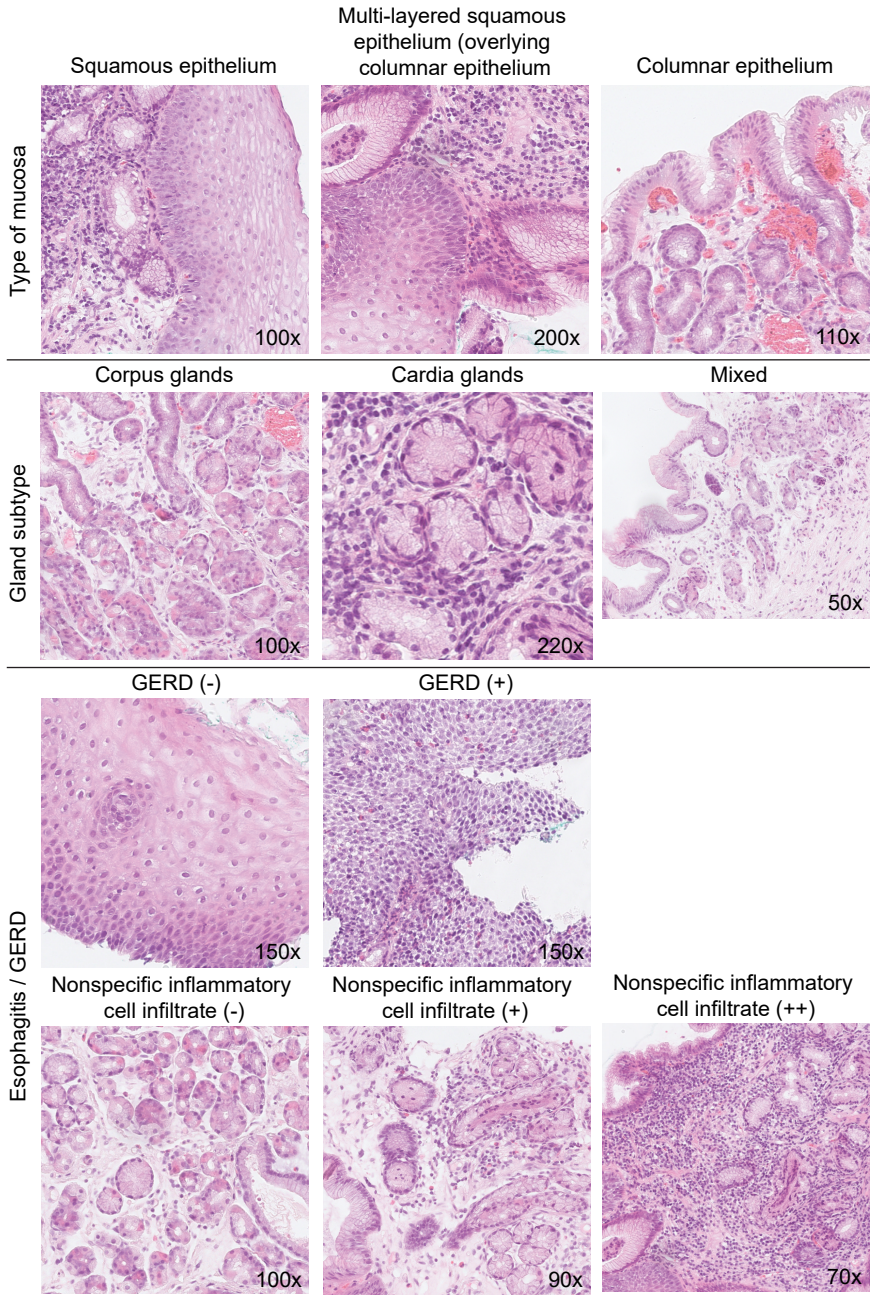
Supplementary Table S1.1. Basic characteristics of selected patients and controls for RNA sequencing of the esophageal biopsy specimen (upper), and for the SNP array genotyping (under). Data is presented as n (%) or median (range). Asterisk indicates significance (p<0.05). EA = esophageal atresia, BE = Barrett's esophagus, N/E = not evaluated. [A] Mann-Whitney test, group A versus group B. [B] Mann-Whitney test, group A versus group C. [C] According to the Gross classification.[1] [D] According to the Los Angeles criteria.[2] EA/BE patients were significantly younger than BE only patients (median age 39.3 versus 59.1 years, p=0.003). BE only patients had more often long segment BE (≥3 cm) compared with EA/BE patients (p=0.023).

Selected patients for RNA sequencing		Group A (EA/BE, n=11)	Group B (EA only, n=10)	*p-value* [A]	Group C (BE only, n=10)	*p-value* [B]
	Male (%)	10 (90.9)	6 (60.0)	0.114	7 (70.0)	0.221
	Type of EA [C]					
	Type A	1 (9.1)	0	0.563		
	Type C	8 (72.7)	6 (60.0)	0.525		
	Type E	0	1 (10.0)	0.437		
	Unknown	2 (18.2)	3 (30.0)	N/E		
	Staged repair	1 (9.1)	0 (0%)	0.242		
	Fundoplication surgery in history	4 (36.4)	2 (20.0)	0.274	1 (10.0)	0.162
	Median age at time of biopsy (range)	39.3 (20.5-58.7)	29.4 (21.8-49.3)	0.099	59.1 (45.2-66.7)	**0.003 ***
	Tobacco smoking					
	No	5 (45.5)	7 (70.0)	0.189	6 (60.0)	0.231
	Former smoker	4 (36.4)	1 (10.0)	0.162	2 (20.0)	0.307
	Yes, active smoker	2 (18.2)	2 (20.0)	0.414	1 (10.0)	0.434
	Missing	0	0	N/E	1 (10.0)	N/E
	Alcohol consumption					
	No alcohol	2 (18.2)	2 (20.0)	0.409	1 (10.0)	0.485
	≤7 units/week	8 (72.7)	8 (80.0)	0.383	4 (40.0)	0.400
	≥8 units/week	1 (9.1)	0	0.550	1 (10.0)	0.485
	Missing	0	0	N/E	4 (40.0)	N/E
	Endoscopic esophagitis [D]					
	No	6 (54.5)	10 (100.0)	**0.023 ***	9 (90.0)	0.085
	Grade A	4 (36.4)	0	0.055	0	0.055
	Grade B	1 (9.1)	0	0.524	1 (10.0)	0.524
	Length of BE					
	Short segment, <3 cm	5 (45.5)			0	**0.023 ***
	Long segment, ≥3 cm	6 (54.5)			10 (100.0)	**0.023 ***
	Dysplasia	0			0	

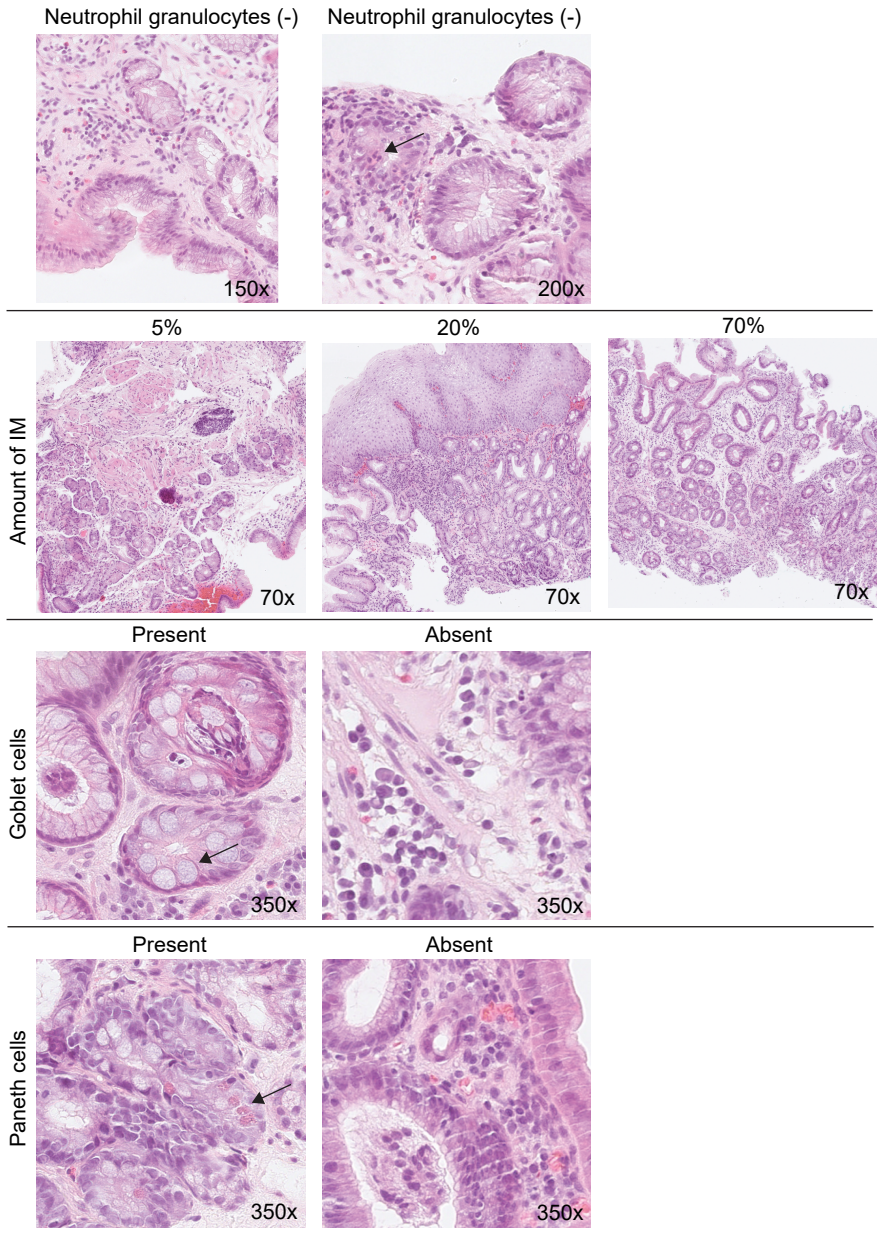
Selected patients for SNP array genotyping		Group A (EA/BE, n=19)	Group B (EA only, n=44)	Group C (BE only, n=10)	Controls (n=730)
	Male (%)	14 (73.3)	26 (59.1)	7 (70.0)	340 (46.6)
	Type of EA				
	Type A	1 (5.3)	2 (4.5)		
	Type C	16 (84.2)	37 (84.1)		
	Type D	0	1 (2.3)		
	Type E	0	1 (2.3)		
	Unknown	2 (10.5)	3 (6.8)		
	Length of BE				
	Short segment, <3 cm	9 (47.4)		0	
	Long segment, ≥3 cm	10 (52.6)		10 (100.0)	

Supplementary Table S1.2. Phenotype description. EA = esophageal atresia, TEF = tracheoesophageal fistula, BE = Barrett's esophagus, IHPS = infantile pyloric stenosis, VACTERL = vertebral, anorectal, cardiac, tracheoesophageal, renal and limb malformations. [A] According to Gross classification[1] [B] Patients included in acid exposure experiments.

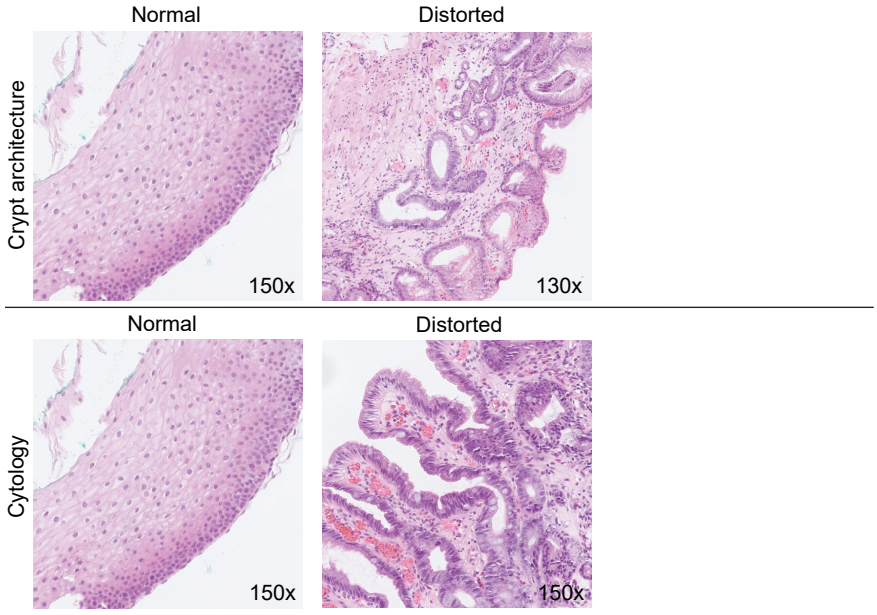
	Individual	Gender	EA type [A]	Phenotype	Remarks
Group A (EA/BE)	BBO-007	male	C	EA/TEF, inguinal hernia	-
	BBO-021	male	C	EA/TEF, congenital hiatal hernia	-
	BBO-027	male	C	EA/TEF, IHPS, extra ribs, fusion of vertebrae, macrocephaly, bulbar dermoid cyst, auricular tags, short thick/broad neck	Oculo-auriculo-vertebral spectrum, Klippel-Feil
	BBO-038	male	C	EA/TEF, anorectal malformation, ureteral duplication, aplasia left kidney, dysplasia right kidney, hypospadias, hip luxation	VACTERL
	BBO-053	female	unknown	EA/TEF	-
	BBO-058	male	C	EA/TEF, abnormal thoracic and lumbal vertebrae, congenital scoliosis, undescended testicles, inguinal hernia	-
	BBO-060	male	C	EA/TEF	-
	BBO-061	male	A	EA, adduction pollex	-
	BBO-063	male	C	EA/TEF	-
	BBO-074	male	unknown	EA/TEF#	-
	BBO-080	male	C	EA/TEF, atrial septal defect type II, perimembranous ventricular septal defect	
Group B (EA only)	BBO-065	male	C	EA/TEF	-
	BBO-066	male	E	TEF, hemivertebrae, fusion of vertebrae, 13 costae, anorectal malformation	-
	BBO-070	female	unknown	EA/TEF	-
	BBO-072	female	unknown	*Patient records could not be retrieved from archives*	-
	BBO-077	male	C	EA/TEF	-
	BBO-086	male	C	EA/TEF, microcephaly, microdactylia	Feingold syndrome
	BBO-087	male	C	EA/TEF, IHPS, mild left sided expansion of the pyelocaliceal system , breath holding spells	-
	BBO-090	female	C	EA/TEF, anorectal malformation (vestibular anus), atrial septal defect type II, perimembranous ventricular septal defect, hypertelorism, protruding ears, sacral dimple	VACTERL, Townes Brocks (Sall1)
	BBO-092	female	C	EA/TEF, dysmaturity	-
	BBO-094	male	unknown	EA/TEF#	-
EA [B]	Patient 1	male	C	EA/TEF, anorectal malformation, double outlet ventricle right, aplasia right thumb, cleft palate	VACTERL
	Patient 2	female	C	EA/TEF, anorectal malformation, duodenal atresia, aplasia sacrum, vertebral anomalies, aplasia right kidney, cleft of lip and palate,	VACTERL
	Patient 3	female	C	EA/TEF, perimembranous ventricular septal defect	-



Supplementary Figure S1.3 – part A. Examples of the review-based checklist[3], used for the histopathological assessments of pathology slides of esophageal biopsy specimens, with the magnification of each image.



Supplementary Figure S1.3 – part B. Examples of the review-based checklist[3], used for the histopathological assessments of pathology slides of esophageal biopsy specimens, with the magnification of each image.



Supplementary Figure S1.3 – part C. Examples of the review-based checklist[3], used for the histopathological assessments of pathology slides of esophageal biopsy specimens, with the magnification of each image.

Supplementary Table S1.4. Summarized results of reassessments of pathology slides of esophageal biopsy specimens that were included in the differential expression analysis. Plus (+) indicates the presence of a criteria in the majority of the slides. Minus (-) indicates the absence of a criteria in all slides. Plus-minus (+/-) indicate the presence of a criteria in a few slides. EA = esophageal atresia, BE = Barrett's esophagus, GERD = gastroesophageal reflux disease, N/A = not applicable. [A] Including one patient with pseudo-pancreatic metaplasia. Columnar epithelium was present in all groups, but not in every individual patient in group B (EA only). In group B (EA only), neutrophil granulocytes were absent while a nonspecific inflammatory cell infiltrate was present in all groups. Focusing on the characteristics of BE, IM with the presence of goblet cells was similarly present in group A (EA/BE) and group C (BE only). The amount of IM was larger in group C (BE only).

	Group A (EA/BE, n=11)	**Group B (EA only, n=10)**	**Group C (BE only, n=10)**
Type of mucosa			
Squamous epithelium	+	+	+
Multi-layered squamous epithelium (overlying) columnar epithelium	-	-	-
Columnar epithelium	+	+/-	+
Gland subtype			
Corpus glands, cardia glands or mixed	mixed	mixed [A]	mixed
Esophagitis / GERD			
GERD present	-	-	-
Nonspecific inflammatory cell infiltrate	+	+	+
Neutrophil granulocytes	+	-	+
Intestinal metaplasia			
Amount of intestinal metaplasia (average per slide)	20%	N/A	50%
Goblet cells present	+	-	+
Paneth cells present	-	+/-	+/-
Dysplasia			
Dysplasia present	-	-	-
Crypt architecture distorted	-	-	-
Cytology distorted	-	-	-

Supplementary Table S1.5. Complete results of reassessments of pathology slides of esophageal biopsy specimens that were included in the differential expression analysis. Plus (+) indicates the presence of a criteria in the majority of the slides. Minus (-) indicates the absence of a criteria in all slides. Plus-minus (+/-) indicate the presence of a criteria in a few slides. * Combined with pseudo-pancreatic metaplasia. EA = esophageal atresia, BE = Barrett's esophagus, GERD = gastroesophageal reflux disease, M = mixed glands, C = cardia glands, Co = corpus glands, N/A = not applicable

	Group A (EA/BE)										
	BBO-008	BBO-021	BBO-027	BBO-038	BBO-053	BBO-058	BBO-060	BBO-061	BBO-063	BBO-074	BBO-080
Type of mucosa											
Squamous epithelium	+	+	+	+	+	+	+	+	+	+	+
Multi-layered squamous epithelium (overlying) columnar epithelium	-	-	-	+	-	-	-	-	-	-	-
Columnar epithelium	+	+	+	+	+	+	+	+	+	+	+
Gland subtype											
Corpus glands, cardia glands or mixed	M	M	M	C	M	C	M	C	Co	M	C
Esophagitis / GERD											
GERD present	-	-	-	-	-	-	-	-	-	-	-
Nonspecific inflammatory cell infiltrate	+	+	+	++	+	+	+	++	+	+	+
Neutrophil granulocytes	+	+	+	+	+	-	+	+	+	+	-
Intestinal metaplasia											
Amount of intestinal metaplasia (%)	5	20	40	5	20	70	20	5	30	5	10
Goblet cells present	+	+	+	+	+	+	+	+	+	+	+
Paneth cells present	-	+	+	-	-	-	-	-	-	-	-
Dysplasia											
Dysplasia present	-	-	-	-	-	-	-	-	-	-	-
Crypt architecture distorted	+	+	+	-	-	-	-	-	-	-	-
Cytology distorted											

	Group B (EA only)									
	BBO-065	BBO-066	BBO-070	BBO-072	BBO-077	BBO-086	BBO-087	BBO-090	BBO-092	BBO-094
Type of mucosa										
Squamous epithelium	+	+	+	+	+	+	+	+	+	+
Multi-layered squamous epithelium (overlying) columnar epithelium	-	-	-	-	-	-	-	-	-	-
Columnar epithelium	+	-	+	+	+	+	+	+	+	-
Gland subtype										
Corpus glands, cardia glands or mixed	C	N/A	M	C *	M	M	M	M *	M	N/A

Esophagitis / GERD										
GERD present	-	-	-	-	-	-	+	+/-	-	-
Nonspecific inflammatory cell infiltrate	+	-	++	+	+	+	+	++	++	-
Neutrophil granulocytes	+	-	-	-	-	-	-	+	-	-
Intestinal metaplasia	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
Amount of intestinal metaplasia (%)	-	-	-	-	-	-	-	-	-	-
Goblet cells present	-	-	-	-	-	-	-	-	-	-
Paneth cells present										
Dysplasia	-	-	-	-	-	-	-	-	-	-
Dysplasia present	-	-	-	-	-	-	-	-	-	-
Crypt architecture distorted	-	-	-	-	-	-	-	-	-	-
Cytology distorted	-	-	-	-	-	-	+	+/-	-	-
	Group C (BE only)									
	BBE-001	BBE-017	BBE-021	BBE-023	BBE-028	BBE-043	BBE-053	BBE-079	BBE-080	BBE-098
Type of mucosa										
Squamous epithelium	+	-	+	+	+	+	+	+	+	+
Multi-layered squamous epithelium (overlying) columnar epithelium	-	-	-	+	-	-	-	-	-	-
Columnar epithelium	+	+	+	+	+	+	+	+	+	+
Gland subtype										
Corpus glands, cardia glands or mixed	M	C	C	M	C	C	C	M	M	M
Esophagitis / GERD										
GERD present	-	-	-	-	-	-	-	-	-	-
Nonspecific inflammatory cell infiltrate	+	+	+	+	+	++	+	+	+	++
Neutrophil granulocytes	-	+	-	+	+	+	-	+	-	+
Intestinal metaplasia										
Amount of intestinal metaplasia (%)	60	40	65	50	15	60	70	30	45	70
Goblet cells present	+	+	+	+	+	+	+	+	+	+
Paneth cells present	+	-	-	+	+	+	+	-	+	-
Dysplasia										
Dysplasia present	-	-	-	-	-	-	-	-	-	-
Crypt architecture distorted	-	-	-	-	-	-	-	-	-	-
Cytology distorted	-	-	-	-	-	-	-	-	-	-

S2. Genes and polymorphisms associated with Barrett's esophagus and esophageal carcinoma

Table S2.1. Genes and polymorphisms associated with Barrett's esophagus (BE), with selected proxy SNPs used for SNP array genotyping. OR = odds ratio, CI = confidence interval, SE = standardized error. * = Genes involved in foregut development. All odds ratios (OR) are presented for heterozygote risk alleles. [A] Homozygote A/A: OR 3.69 (1.46-9.29), [B] Homozygote G/G: OR 3.65 (1.73-7.69), [C] Homozygote C/C: OR 2.56 (1.10-5.94), [D] Homozygote A/A: OR 0.43 (0.24-0.75). [E] Controls were patients with gastroesophageal reflux disease, defined as either endoscopic erosive esophagitis or complaints of substernal chest burning and/or regurgitation. [F] Controls were patients with reflux esophagitis.

Gene	SNP	Position hg19	Risk allele	Associated with	OR (95% CI) ± SE (β)	Type of study	Number of patients (n)	Number of controls (n)	Ref	Proxy SNP	D'	R'
ALDH1A2	rs3784262	chr15:58253106	C	BE, EAC	0.90 (0.87-0.93)	Meta-analysis	10038	27975	4	rs3204689	0.9677	0.9214
BARX1 *	rs11789015	chr9:96716028	G	BE	0.86 (0.81-0.92)	Meta-analysis	5027	15289	4	rs11789015	1	1
					0.85 (0.79-0.91)	GWAS	3175	10117	5			
CCND1	rs9344	chr11:69462910	A	BE	1.40 (0.76-2.56) [A]	Case-control	125	95	6	rs9344	1	1
CDX1	rs717746	chr5:149556558	G	BE	2.07 (1.05-4.08) [B]	Case-control	109	223 [E]	7	rs717746	1	1
CDX2	rs4769585	chr13:28550578	C	BE	2.68 (1.20-5.98) [C]	Case-control	109	223 [E]	7	rs6491244	0.9880	0.9722
CRTC1	rs10423674	chr19:18817903	T	BE	0.89 (0.95-0.93)	Meta-analysis	6605	23888	4	rs10423674	1	1
					0.85 (0.80-0.91)	GWAS	3175	10117	5			
FOXF1 *	rs9936833	chr16:86403118	C	BE	1.14 (1.10-1.19)	GWAS	7838	17997	8	rs9936833	1	1
					1.13 (0.98-1.29)	Case-control	1065	1019	9			
FOXP1 *	rs2687201	chr3:70928930	A	BE	1.16 (1.10-1.23)	Meta-analysis	5027	15289	4	rs287201	1	1
					1.18 (1.10-1.26)	GWAS	3175	10117	5			
GDF7 *	rs3072	chr2:20878406	G	BE	1.14 (1.09-1.18)	GWAS	10158	21062	4	rs9306894	0.9914	0.9662
GSTP1	rs1695	chr11: 67352689	G	BE	2.56 (1.30-5.05)	Case-control	22	173	10	rs1695	1	1
					1.50 (1.16-1.95)	Meta-analysis	434	738	11			
IGF1	rs6214	chr12:102793569	A	BE	0.90 (0.59-1.37) [D]	Case-control	207	244	12	rs6214	1	1
ILI12B	rs3212227	c:158742950	C	BE	1.82 (1.17-2.69)	Case-control	255	247 [F]	13	rs3213094	1	1
KHDRBS2-MTRNR2L9	rs62423175	chr6:62195368	A	BE	1.14 ± 0.03	Meta-analysis	6167	17159	14	rs1516709	0.9149	0.6837
LINC00208-BLK	rs10108511	chr8:11435516	T	BE	1.14 ± 0.02	Meta-analysis	6167	17159	14	rs2898290	0.9959	0.9565
MGST1	rs7312090	chr12:16515945	T	BE	1.16 (1.07-1.25)	Case-control	3288	3203	15	rs6488840	1	0.9928
	rs4149186	chr12:16498700	C	BE	1.11 (1.02-1.21)	Case-control	3288	3203	15	rs7312090	1	1

MHC region	rs9257809	chr6:29356331	A	BE	1.12 (1.13-1.28)	GWAS	7838	17997	[8]	rs9257809	1	1
				BE	1.26 ± 0.04	Meta-analysis	6167	17159	[14]			
MSRA	rs17749155	chr8:10068073	A	BE	1.20 ± 0.03	Meta-analysis	6167	17159	[14]	rs7832976	0.9829	0.9045
SATB2	rs139606545	chr2:200045039	T	BE	0.91 ± 0.02	Meta-analysis	6167	17159	[14]	rs4675343	0.9958	0.9188
TBX5 *	rs2701108	chr12:114674261	C	BE	0.90 (0.86-0.93)	GWAS	10158	21062	[4]	rs2701108	1	1
TMOD1	rs7852462	chr9:100310501	T	BE	0.87 ± 0.02	Meta-analysis	6167	17159	[14]	rs10759765	0.9225	0.824

Table S2.2. Genes and polymorphisms associated with Barrett's esophagus (BE), esophageal adenocarcinoma (EAC) or esophageal squamous cell carcinoma (ESCC). These polymorphisms were not selected for SNP array genotyping because no proxy SNP could be selected, or because they were only associated with EAC or ESCC and not with BE. OR = odds ratio, CI = confidence interval, SE = standardized error. * = Genes involved in foregut development. All odds ratios (OR) are presented for heterozygote risk alleles.. [A] Homozygote A/A: OR 5.99 (1.86-18.96). [B] Controls were patients with gastroesophageal reflux disease, defined as either endoscopic erosive esophagitis or complaints of substernal chest burning and/or regurgitation.

Gene	SNP	Position hg19	Risk allele	Associated with	OR (95% CI) ± SE (β)	Type of study	Number of patients (n)	Number of controls (n)	Ref
ABCC5-HTR3c	rs9823696	chr3:183783353	A	EAC	1.17 (1.11-1.24)	Meta-analysis	4112	17159	14
*BARX1 **	rs11789015	chr9:96716028	G	BE, EAC	0.85 (0.81-0.89)	Meta-analysis	4242	15292	4
				EAC	0.81 (0.75-0.88)	GWAS	2390	10120	5
				EAC	0.87 (0.75-1.02)	Case-control	1065	1019	9
				ESCC	0.77 (0.65-0.90)	Case-control	2119	2463	16
CAMTA1	rs17030152	chr1:7083719	C	EAC	0.87 (0.75-1.02)	Case-control	1065	1019	9
CCND1	rs9344	chr11:69462910	A	EAC [A]	1.37 (0.57-3.26)	Case-control	56	95	6
CDX1	rs3776083	chr5:149567970	A	BE	1.47 (0.61-3.54)	Case-control	109	223 [B]	7
CDX2	rs3812863	chr13:28545268	A	BE	1.95 (0.89-4.24)	Case-control	109	223 [B]	7
CHEK2	rs738722	chr22:28130012	T	ESCC	1.30 (1.19-1.43)	GWAS	2115	3202	17
CFTR	rs17451754	chr7:117256712	A	BE	0.87 ± 0.03	Meta-analysis	6167	17159	14
				EAC	0.80 ± 0.04	Meta-analysis	4112	17159	14
CRTC1	rs10419226	chr19:18803172	A	BE	1.19 (1.12-1.26)	GWAS	3175	10117	5
	rs199620551	chr19:18804295	T	BE	0.90 ± 0.02	Meta-analysis	6167	17159	14
				EAC	0.90 ±0.03	Meta-analysis	4112	17159	14
				EAC	0.85 (0.79-0.91)	GWAS	2390	10120	5
DIO3	rs2895917	chr14:102052775	T	EAC	0.88 (0.76-1.02)	Case-control	1065	1019	9
FOXF1	rs2178146	chr16:86463695	G	BE	0.89 (0.84-0.95)	GWAS	3175	10117	5
	rs3111601	chr16:86400081	G	BE	1.13 (1.05-1.20)	GWAS	3175	10117	5
				EAC	1.16 (1.08-1.24)	GWAS	2390	10120	5
	rs2178146	chr16:86463695	G	EAC	0.85 (0.79-0.91)	GWAS	2390	10120	5
	rs9936833	chr16:86403118	C	EAC	1.21 (0.99-1.47)	Case-control	318	605	18
*FOXF1-LOC732275 **	rs1979654	chr3:86396835	C	BE	0.90 ± 0.02	Meta-analysis	6167	17159	14
				EAC	0.90 ± 0.03	Meta-analysis	4112	17159	14
*FOXP1 **	rs2687202	chr3:70929983	T	BE	1.13 ± 0.02	Meta-analysis	6167	17159	14
				EAC	1.13 ± 0.03	Meta-analysis	4112	17159	14
	rs9837992	chr3:70959438	A	EAC	1.23 (1.07-1.42)	Case-control	1065	1019	9

*FOXP1 **	rs2687201	chr3:70928930	A	BE, EAC	1.17 (1.11-1.23)	Meta-analysis	4242	15292	4
				EAC	1.20 (1.12-1.29)	GWAS	2390	10120	5
				EAC	1.26 (1.09-1.46)	Case-control	1065	1019	9
GATA6	rs4800353	chr18:19654137	G	EAC	0.83 (0.69-1.01)	Case-control	1065	1019	9
*GDF7-LDAH**	rs7255	chr2:20878820	C	BE	1.12 ± 0.02	Meta-analysis	6167	17159	14
				EAC	1.17 ± 0.03	Meta-analysis	4112	17159	14
GHR	rs6898743	chr5:42602492	G	EAC	0.42 (0.23-0.76)	Case-control	210	240	12
GSTP1	rs1695	chr11: 67352689	G	EAC	1.73 (0.75-4.02)	Case-control	12	21	19
				EAC	1.20 (0.94-1.54)	Meta-analysis	432	1086	11
KHDRBS2-MTRNR2L9	rs62423175	chr6:62195368	A	EAC	1.23 ± 0.0377	Meta-analysis	4112	17159	14
LINC00208-BLK	rs10108511	chr8:11435516	T	EAC	1.08 ± 0.03	Meta-analysis	4112	17159	14
MFHAS1	rs4523255	chr8:8713038	T	EAC	1.14 (0.99-1.31)	Case-control	1065	1019	9
MGST1	rs4149203	chr12:16514921	T	BE	1.16 (1.08-1.26	Case-control	3295	3207	15
	rs3852575	chr12:16516260	T	BE	1.16 (1.08-1.25)	Case-control	3295	3207	15
	rs1419204	chr12:16515062	C	BE	1.16 (1.08-1.25)	Case-control	3295	3207	15
	rs4149207	chr12:16517491	T	BE	1.14 (1.06-1.23)	Case-control	3295	3207	15
	rs4149208	chr12:16517581	T	BE	1.14 (1.06-1.23)	Case-control	3295	3207	15
	rs3759207	chr12:16516710	C	BE	1.14 (1.05-1.23)	Case-control	3295	3207	15
	rs4149195	chr12:16512128	G	BE	1.20 (1.07-1.35)	Case-control	3295	3207	15
	rs2239676	chr12:16500448	G	BE	1.19 (1.06-1.34)	Case-control	3295	3207	15
	rs4149187	chr12:16500071	G	BE	1.18 (1.05-1.32)	Case-control	3295	3207	15
	rs2239677	chr12:16500680	A	BE	1.38 (1.09-1.75)	Case-control	3295	3207	15
	rs2239675	chr12:16500265	G	BE	1.12 (1.02-1.23)	Case-control	3295	3207	15
	rs2975138	chr12:16501551	A	BE	1.10 (1.02-1.20)	Case-control	3295	3207	15
MHC region	rs9257809	chr6:29356331	G	EAC	1.14 ± 0.05	Meta-analysis	4112	17159	14
				ESCC	1.76 (1.16-2.66)	Case-control	107	605	18
MSRA	rs17749155	chr8:10068073	A	EAC	1.13 ± 0.04	Meta-analysis	4112	17159	14
PCDH20	rs2669333	chr13:63574196	A	EAC	1.15 (1.00-1.33)	Case-control	1065	1019	9
PLCE1	rs2274223	chr10:96066314	G	ESCC	1.34 (1.22-1.48)	GWAS	2115	3202	17
	rs3765524	chr10:96058298	T	ESCC	1.35 (1.22-1.49)	GWAS	2115	3202	17
	rs3781264	chr10:96070375	C	ESCC	1.38 (1.23-1.53)	GWAS	2115	3202	17
	rs11187842	chr10:96052511	T	ESCC	1.37 (1.23-1.53)	GWAS	2115	3202	17
	rs753724	chr10:96051417	T	ESCC	1.38 (1.23-1.54)	GWAS	2115	3202	17
SATB2	rs139606545	chr2:200045039	T	EAC	0.88 ± 0.03	Meta-analysis	4112	17159	14

TBX5-LOC105369996 *	rs1247942	chr12:114673723	C	BE	0.88 ± 0.02	Meta-analysis	6167	17159	14
				EAC	0.90 ± 0.03	Meta-analysis	4112	17159	14
TMOD1	rs7852462	chr9:100310501	T	EAC	0.93 ± 0.03	Meta-analysis	4112	17159	14
TPPP-CEP72	rs9918259	chr5:663092	T	BE	1.20 ± 0.04	Meta-analysis	6167	17159	14
				EAC	1.20 ± 0.04	Meta-analysis	4112	17159	14
XRCC2	rs11771429	chr7:153271877	T	EAC	0.85 (0.71-1.02)	Case-control	1065	1019	9

S3. Results of SNP genotyping and polygenic risk score

Supplementary Table S3.1. Odds ratios calculated from the single nucleotide polymorphism (SNP) genotyping data. Group A = patients with esophageal atresia (EA) and Barrett's esophagus (BE) (n=19); Group B = patients with EA without BE (n=44); Group C = patients with BE without EA in history (n=10); controls n=730. OR = odds ratio, CI = confidence interval, X2 = chi-square test. Asterisk indicates significance level p<0.05.

		Group A (EA/BE) vs. controls		**Group B (EA only) vs. controls**		**Group C (BE only) vs. controls**		**Group A (EA/BE) vs. group C (BE only)**	
Gene	**SNP**	*OR (95% CI)*	*p-value X^2*	*OR (95% CI)*	*p-value X^2*	*OR (95% CI)*	*p-value X^2*	*OR (95% CI)*	*p-value X^2*
ALDH1A2	rs3784262	2.28 (1.08-4.83)	**0.028 ***	0.53 (0.22-0.83)	**0.005 ***	0.58 (0.24-1.40)	0.222	3.94 (1.24-12.4)	**0.017 ***
BARX1	rs11789015	1.70 (0.87-3.31)	0.116	1.43 (0.91-2.26)	0.124	0.97 (0.35-2.68)	0.956	1.75 (0.53-5.82)	0.361
CCND1	rs9344	1.04 (0.54-1.97)	0.916	0.80 (0.52-1.23)	0.306	1.41 (0.58-3.40)	0.450	0.74 (0.25-2.17)	0.581
CDX1	rs717746	1.14 (0.60-2.16)	0.695	1.72 (1.11-2.67)	**0.014 ***	0.61 (0.24-1.54)	0.294	1.86 (0.61-5.65)	0.275
CDX2	rs4769585	1.28 (0.66-2.46)	0.463	1.05 (0.68-1.61)	0.832	0.45 (0.18-1.13)	0.082	2.85 (0.93-8.73)	0.064
CRTC1	rs10423674	1.14 (0.59-2.22)	0.693	0.58 (0.35-0.96)	**0.033 ***	1.06 (0.42-2.65)	0.908	1.08 (0.35-3.34)	0.890
FOXF1	rs9936833	0.56 (0.27-1.16)	0.116	0.81 (0.52-1.28)	0.368	0.52 (0.19-1.44)	0.205	1.07 (0.31-3.69)	0.913
FOXP1	rs2687201	1.41 (0.73-2.72)	0.306	0.86 (0.53-1.38)	0.526	4.01 (1.60-10.07)	**0.002 ***	0.35 (0.11-1.08)	0.064
GDF7	rs3072	0.45 (0.21-0.99)	0.044	0.93 (0.59-1.45)	0.740	2.08 (0.86-5.03)	0.098	0.22 (0.07-0.70)	**0.009 ***
GSTP1	rs1695	1.57 (0.82-2.99)	0.170	0.81 (0.51-1.30)	0.387	0.83 (0.32-2.16)	0.705	1.89 (0.60-5.93)	0.276
IGF1	rs6214	0.48 (0.23-0.98)	**0.041 ***	0.76 (0.49-1.18)	0.225	0.80 (0.35-1.83)	0.593	0.60 (0.20-1.77)	0.352
ILI12B	rs3212227	1.21 (0.57-2.56)	0.629	0.67 (0.37-1.23)	0.195	1.29 (0.47-3.57)	0.619	0.93 (0.27-3.26)	0.911
KHDRBS2-MTRNR2L9	rs62423175	1.40 (0.69-2.84)	0.352	0.76 (0.44-1.33)	0.341	1.14 (0.42-3.16)	0.793	1.22 (0.36-4.16)	0.749
LINC00208-BLK	rs10108511	1.31 (0.69-2.49)	0.415	1.06 (0.69-1.63)	0.793	1.06 (0.44-2.55)	0.898	1.24 (0.42-3.64)	0.703
MGST1	rs4149186	1.51 (0.76-3.02)	0.238	1.30 (0.81-2.10)	0.279	1.09 (0.40-3.02)	0.863	1.38 (0.41-4.66)	0.601
MGST1	rs7312090	0.57 (0.26-1.25)	0.158	1.00 (0.63-1.58)	0.995	0.54 (0.18-1.60)	0.258	1.07 (0.28-4.07)	0.925
MHC region	rs9257809	0.67 (0.26-1.73)	0.404	0.89 (0.44-1.80)	0.741	0.91 (0.21-3.93)	0.890	0.73 (0.13-4.13)	0.726
MSRA	rs17749155	0.51 (0.18-1.43)	0.194	0.82 (0.46-1.46)	0.494	0.11 (0.36-3.23)	0.895	0.47 (0.11-2.11)	0.320
SATB2	rs139606545	0.73 (0.37-1.41)	0.347	0.75 (0.48-1.16)	0.198	0.42 (0.15-1.14)	0.081	1.75 (0.53-5.82)	0.361
TBX5	rs2701108	1.45 (0.76-2.79)	0.260	1.45 (0.94-2.24)	0.094	0.67 (0.24-1.83)	0.431	2.18 (0.66-7.20)	0.198
TMOD1	rs7852462	0.96 (0.50-1.83)	0.898	0.96 (0.62-1.47)	0.839	1.08 (0.45-2.61)	0.868	0.89 (0.30-2.63)	0.832

Supplementary Table S3.2. Overview of polygenic risk scores (PGRS) for all groups, based on odds ratios (ORs) selected from the literature (left) and ORs calculated from the SNP array (right). See also Supplementary Table S3.3. Group A = patients with esophageal atresia (EA) and Barrett's esophagus (BE); group B = patients with EA without BE; and group C = patients with BE without EA in history. IQR = interquartile range. Asterisk indicates significance level p<0.05.

Group	**n**	**PGRS based on ORs from literature**			**PGRS based on ORs calculated from our study population**		
		Median (range)	*IQR*	*Kruskal-Wallis test*	*Median (range)*	*IQR*	*Kruskal-Wallis test*
Group A (EA/BE)	19	3.24 (1.39-4.68)	1.40	0.495	3.05 (0.14-6.04)	1.70	**0.001 ***
Group B (EA only)	44	2.98 (1.19-4.74)	1.28		2.52 (-2.73-5.72)	3.38	
Group C (BE only)	10	2.63 (1.85-3.53)	1.17		-0.24 (-2.83-2.15)	2.42	
Controls	730	3.00 (-0.28-5.78)	1.65		2.21 (-4.44-7.83)	2.21	

Supplementary Table S3.3. Overview of the selected odds ratios (OR) used for the polygenic risk score. In case multiple studies published an OR for a certain SNP, the study with the largest sample size was included in the PGRS. For *MGST1* two SNPs were described, not in linkage disequilibrium with each other, for which both SNPs were included. OR = odds ratio, CI = confidence interval, SE = standardized error.

Gene	**SNP**	**Proxy SNP**	**Literature** *OR (95% CI) ± SE (β)*	**SNP array data (n=29)** *OR (95% CI)*
ALDH1A2	rs3784262	rs3204689	0.90 (0.87-0.93)[4]	3.94 (1.24-12.4)
BARX1	rs11789015	rs11789015	0.86 (0.81-0.92)[4]	1.75 (0.53-5.82)
CCND1	rs9344	rs9344	1.40 (0.76-2.56)[6]	0.74 (0.25-2.17)
CDX1	rs717746	rs717746	2.07 (1.05-4.08)[7]	1.86 (0.61-5.65)
CDX2	rs4769585	rs6491244	2.68 (1.20-5.98)[7]	2.85 (0.93-8.73)
CRTC1	rs10423674	rs10423674	0.89 (0.95-0.93)[4]	1.08 (0.35-3.34)
FOXF1	rs9936833	rs9936833	1.14 (1.10-1.19)[8]	1.07 (0.31-3.69)
FOXP1	rs2687201	rs2687201	1.16 (1.10-1.23)[4]	0.35 (0.11-1.08)
GDF7	rs3072	rs9306894	1.14 (1.09-1.18)[4]	0.22 (0.07-0.70)
GSTP1	rs1695	rs1695	1.50 (1.16-1.95)[11]	1.89 (0.60-5.93)
IGF1	rs6214	rs6214	0.90 (0.59-1.37)[12]	0.60 (0.20-1.77)
ILI12B	rs3212227	rs3213094	1.82 (1.17-2.69)[13]	0.93 (0.27-3.26)
KHDRBS2-MTRNR2L9	rs62423175	rs1516709	1.14 ± 0.03[14]	1.22 (0.36-4.16)
LINC00208-BLK	rs10108511	rs2898290	1.14 ± 0.02[14]	1.24 (0.42-3.64)
MGST1	rs4149186	rs6488840	1.11 (1.02-1.21)[15]	1.38 (0.41-4.66)
MGST1	rs7312090	rs7312090	1.16 (1.07-1.25)[15]	1.07 (0.28-4.07)
MHC region	rs9257809	rs9257809	1.26 ± 0.04[14]	0.73 (0. 13-4.13)
MSRA	rs17749155	rs7832976	1.20 ± 0.03[14]	0.47 (0.11-2.11)
SATB2	rs139606545	rs4675343	0.91 ± 0.02[14]	1.75 (0.53-5.82)
TBX5	rs2701108	rs2701108	0.90 (0.86-0.93)[4]	2.18 (0.66-7.20)
TMOD1	rs7852462	rs10759765	0.87 ± 0.02[14]	0.89 (0.30-2.63)

Supplementary Table S3.4. Comparison of all groups separately for the polygenic risk score (PGRS) based on odds ratios (ORs) selected from the literature (left) and ORs calculated from the SNP array (right), using Mann-Whitney tests. Group A = patients with esophageal atresia (EA) and Barrett's esophagus (BE) (n=19); Group B = patients with EA without BE (n=44); Group C = patients with BE without EA in history (n=10); controls n=730.

Group	**PGRS based on ORs from literature** *p-value*	**PGRS based on ORs calculated from our study population** *p-value*
Group A (EA/BE) vs. group B (EA only)	0.500	0.274
Group A (EA/BE) vs. group C (BE only)	0.069	**<0.001 ***
Group A (EA/BE) vs. controls	0.381	0.055
Group B (EA only) vs. group C (BE only)	0.124	**0.001 ***
Group B (EA only) vs. controls	0.694	0.568
Group C (BE only) vs. controls	0.251	**<0.001 ***

S4. Overview of patient material and data quality

Supplementary Table S4.1. Results of RNA and DNA isolation from esophageal biopsy specimens, blood and skin fibroblasts in terms of quantity and quality. Group A = patients with esophageal atresia (EA) and Barrett's esophagus (BE); group B = patients with EA without BE; and group C = patients with BE without EA in history. SQ = squamous cell epithelium, GEJ = gastroesophageal junction, RIN = RNA integrity number. A The polygenetic risk score of these individuals was respectively 1.19, 3,18 and 3.26 for the patients, and 1.73, 3.30 and 4.80 for the controls. RNA amount is presented as non-exposed / acid-exposed. B Two outliers were excluded for the differential expression and pathway enrichment analysis (BBE-017 and BBE-079). See Supplementary Table S1.2 for a phenotypical description of these patients.

	Patient	**RNA**						**DNA**	**Experiments that patient was included in**
		SQ		**GEJ**		**Fibroblasts**		**Blood**	**Fibroblasts**	
		(ng/ul)	*RIN*	*(ng/ul)*	*RIN*	*(ng/ul)*	*RIN*	*(ng/ul)*	*(ng/ul)*	
Group A (EA/BE)	BBO-007	162	10	262	7.2			60.1		RNAseq of biopsy specimens, SNP genotyping
	BBO-018							57.3		SNP genotyping
	BBO-021			27	8.4			55.5		RNAseq of biopsy specimens, SNP genotyping
	BBO-027			86	8.9			56.1		RNAseq of biopsy specimens, SNP genotyping
	BBO-038	23	9.8	346	8.2			58.6		RNAseq of biopsy specimens, SNP genotyping
	BBO-053	86	9.7	341	9.1			60.1		RNAseq of biopsy specimens, SNP genotyping
	BBO-058	80	10	924	9.2			72.5		RNAseq of biopsy specimens, SNP genotyping
	BBO-060	556	9.8	699	9.1			57.0		RNAseq of biopsy specimens, SNP genotyping
	BBO-061			341	9.5			62.9		RNAseq of biopsy specimens, SNP genotyping
	BBO-063	131	10	839	9.0			61.4		RNAseq of biopsy specimens, SNP genotyping
	BBO-064							58.6		SNP genotyping
	BBO-069							59.5		SNP genotyping
	BBO-074	313	10	132	9.9			58.7		RNAseq of biopsy specimens, SNP genotyping
	BBO-080	206	9.9	388	7.6			57.3		RNAseq of biopsy specimens, SNP genotyping
	BBO-134							50.0		SNP genotyping
	BBO-142							50.0		SNP genotyping
	BBO-149							50.0		SNP genotyping
	BBO-160							50.0		SNP genotyping
	H1							158.3		SNP genotyping
	H2							111.5		SNP genotyping
	H3							107.2		SNP genotyping
	H4							151.4		SNP genotyping
	H5							101.5		SNP genotyping
	H6							86.0		SNP genotyping
	H7							246.0		SNP genotyping

Group B (EA only)	BBO-002							66.9	SNP genotyping
	BBO-003							59.9	SNP genotyping
	BBO-013							55.7	SNP genotyping
	BBO-014							56.2	SNP genotyping
	BBO-015							53.8	SNP genotyping
	BBO-020							56.3	SNP genotyping
	BBO-026							64.9	SNP genotyping
	BBO-034							72.5	SNP genotyping
	BBO-036							59.9	SNP genotyping
	BBO-050							58.1	SNP genotyping
	BBO-055							63.8	SNP genotyping
	BBO-065	34	9.6	274	10			48.0	RNAseq of biopsy specimens, SNP genotyping
	BBO-066	95	10	1035	9.9			62.9	RNAseq of biopsy specimens, SNP genotyping
	BBO-070	127	9.9	201	9.6			57.4	RNAseq of biopsy specimens, SNP genotyping
	BBO-072	165	9.9	71	9.7			55.8	RNAseq of biopsy specimens, SNP genotyping
	BBO-077	47	10	408	8.7			63.9	RNAseq of biopsy specimens, SNP genotyping
	BBO-086	125	10	385	8.1			59.4	RNAseq of biopsy specimens, SNP genotyping
	BBO-087	126	9.9	509	8.1			63.1	RNAseq of biopsy specimens, SNP genotyping
	BBO-090	99	9.6	159	9.3			54.5	RNAseq of biopsy specimens, SNP genotyping
	BBO-092			188	7.0				RNAseq of biopsy specimens
	BBO-094	62	10	617	6.7			71.9	RNAseq of biopsy specimens, SNP genotyping
	08C0457							57.6	SNP genotyping
	04E0970							71.8	SNP genotyping
	19E0874							42.6	SNP genotyping
	92E0317							42.5	SNP genotyping
	97E0599							54.7	SNP genotyping
	92E0437							67.3	SNP genotyping
	18E0535							47.8	SNP genotyping
	00E0622							43.0	SNP genotyping
	95E0710							48.2	SNP genotyping
	02E0181							89.2	SNP genotyping
	92E0275							73.2	SNP genotyping
	82E0136							69.0	SNP genotyping
	02E0260							67.6	SNP genotyping
	Patient 1 [A]					239 / 30	9.3 / 7.3		Acid exposure experiment, including RNAseq
	Patient 2 [A]					206 / 70	9.3 / 9.2		Acid exposure experiment, including RNAseq
	Patient 3 [A]					141 / 30	9.8 / 8.3		Acid exposure experiment, including RNAseq

Group C (BE only)	BBE-001			260	8.4			39.3		RNAseq of biopsy specimens, SNP genotyping
	BBE-017	39	7.8	14 [B]	6.3			35.2		RNAseq of biopsy specimens, SNP genotyping
	BBE-021	209	9.3	756	8.3			82.3		RNAseq of biopsy specimens, SNP genotyping
	BBE-023	163	9.1	382	7.4			44.0		RNAseq of biopsy specimens, SNP genotyping
	BBE-028	71	9.1	132	7.9			41.6		RNAseq of biopsy specimens, SNP genotyping
	BBE-043	162	9.1	264	7.1			39.3		RNAseq of biopsy specimens, SNP genotyping
	BBE-053	65	9.4	112	7.5			78.3		RNAseq of biopsy specimens, SNP genotyping
	BBE-079	16 [B]	8.0	88	8.5			37.3		RNAseq of biopsy specimens, SNP genotyping
	BBE-080			376	9.4			31.0		RNAseq of biopsy specimens, SNP genotyping
	BBE-098	92	9.0	97	7.3			39.2		RNAseq of biopsy specimens, SNP genotyping
Controls	90E1033								22.0	SNP genotyping
	85E0344								47.8	SNP genotyping
	86RD677								93.2	SNP genotyping
	Control 1 [A]					31 / 13	9.0 / 7.2			Acid exposure experiment, including RNAseq
	Control 2 [A]					223 / 66	9.3 / 8.3			Acid exposure experiment, including RNAseq
	Control 3 [A]					173 / 48	9.0 / 8.1			Acid exposure experiment, including RNAseq

Supplementary Table S4.2. Quality report of RNA sequencing data from esophageal biopsy specimens.

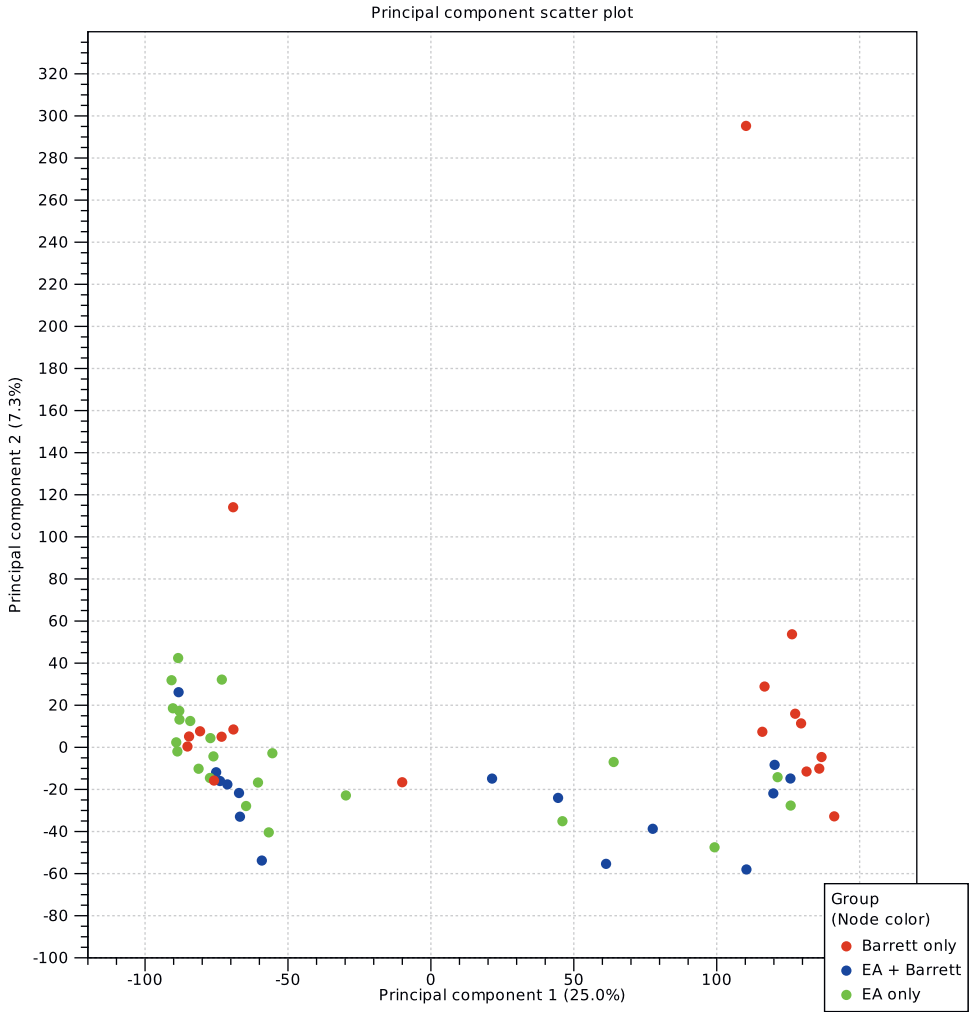
Sample	Patient	Read count	Mapped to genes (%)
103650-001-026	BBO-021	89,974,680	97.17
103650-001-027	BBO-027	80,585,282	97.48
103650-001-028	BBO-038	80,968,668	96.93
103650-001-029	BBO-053	81,423,510	97.22
103650-001-030	BBO-058	80,364,912	97.68
103650-001-031	BBO-060	80,402,720	97.68
103650-001-032	BBO-061	80,505,706	96.79
103650-001-033	BBO-063	79,694,064	97.46
103650-001-034	BBO-074	80,475,762	97.70
103650-001-035	BBO-080	81,389,838	94.77
103650-001-036	BBO-065	80,280,398	97.89
103650-001-037	BBO-066	81,411,852	98.35
103650-001-038	BBO-070	80,666,032	97.85
103650-001-039	BBO-072	80,447,282	98.00
103650-001-040	BBO-077	95,542,940	97.57
103650-001-041	BBO-086	82,468,060	97.09
103650-001-042	BBO-087	101,875,962	97.40
103650-001-043	BBO-090	80,153,816	97.66
103650-001-044	BBO-092	79,295,354	97.30
103650-001-045	BBO-094	114,411,054	98.01
103650-001-046	BBE-001	81,928,370	97.76
103650-001-047	BBE-017	87,733,270	96.65
103650-001-048	BBE-021	104,996,190	97.70
103650-001-049	BBE-023	88,391,266	97.54
103650-001-050	BBE-028	80,379,380	97.38
103650-001-051	BBE-043	123,662,676	97.63
103650-001-052	BBE-053	81,052,774	98.13
103650-001-053	BBE-079	96,697,812	97.23
103650-001-054	BBE-080	89,275,284	97.50
103650-001-055	BBE-098	79,081,692	97.38
103650-001-056	BBO-007	79,711,864	97.53
103650-001-057	BBO-038	91,284,342	97.27
103650-001-058	BBO-053	99,939,582	97.49
103650-001-059	BBO-058	80,855,640	97.90
103650-001-060	BBO-060	80,017,504	97.40
103650-001-061	BBO-063	91,121,504	97.70
103650-001-062	BBO-074	79,902,188	97.61
103650-001-063	BBO-080	79,890,396	98.28
103650-001-064	BBO-065	122,715,740	97.88
103650-001-065	BBO-066	80,617,726	97.72
103650-001-066	BBO-070	98,784,886	97.65
103650-001-067	BBO-072	81,879,960	98.12
103650-001-068	BBO-077	81,009,706	98.08
103650-001-069	BBO-086	165,874,334	98.09
103650-001-070	BBO-087	81,516,230	97.80
103650-001-071	BBO-090	105,864,940	98.14
103650-001-072	BBO-094	109,429,738	98.18
103650-001-073	BBE-017	81,199,760	97.79
103650-001-074	BBE-021	89,550,670	97.66
103650-001-075	BBE-023	81,142,760	97.45
103650-001-076	BBE-028	83,039,748	97.36

103650-001-077	BBE-043	86,493,406	97.89
103650-001-078	BBE-053	80,517,098	97.82
103650-001-079	BBE-079	62,471,354	96.87
103650-001-080	BBE-098	80,434,098	97.58

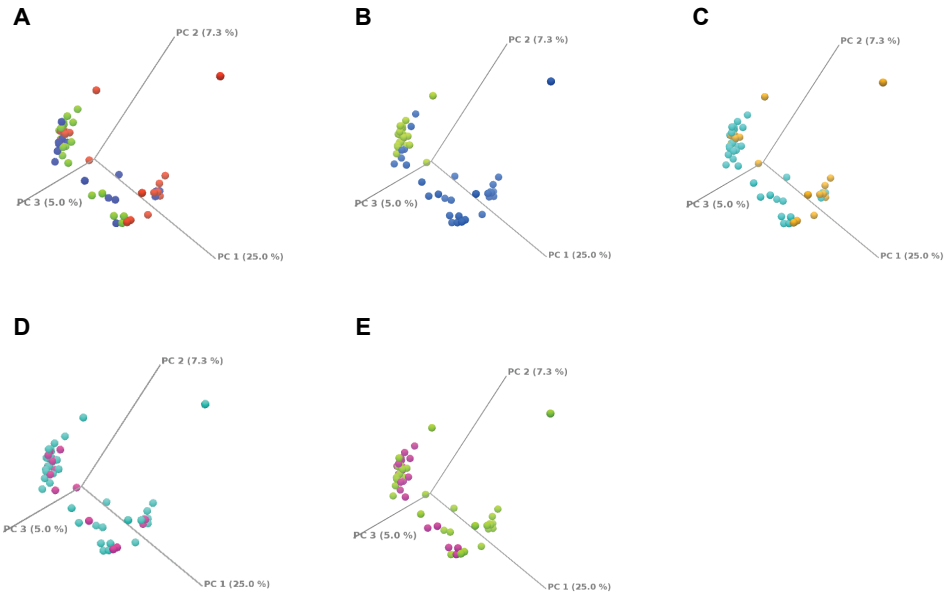
Supplementary Table S4.3. Results of RNA isolation from fibroblast of the acid exposure experiments in terms of quantity and quality, plus a quality report of RNA sequencing data. RIN = RNA integrity number. See Supplementary Table S1.2 for a phenotypical description of these patients.

Sample	Concentration (ng/ul)	RIN	Read count	Mapped to genes (%)
Acid-exposed patient 1	30	7.3	128,303,494	98.93
Acid-exposed patient 2	70	9.2	103,308,260	98.99
Acid-exposed patient 3	30	8.3	87,959,820	98.93
Acid-exposed control 1	17	7.2	80,815,128	99.07
Acid-exposed control 2	66	8.3	80,630,850	99.06
Acid-exposed control 3	48	8.1	155,906,284	98.78
Non-exposed patient 1	239	9.3	101,489,322	94.55
Non-exposed patient 2	206	9.3	162,746,478	94.87
Non-exposed patient 3	141	9.8	121,773,766	94.59
Non-exposed control 1	31	9.0	131,980,322	95.82
Non-exposed control 2	223	9.3	134,581,046	95.67
Non-exposed control 3	173	9.0	124,560,450	95.10

S5. Principle component analysis of gene expression data



Supplementary Figure S5.1. Two-dimensional scatter plot of principal component analysis (PCA), clustered for group. The percentages represent the proportion of variant explained by that specific principal component. Two outliers (BBE-017 and BBE-079) were excluded for further analysis. EA = esophageal atresia.



Supplementary Figure S5.2. Tree-dimensional scatter plot of principal component analysis (PCA), clustered for A = group (esophageal atresia (EA) with Barrett's esophagus (BE); EA only; and BE only), B = origin of biopsy, C = EA in history, D = sex, and E = presence of BE.

S6. Results of differential expression and pathway analysis of esophageal biopsy specimen

Supplementary Table S6.1. Number of significantly differently expressed genes when comparing the different subgroups. Settings: max group mean >2, fold change (FC) <-1.5 or >1.5, false discovery rate (FDR) p-value <0.05. [A] Associated polymorphisms, previously found with genome wide association studies (see Supplementary Material S2) [B] Uploaded to Ingenuity Pathway Analysis. Group A = patients with esophageal atresia (EA) and Barrett's esophagus (BE), group B = patients with EA without BE, group C = patients with BE without EA in history, I = squamous cell epithelium (SQ) samples from group A, II = gastroesophageal junction (GEJ) samples from group A, III = SQ samples from group B, IV = GEJ samples from group B, V = SQ samples from group C, VI = GEJ samples from group C

Comparison	Significant genes (n)	Alternative settings	Overlapping disease and developmental genes [A]
I vs. II	3191		*BMP4, CDX1, CDX2, CFTR, EGFR, FOXF1, GATA6, HOXA13, MYC, PLCE1, SOX2*
I vs. III	20 [B]		-
I vs. IV	911		*BMP4, FOXF1, GATA6*
I vs. V	80 [B]		-
I vs. VI	5617		*BMP4, CCND1, CDX1, CDX2, CFTR, EGFR, FOXF1, GATA6, HOXA13, MYC, PLCE1, SOX2*
II vs. III	4446		*ABCC5, BMP4, CCND1, CDX1, CDX2, CFTR, EGFR, FOXF1, GATA6, HOXA13, MYC, PLCE1, SOX2*
II vs. IV	631 [B]		*CDX1, CDX2, CFTR, HOXA13, SOX2*
II vs. V	3657		*BMP4, CDX1, CDX2, CFTR, FOXF1, GATA6, HOXA13, MYC, PLCE1*
II vs. VI	981	FC <-3 or >3 à n=514 [B]	-
III vs. IV	1677		*ABCC5, BMP4, FOXF1, GATA6*
III vs. V	165		-
III vs. VI	6090		*ABCC5, BMP4, CCND1, CDX1, CDX2, CFTR, EGFR, FOXF1, GATA6, GSTP, HOXA13, MYC, PLCE1, SOX2*
IV vs. V	973	FC <-3 or >3 à n=521 [B]	*BMP4, FOXF1, GATA6*
IV vs. VI	3654		*BMP4, CCND1, CDX1, CDX2, CFTR, EGFR, FOXF1, GSTP, HOXA13, MYC, PLCE1, SOX2*
V vs. VI	5599		*BMP4, CDX1, CDX2, CFTR, FOXF1, GATA6, GSTP, HOXA13, MYC, PLCE1, SOX2*

Supplementary Table S6.2. Canonical pathways, significantly enriched by differentially expressed genes, and corresponding diseases and bio functions, with a significantly increased or decreased activations. Settings: p-value <0.05 (= -log(p-value)>1.3), Z-score <-2 or >2 (only for diseases and bio functions). n=total number of canonical pathways significantly enriched by differentially expressed genes, N/A = not applicable, z-score could not be calculated. Group A = patients with esophageal atresia (EA) and Barrett's esophagus (BE), group C = patients with BE without EA in history, I = squamous cell epithelium (SQ) samples from group A, II = gastroesophageal junction (GEJ) samples from group A, III = SQ samples from group B, IV = GEJ samples from group B, V = SQ samples from group C, VI = GEJ samples from group C. * = involved in oncological processes, # = involved inflammatory processes

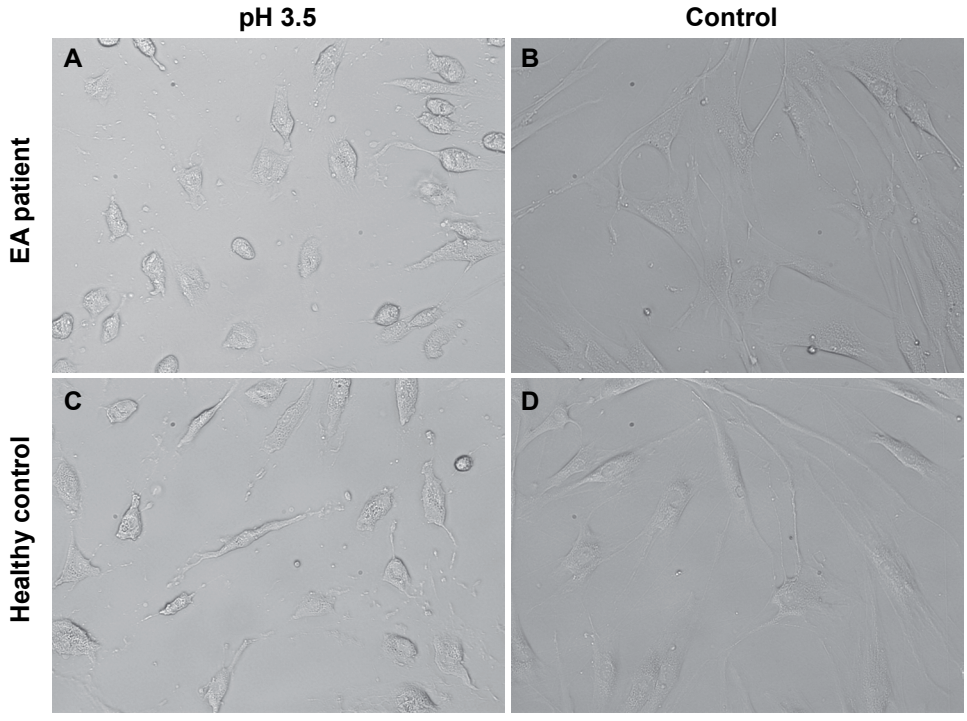
	n=353	-log(p-value)	z-score
Canonical pathways	SPINK1 Pancreatic Cancer Pathway *	15.7	0
	Intrinsic Prothrombin Activation Pathway	7.92	1.897
	Neuroprotective Role of THOP1 in Alzheimer's Disease	6.83	3.207
	Cholecystokinin/Gastrin-mediated Signaling	4.38	2.111
	LXR/RXR Activation *	4.31	-2.111
	Toll-like Receptor Signaling #	3.73	1.89
	p38 MAPK Signaling #	3.72	1.897
	Adrenomedullin signaling pathway	3.56	2.309
	Acute Phase Response Signaling #	3.39	0.632
	IL-6 Signaling #	2.89	1.667
	PPAR Signaling	2.81	-1.414
	Production of Nitric Oxide and Reactive Oxygen Species in Macrophages #	2.68	0.302
	Dendritic Cell Maturation #	2.27	2.333
	Retinoate Biosynthesis I #	2.27	2
	HMGB1 Signaling #	2.09	1.633
	HIPPO signaling *	2.05	-1
	Retinol Biosynthesis #	1.95	-1
	Aryl Hydrocarbon Receptor Signaling *	1.51	-1
	Signaling by Rho Family GTPases #	1.49	2.333
	NF-κB Signaling #	1.45	2.121
	Sphingosine-1-phosphate Signaling # *	1.44	2.236
	Osteoarthritis Pathway #	1.44	-0.378
	Nicotine Degradation II	1.33	2

Diseases and bio functions	Migration of cells	2,00E-10	4.741
	Cell movement	9,38E-10	4.737
	Migration of tumor cell lines	4,85E-07	4.421
	Cell movement of tumor cell lines	6,48E-08	3.832
	Organization of cytoskeleton	3,57E-04	3.733
	Organization of cytoplasm	2,07E-03	3.733
	Invasion of tumor cell lines	6,79E-06	3.658
	Cell movement of blood cells	1,42E-04	3.430
	Leukocyte migration	2,01E-04	3.344
	Cell movement of leukocytes	8,70E-05	3.331
	Chemotaxis	6,96E-05	3.209
	Chemotaxis of leukocytes	1,13E-04	3.108
	Cell movement of myeloid cells	8,49E-04	3.071
	Invasion of cells	2,53E-08	3.057
	Homing of cells	9,80E-05	3.054
	Formation of skin	5,53E-40	3.042
	Chemotaxis of myeloid cells	1,92E-04	2.939
	Inflammatory response	4,46E-07	2.803
	Chemotaxis of phagocytes	1,08E-04	2.690
	Cell movement of breast cancer cell lines	2,56E-04	2.573
	Formation of epidermis	5,06E-24	2.433
	Neoplasia of tumor cell lines	2,69E-05	2.384
	Cell movement of mononuclear leukocytes	1,69E-03	2.280
	Differentiation of epithelial cells	3,23E-19	2.269
	Cell movement of granulocytes	3,01E-05	2.263
	Chemotaxis of granulocytes	3,52E-05	2.260
	Chemotaxis of neutrophils	3,05E-04	2.254
	Cell proliferation of carcinoma cell lines	2,31E-05	2.186
	Advanced malignant tumor	9,25E-04	2.161
	Neoplasia of cells	2,25E-04	2.144
	Activation of phagocytes	2,94E-05	2.119
	Cancer of cells	5,83E-04	2.114
	Differentiation of skin	1,11E-24	2.064
	Metabolism of eicosanoid	5,05E-04	2.026
	Allergy	2,20E-13	2.019
	Weight loss	7,35E-04	-2.030
	Blister	1,71E-04	-2.219
	Apoptosis of skin	3,39E-04	-2.595
	Congenital anomaly of digit	6,28E-06	-2.949
	Limb defect	1,70E-05	-3.110
	Congenital anomaly of limb	2,57E-07	-3.110

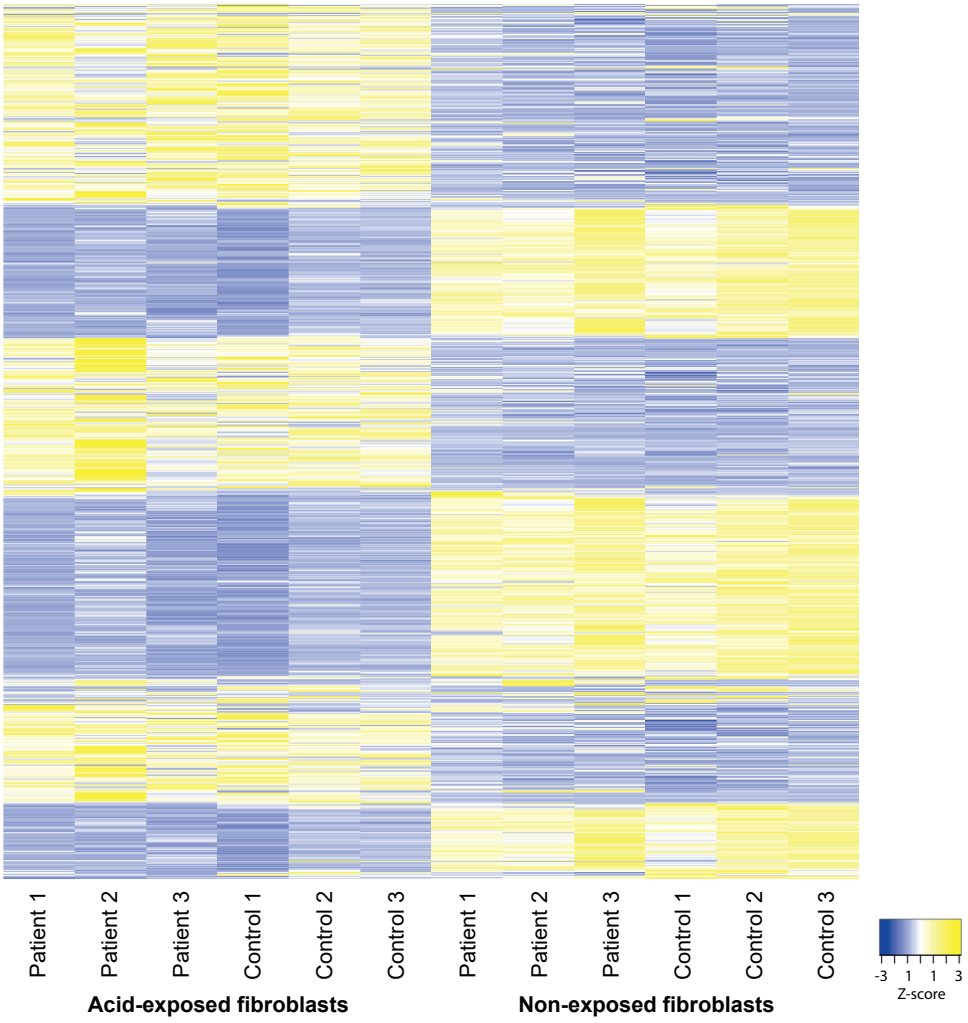
SPINK1 is a potential biomarker for Barrett's esophagus

SPINK1 Pancreatic Cancer Pathway was downregulated in EA/BE patients compared with BE only patients (Z-score=-3, $p<0.0001$). SPINK1 is an enzyme secreted by pancreatic acinar cells, and it is a clinical indicator of malignant disease.[20] The pancreas is derived from the distal foregut, and some of the involved transcription factors during overlap with those of esophageal development.[21] SPINK1 is expressed in the liver, pancreas and the gastrointestinal tract, with increased expression in gastrointestinal tumors[20]. Although it does not seem to be expressed in unaffected esophagus,[22] SPINK1 was expressed in the majority of the patients with highest TPM levels in BE only patients. Of note: SPINK1 is somewhat expressed in the stomach.[22] Biopsies of EA only patients were taken from the gastroesophageal junction, which means that biopsies could contain some stomach tissue as well.

S7. Results of differential expression and pathway analysis of acid-exposed fibroblast cells



Supplementary Figure S7.1. Morphology (20x magnification) of fibroblast cells after 30 minutes exposure to acid Dulbecco's Modified Eagle's Medium with pH 3.5 and control medium, of a patient with esophageal atresia (EA, A and B) and a healthy control (C and D).

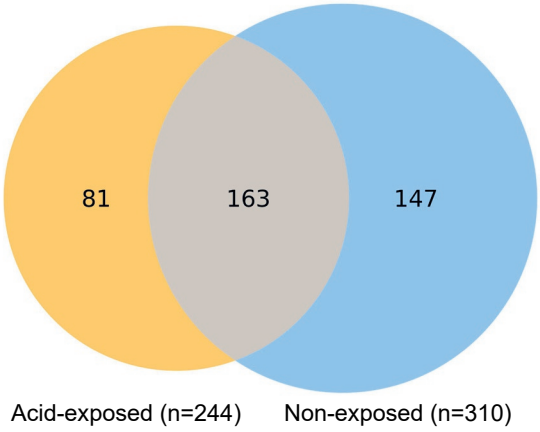


Supplementary Figure S7.2. Heat map of mean transcript per million (TPM) for fibroblast cells after the in vitro experiment, for a selected gene panel (n=2344). We evaluated all genes (n=2344) of the enriched pathways between GEJ samples of EA/BE patients and GEJ samples of BE only patients. For these 2344 genes, there was a clear difference between upregulated and downregulated genes in fibroblasts after acid exposure, in both EA patients and healthy controls. Gene panel was extracted from the significantly enriched pathways between gastroesophageal junction (GEJ) samples of group A (esophageal atresia (EA) with Barrett's esophagus (BE)) and GEJ samples of group C (BE only), see Supplementary Table S6.4. Settings: maximum group mean >2, fold change <-1.5 or >1.5, false discovery rate p-value <0.05, hierarchical clustering by average linkage, distance between rows and columns by Eucledian method.

Supplementary Table S7.3. Canonical pathways, enriched by differentially expressed genes. [A] Settings: p-value <0.05 (=-log(p-value)>1.3), z-score <-2 or >2. [B] Settings: p-value <0.05 (=-log(p-value)>1.3), top 10 pathways presented. n=total number of canonical pathways significantly enriched by differentially expressed genes. N/A = not applicable, z-score could not be calculated.

	Canonical Pathways	-log(p-value)	z-score
Acid-exposed vs. non-exposed (all samples) (n=578) [A]	EIF2 Signaling	43.3	5.338
	Coronavirus Pathogenesis Pathway	19	-2.722
	Oxidative Phosphorylation	15.8	5.642
	Kinetochore Metaphase Signaling Pathway	14.8	-3.452
	Sirtuin Signaling Pathway	9.8	-3
	Cell Cycle: G2/M DNA Damage Checkpoint Regulation	7.99	2.982
	Unfolded protein response	6.84	2.183
	NRF2-mediated Oxidative Stress Response	6.51	2.683
	IL-6 Signaling	5.02	2.117
	ILK Signaling	3.41	3.413
	RAN Signaling	3.17	-2.646
	Death Receptor Signaling	2.89	-2.065
	Hypoxia Signaling in the Cardiovascular System	2.74	2.646
	BAG2 Signaling Pathway	264	2.111
	PPAR Signaling	2.57	-2.524
	PCP pathway	2.33	2.309
	Cell Cycle Control of Chromosomal Replication	2.15	-3.464
	IL-17 Signaling	2.07	3.157
	Role of PKR in Interferon Induction and Antiviral Response	1.98	-2.236
	Inhibition of ARE-Mediated mRNA Degradation Pathway	1.83	3.130
	MIF Regulation of Innate Immunity	1.74	2.333
	Angiopoietin Signaling	1.53	-2.121
	Regulation Of The Epithelial Mesenchymal Transition In Development Pathway	1.48	2.496
Patients vs. controls (acid-exposed) (n=258) [B]	Dendritic Cell Maturation	4.6	-0.707
	Type I Diabetes Mellitus Signaling	4.09	N/A
	T Helper Cell Differentiation	3.97	N/A
	Role of Macrophages, Fibroblasts and Endothelial Cells in Rheumatoid Arthritis	3.71	N/A
	Hepatic Fibrosis / Hepatic Stellate Cell Activation	3.7	N/A
	Graft-versus-Host Disease Signaling	3.6	N/A
	Role of Osteoblasts, Osteoclasts and Chondrocytes in Rheumatoid Arthritis	3.28	N/A
	PD-1, PD-L1 cancer immunotherapy pathway	3.22	1.342
	Axonal Guidance Signaling	2.88	N/A
	Coagulation System	2.84	N/A

Patients vs. controls (non-exposed) (n=314)[B]	Osteoarthritis Pathway	6.98	-1.265
	Hepatic Fibrosis / Hepatic Stellate Cell Activation	5.99	N/A
	Tumor Microenvironment Pathway	5.34	0
	Axonal Guidance Signaling	4.96	N/A
	HOTAIR Regulatory Pathway	4.82	-1
	Role of Osteoblasts, Osteoclasts and Chondrocytes in Rheumatoid Arthritis	4.53	N/A
	Colorectal Cancer Metastasis Signaling	3.99	-0.333
	Role of Macrophages, Fibroblasts and Endothelial Cells in Rheumatoid Arthritis	3.87	N/A
	Caveolar-mediated Endocytosis Signaling	3.29	N/A
	HIF1α Signaling	3.26	-0.707

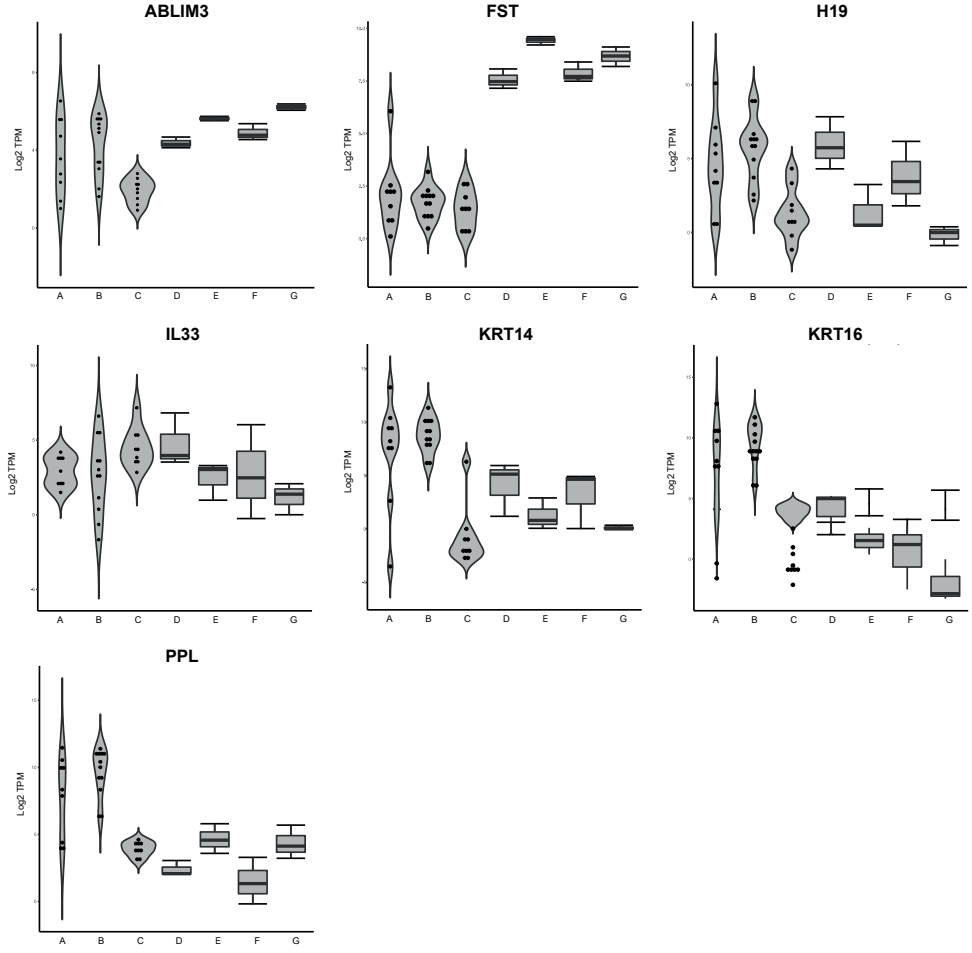


Supplementary Figure S7.4. Number of differentially expressed genes between patients with EA and healthy controls after acid-exposure (yellow, n=244), and number of differentially expressed genes between patients and controls without exposure (blue, n=310). This leaves 81 genes that were differentially expressed between patients and controls after acid exposure but NOT without exposure.

Supplementary Table S7.5. Canonical pathways, significantly enriched by differentially expressed genes. Settings: p-value <0.05 (=-log(p-value)>1.3), n=total number of canonical pathways significantly enriched by differentially expressed genes. N/A = not applicable, z-score could not be calculated. * = involved in oncological processes, # = involved inflammatory processes

Canonical Pathways (n=173)	-log(p-value)	z-score
Communication between Innate and Adaptive Immune Cells #	4.85	N/A
Graft-versus-Host Disease Signaling #	4.77	N/A
Dendritic Cell Maturation #	4.56	0.816
LXR/RXR Activation *	4.36	-2.236
Role of Hypercytokinemia/hyperchemokinemia in the Pathogenesis of Influenza #	3.77	0
Altered T Cell and B Cell Signaling in Rheumatoid Arthritis #	3.70	N/A
Hepatic Fibrosis Signaling Pathway	3.69	1.89
Granulocyte Adhesion and Diapedesis #	3.63	N/A
PPAR Signaling	3.44	-2
Airway Pathology in Chronic Obstructive Pulmonary Disease	3.25	N/A
IL-6 Signaling #	3.14	2
Atherosclerosis Signaling	3.13	N/A
LPS/IL-1 Mediated Inhibition of RXR Function #	3.11	N/A
IL-10 Signaling #	2.83	N/A
T Helper Cell Differentiation #	2.78	N/A
TR/RXR Activation #	2.60	N/A
Hepatic Cholestasis	2.52	N/A
Hepatic Fibrosis / Hepatic Stellate Cell Activation	2.52	N/A
PD-1, PD-L1 cancer immunotherapy pathway *	2.32	N/A
Role of Osteoblasts, Osteoclasts and Chondrocytes in Rheumatoid Arthritis	2.28	N/A
Type I Diabetes Mellitus Signaling	2.26	N/A
Coagulation System	2.25	N/A
B Cell Development #	2.23	N/A
p38 MAPK Signaling #	2.19	N/A
Antigen Presentation Pathway #	2.16	N/A
FXR/RXR Activation # *	2.11	N/A
Intrinsic Prothrombin Activation Pathway	2.10	N/A
Th2 Pathway #	2.02	N/A
Autoimmune Thyroid Disease Signaling #	1.97	N/A
Role of Cytokines in Mediating Communication between Immune Cells #	1.89	N/A
Neuroinflammation Signaling Pathway #	1.80	-2
HMGB1 Signaling #	1.80	N/A
Th1 and Th2 Activation Pathway #	1.76	N/A
Role of Macrophages, Fibroblasts and Endothelial Cells in Rheumatoid Arthritis	1.74	N/A
Calcium-induced T Lymphocyte Apoptosis # *	1.72	N/A
NF-κB Signaling #	1.70	N/A
Acute Phase Response Signaling #	1.70	N/A
Role of NFAT in Regulation of the Immune Response #	1.69	N/A
Axonal Guidance Signaling	1.68	N/A
Regulation Of The Epithelial Mesenchymal Transition By Growth Factors Pathway *	1.65	N/A
Clathrin-mediated Endocytosis Signaling *	1.62	N/A
Agranulocyte Adhesion and Diapedesis #	1.62	N/A
TREM1 Signaling #	1.62	N/A
Toll-like Receptor Signaling #	1.61	N/A
BEX2 Signaling Pathway *	1.58	N/A
Pathogenesis of Multiple Sclerosis	1.55	N/A
FGF Signaling *	1.53	N/A

IL-4 Signaling #	1.52	N/A
Allograft Rejection Signaling #	1.51	N/A
Crosstalk between Dendritic Cells and Natural Killer Cells #	1.48	N/A
Osteoarthritis Pathway #	1.47	N/A
OX40 Signaling Pathway #	1.47	N/A
Bladder Cancer Signaling *	1.41	N/A
Bile Acid Biosynthesis, Neutral Pathway	1.39	N/A
iCOS-iCOSL Signaling in T Helper Cells #	1.31	N/A



Supplementary Figure S7.6. Violin plots of gastroesophageal (GEJ) samples (left) and box plots of acid-exposed fibroblasts (right). Of the overlapping enriched pathways of GEJ samples of patients with esophageal atresia (EA) who have developed Barrett's esophagus (BE) compared to BE only patients and acid-exposed fibroblasts of patients compared to controls, seven genes were differentially expressed in both GEJ samples and in fibroblasts. A = EA/BE patients, B = EA only patients, C = BE only, D = EA patients (acid-exposed), E = controls (acid-exposed), F = EA patients (non-exposed), G = Controls (non-exposed). TPM = transcripts per million.

SUPPLEMENTARY METHODS

SM1: Sample extraction protocol and storage

All materials used in this study were retrieved from the Biobank Esophageal Atresia (MEC-2015-645) and the Biobank Barrett (MEC-2010-094), in which samples have been stored after written informed consent. During surveillance endoscopies, 2x2 mucosal biopsies were taken from two esophageal sites, one for histological evaluation and one for RNA extraction. The first from the unaffected esophageal squamous cell epithelium (SQ). In patients with EA this biopsy was taken above the original anastomosis. The second set of biopsies was taken from the GEJ or, if present, from Barrett's mucosa (see Figure 1 in main manuscript). All biopsies either have been snap frozen in liquid nitrogen directly and stored at -80°C, or have been transferred in a RNAlater™ Stabilization Solution (Thermo Fisher Scientific, Waltham, USA) overnight at 4°C, after which the RNAlater was removed and the biopsies were stored at -80°C. EDTA blood samples have been collected and stored at -20°C before extraction of genomic DNA.

SM2: RNA and DNA isolation

DNA was extracted from peripheral blood and fibroblasts using the DNA Mini Kit (Qiagen, Venlo, the Netherlands). DNA quality and quantity was determined with the Thermo Scientific Nano Drop 2000 (ThermoFisher Scientific Inc., Waltham, USA) and Quant-iT™ PicoGreen® dsDNA kit (Invitrogen, Carlsbad, CA, USA). Total RNA was isolated from the biopsies and fibroblasts using the AllPrep DNA/RNA Mini Kit (Qiagen, Venlo, the Netherlands) or the RNeasy Mini Kit (Qiagen, Venlo, the Netherlands) or the E.Z.N.A.® Total RNA Kit I (Omega Bio-tek Inc., Norcross, Georgia, USA), and stored at-80°C. The quantity and quality of the RNA was determined with the Lab-on-Chip RNA 6000 Nano (Agilent Technologies, Santa Clara, USA). Samples with an RNA integrity number (RIN) >6 were prepared and processed for RNA sequencing.

SM3: SNP genotyping

A total of 200 ng dsDNA was used for single nucleotide polymorphism (SNP) array genotyping analysis using the Infinium Global Screening Array v1.0 or v3.0 (Illumina, Inc. San Diego, USA), according to the manufacturer's standard protocol. Output was generated using Illumina Genome studio v2.0 (Illumina, San Diego, CA, USA). Using SNP-array, we also evaluated DNA copy number variations (CNV) profiles of the biopsies of EA/BE patients and BE patients, if sufficient amounts and quality of DNA was present. We determined genomic stability (the presence or absence of large de novo gains or losses) by inspecting these visually in Biodiscovery Nexus CN10.0 (Biodiscovery Inc., Hawthorne, CA, USA) and comparing them to their germline counterpart. Predisposition loci (and corresponding lead or proxy SNPs) associated with BE, EAC and/or ESCC were derived from the literature (see Supplementary Material S2.[4-19] We used SNP array genotyping data from patients of group A (EA/BE, n=19),

patients of group B (EA only, n=44) and patients of group C (BE only, n=10) to see if previously BE associated SNPs and/or haplotypes were more prevalent in patients with EA and BE. If the associated SNP was not present on the genotyping platform, a proxy was selected using the LD proxy and LD pair Tool (https://ldlink.nci.nih.gov), with a cut-off level of D'>0.9 and R'>0.6. We compared these genotypes to unaffected controls (n=730), sequenced on the same platform as our patients, with a chi-square test and calculated odds ratios (ORs). Admixture was used to infer ancestry.[23] We used the allele counts and published ORs of the associated SNPs to calculate a polygenic risk score (PGRS) using an additive model: PGRS=$\sum$*Ln (OR risk allele)*allele count*.[24] If multiple studies published an OR for a SNP, the OR from the study with the largest sample size was included in the PGRS. In a second calculation, we used the ORs of the associated SNPs as calculated from our study population. A Kruskal-Wallis test and Mann-Whitney tests were used to compare the PGRS between the different groups. All statistical analyses were performed in SPSS V.25.0 (IBM, Chicago, Illinois, USA), with a significance level of $p<0.05$.

SM4: RNA sequencing

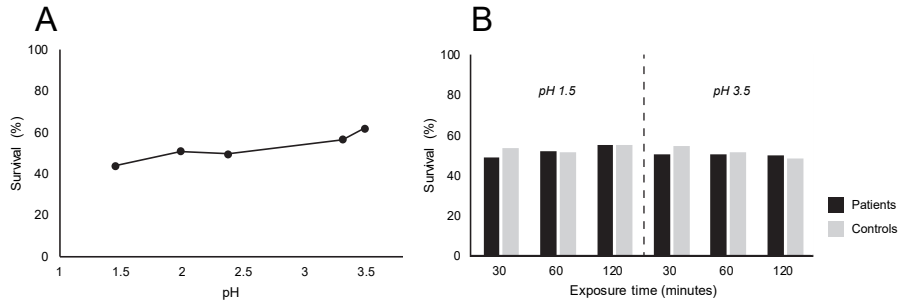
First, strand cDNA libraries were made with the strand-specific NEBNext Ultra II Directional RNA Library Prep Kit protocol and polyA mRNA workflow (NEB #E7760S/L) on an Illumina NovaSeq6000 (Illumina, San Diego, USA). Quality control, read trimming, read alignment, transcript quantification and differential expression analysis were performed using CLC Genomics Workbench version 20 (Qiagen, Venlo, The Netherlands). Reads were aligned to the human reference genome (hg19) according to the following settings: mismatch cost 2, insertion/deletion cost 3, length fraction 0.8, similarity fraction 0.8, alignment to gene regions only. Paired reads were counted as one. Trimmed mean per million (TMM) values was used to normalize for sequencing depth across samples. For each gene, counts per million (CPM) and transcripts per million (TPM) were calculated. Read counts were normalized for transcript length and total number of mapped reads (RPKM). Principal component analysis (PCA) was performed to explore cluster separation and identify outlying samples.[25] Counts for each individual gene were transformed to a smaller set of orthogonal principal components, in which the first component specifies the direction with the largest variability in the data. Two-dimensional and three-dimensional plots are produced. For each gene, log CPM values and a Z-score were calculated using all samples. Our ethics committee does not allow sharing of individual patient or control genotype information in the public domain, including sequencing reads.

SM5: Acid exposure experiments

To study the effect of GER on RNA level, we simulated a reflux episode in an in vitro experiment (see Figure 1 in main manuscript). The pH level of the stomach normally varies between 1.0 and 3.5.[26] We evaluated the survival of fibroblasts in medium with a pH level between pH 1.5 and 3.5. All experiments were performed in duplo. Survival rate differences were determined

using a paired t-test or a one-way analysis of variance (ANOVA). Next, comparison of an exposure time of 30, 60 and 120 minutes in both patient and control cell lines using these pH levels did not show a significant difference (see Figure and Table SM5.1). Since a reflux episode is usually several minutes, we continued with an exposure time of 30 minutes.

For the final experiment, we exposed human fibroblasts from three patients with EA and three healthy controls for 30 minutes to medium with a pH level of 3.5 or to normal medium (control). Human fibroblasts were cultured in DMEM (Dulbecco's Modified Eagle's Medium; Invitrogen, Carlsbad, CA, USA), supplemented with 10% fetal calf serum (FCS) and 1% penicillin/streptomycin at 37°C under a humidified 5% CO2 atmosphere. Hydrochloric acid was added to culture medium until the desired pH level was reached. We selected an exposure time of 30 minutes to pH 3.5 adjusted medium. Subsequently, cells were washed with phosphate buffered saline (PBS) and given standard medium. After 24 hours, survival was measured with the TC20™ Automated Cell Counter (Bio-Rad Laboratories B.V., Veenendaal, The Netherlands). Cell morphology was evaluated with the Olympus IX70-S8F Inverted Fluorescence Microscope (Olympus Corporation, Tokyo, Japan).



Supplementary Figure and Table SM5.1. Overview of survival rates of fibroblast cells after exposure to pH adjusted medium. * = 30 minutes. Experiment A are the pooled results of a duplo experiment on three control cell lines. Experiment B are the pooled results of a duplo experiment on three patient cell lines and three control cell lines.

Experiment A		**Experiment B**		
Exposure * (pH)	**Survival (%)**	**Exposure (pH – minutes)**	**Survival (%)**	
	Controls		*Patients*	*Controls*
1.46	43.9	1.47 – 30	48.8	53.8
1.99	50.9	1.47 – 60	52.3	51.5
2.38	49.7	1.47 – 120	55.1	55.2
3.31	56.7	3.46 – 30	50.4	54.5
3.49	62.0	3.46 – 30	50.7	51.6
		3.46 – 30	50.0	48.7
		7.70- 30	80.0	76.7

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MANAGEMENT AND INTERVENTIONS

CHAPTER 4

An international survey on anastomotic stricture management after esophageal atresia repair: considerations and advisory statements

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ABSTRACT

Background

Endoscopic dilatation is the first-line treatment of stricture formation after esophageal atresia (EA) repair. However, there is no consensus on how to perform these dilatation procedures which may lead to a large variation between centers, countries and doctor's experience. This is the first cross-sectional study to provide an overview on differences in endoscopic dilatation treatment of pediatric anastomotic strictures worldwide.

Methods

An online questionnaire was sent to members of five pediatric medical networks, experienced in treating anastomotic strictures in children with EA. The main outcome was the difference in endoscopic dilatation procedures in various centers worldwide, including technical details, dilatation approach (routine or only in symptomatic patients), and adjuvant treatment options. Descriptive statistics were performed with SPSS.

Results

Responses from 115 centers from 32 countries worldwide were analyzed. The preferred approach was balloon dilatation (68%) with a guidewire (66%), performed by a pediatric gastroenterologist (n=103) or pediatric surgeon (n=48) in symptomatic patients (68%). In most centers, hydrostatic pressure was used for balloon dilatation. The insufflation duration was standardized in 59 centers with a median duration of 60 (range 5-300) seconds. The preferred first-line adjunctive treatments in case of recurrent strictures were intralesional steroids and topical mitomycin C, in respectively 47% and 31% of the centers.

Conclusions

We found a large variation in stricture management in children with EA, which confirms the current lack of consensus. International networks for rare diseases are required for harmonizing and comparing the procedures, for which we give several suggestions.

INTRODUCTION

Despite improved treatment strategies, up to 60% of children with esophageal atresia (EA) develop an anastomotic stricture after surgical correction, mostly in the first year of life.[1] Based on a recent guideline for the management of complications in children with EA of the European Society for Pediatric Gastroenterology, Hepatology and Nutrition (ESPGHAN) and North American Society for Pediatric Gastroenterology, Hepatology and Nutrition (NASPGHAN), the first-line treatment for anastomotic strictures is endoscopic dilatation under general anesthesia.[2]

Currently, there is no consensus on how dilatation should be performed.[3] Two different methods of endoscopic dilatation are used: balloon dilatation and semi-rigid dilatation, i.e., bougienage. The primary goals of esophageal dilatation are symptom relief, maintenance of age-appropriate oral nutrition, and reduction of pulmonary aspiration risk. Balloon dilatation applies a radial force over the entire length of the esophageal stricture, while bougies generate shearing axial forces as they pass across the stenosis (see Figure 1).[4] Currently, there are no randomized controlled trials comparing the efficacy and safety of balloon dilatation and bougienage for the treatment of anastomotic strictures in children with EA. Data from published studies on pediatric esophageal strictures of varying etiology show conflicting results.[5–7] A recent meta-analysis included five randomized controlled trials that have compared the two techniques in adults with benign esophageal strictures; the results indicated no significant differences in efficacy and safety.[8–13]

Due to the lack of strong evidence, the choice of dilatation method is currently based on the center's and operator's experience and preference. To come to consensus and guidelines, it is important to know how management dilatation strategies are currently applied in different centers. Therefore, we performed a survey study with the aim to provide an overview on differences in endoscopic dilatation treatment of pediatric anastomotic strictures worldwide.

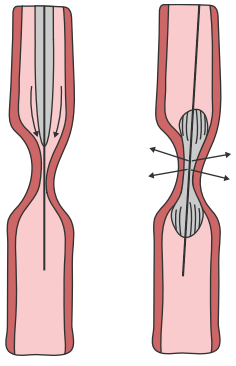

Figure 1. Bougienage (left) creates axial forces; balloon dilatation (right) creates mainly radial forces, as shown by the arrows.

PARTICIPANTS AND METHODS

We conducted a cross-sectional survey study from November 2018 up to and including March 2019. The study was approved by the institutional review board of the Erasmus University Medical Center Rotterdam, the Netherlands (MEC-2018-1463). A free-access online questionnaire (LimeSurvey GmbH version 2.06lts, Hamburg, Germany) consisting of 38 questions in the English language was distributed via e-mail and newsletters to all members of the ESPGHAN EA Working Group, the NASPGHAN, the Australian Society of Paediatric Gastroenterology Hepatology and Nutrition (AuSPGHAN), the European Pediatric Surgeons' Association (EUPSA), and the International Network of Esophageal Atresia (INoEA). Distribution via other pediatric associations was not possible since we were not able to initiate collaboration. Members of these associations were asked to forward the online questionnaire to colleagues in the field. A reminder was sent one month after initial contact.

Participants could be of any specialty, as long as they had experience in the treatment of anastomotic strictures in children with EA. In case of multiple responses per center, responses were pooled to an average. If no average could be calculated, we included the most comprehensive answer. If less than 80% of the questions was answered, the questionnaire was excluded from the analysis.

A draft questionnaire was made based on both literature and expert opinion from the ESPGHAN EA Working Group meeting in Geneva on May 2018. This draft was reviewed and approved by the EUPSA Network Office. All members of the research team were invited to comment on the draft version; comments were accounted for in the final version. In brief, the survey questions concerned: the center the respondent was working at, the number of dilatation procedures performed in the center per month, the preferred dilatation technique (balloon dilatation or bougienage) and the use of alternative or adjuvant treatment options for recurrent strictures. The complete questionnaire can be found in Supplementary File 1.

The main outcome was the difference in endoscopic dilatation treatment of stricture formation after EA repair in various centers worldwide, including technical details (e.g., insufflation material and duration, use of a guidewire or fluoroscope), dilatation approach and adjuvant treatment options. Descriptive statistics were applied to the data. Answers were mainly categorical. Data are represented as number (%). All data was analyzed using SPSS V.24.0 (IBM, Chicago, Illinois, USA). The CHERRIES (Checklist for Reporting Results of Internet E-Surveys) checklist was used as a reporting framework.[14]

RESULTS

Response

In total, 232 questionnaires were filled out. Since the initial recipients had been asked to forward the questionnaire to other clinicians, a response rate could not be calculated. Responses came from 32 countries worldwide (see Figure 2). Sixty percent of the responses came from European countries, 24.3% from North American countries and 15.7% from other continents. After excluding 103 incomplete questionnaires with <80% of the questions answered and pooling 25 duplicate responses of 11 centers, data from 115 centers remained for analysis (see Figure 3). The majority of the centers (87.8%) were academic centers. The majority of the responses came from departments of Pediatric Gastroenterology (n=57) and Pediatric Surgery (n=45).

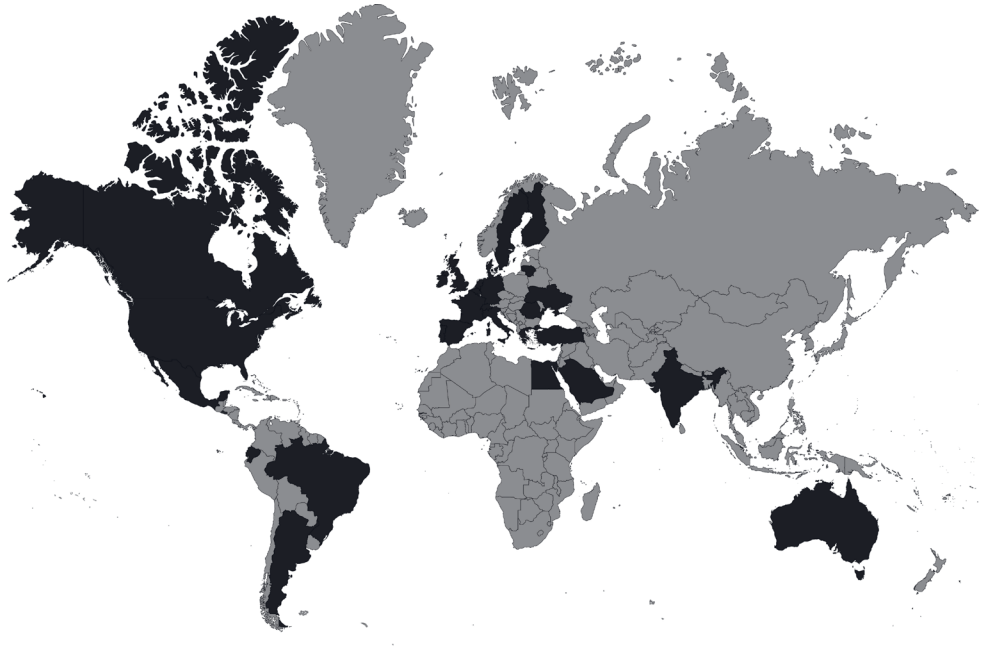

Figure 2. Participating centers (n=115 in dark grey) in 32 countries spread over six continents. Figure created with: https://www.amcharts.com/visited_countries/.

Physicians performing endoscopies

The majority of the centers performed 10-30 pediatric endoscopies per month, but less than five pediatric esophageal dilatation procedures per month. Half of the centers performed less than three dilatation procedures for anastomotic strictures in patients with EA per month. All center demographics are listed in Table 1.

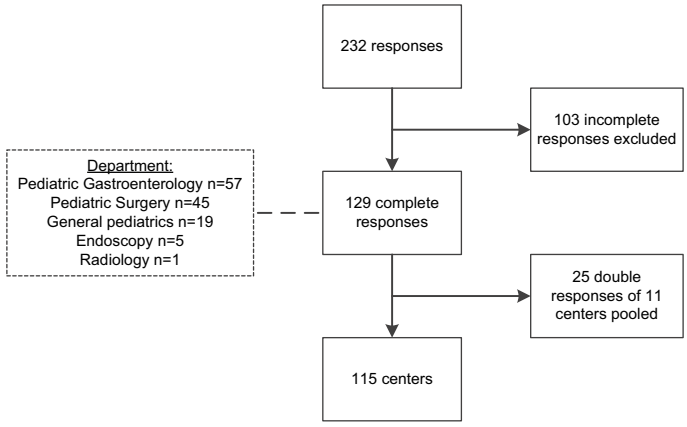

Figure 3. Flowchart of the responses.

Approach for anastomotic strictures

Seventy-eight centers (68.4%) performed selective dilatations, meaning they performed a dilatation procedure only in symptomatic patients. In 36 centers (31.6%) routine dilatations were performed to prevent symptoms to occur; these centers planned subsequent dilatations in advance. Balloon dilatation was the preferred technique to treat anastomotic strictures in patients with EA in 78 centers (67.8%). Twenty centers (17.4%) preferred semi-rigid dilatation or bougienage; seventeen centers (14.8%) applied both techniques. In total, balloon dilatation was applied in 95 centers – regardless if it was the preferred technique or not. In 88 of those 95 centers, this was done endoscopically. Twenty-nine of those 95 centers used a radiologically guided approach; sixty-three centers routinely used a guidewire. See also Figure 4.

Balloon and semi-rigid dilatation

For balloon dilatation, the Controlled Radial Expansion (CRE) balloon dilatator from Boston Scientific™ was used most often (n=66). Alternatively, twenty-two centers used the Rigiflex dilatator (Boston Scientific™), eight centers used the Ultra-Thin Diamond dilatator (Boston Scientific™), and six centers used the Maxforce (Boston Scientific™). The Hercules dilatator (Cook Medical®) and the Percutaneous Transluminal Valvuloplasty Balloon Catheter (VACS®, B. Braun Medical B.V.) were the least used, in two and one centers, respectively.

Forty-nine centers used water or 0.9% natrium chloride to insufflate the balloon, forty-six centers used contrast fluid, and 16 centers used air. Some centers used multiple types of insufflation material since they also used multiple types of dilators. The design of the questionnaire did not permit correlating the type of insufflation with each type of dilatator. The insufflation duration was standardized in 59 centers (51.3%). Across the centers, the median insufflation duration was 60 seconds (range 5-300).

Table 1. Demographics of the participating centers (n=115). EA = esophageal atresia. [a] Both diagnostic and therapeutic, in all pediatric patients. [b] In all pediatric patients

Characteristic	n (%)
Continent	
Europe	69 (60)
North America	28 (24.3)
South America	6 (5.2)
Africa	5 (4.3)
Asia	4 (3.5)
Oceania	3 (2.6)
Total number of pediatric upper endoscopies [a] (per month)	
<10	8 (7.0)
10-30	51 (44.3)
31-50	25 (21.7)
51-70	9 (7.8)
>70	22 (19.1)
Number of pediatric esophageal dilatation procedures [b] (per month)	
<5	61 (53.0)
5-10	33 (28.7)
11-15	6 (5.2)
16-20	2 (1.7)
>20	5 (4.3)
Unknown	8 (7.0)
Number of patients with EA <18 years under follow-up	
<20	27 (23.5)
20-40-	34 (29.6)
41-60	14 (12.2)
61-80	12 (10.4)
81-100	4 (3.5)
>100	21 (18.3)
Unknown	3 (2.6)
Number of dilatation procedures for anastomotic strictures in patients with EA (per month)	
<3	60 (52.2)
3-5	34 (29.6)
6-7	9 (7.8)
8-10	3 (2.6)
>10	3 (2.6)
Unknown	6 (5.2)

Endoscopies were most frequently performed by pediatric gastroenterologists (n=103) and pediatric surgeons (n=48), and less often by adult gastroenterologists (n=24) or adult surgeons (n=12). Two centers had employed a specialized pediatric endoscopist. In 85 of the 101 academic centers (84.2%) trainees performed endoscopies as well.

For bougienage, twenty-seven centers used the Savary-Gilliard dilatator (Cook Medical®). Other semi-rigid dilatators used were the Tucker dilatator (Teleflex®) in seven centers, the Maloney dilatator (Pillings®) in seven centers, the American Dilatation System dilatator (Bard™) in three centers, the Rehbein dilatator (Rush®) in one center, and the Hurst dilatator (Pillings®) in one center.

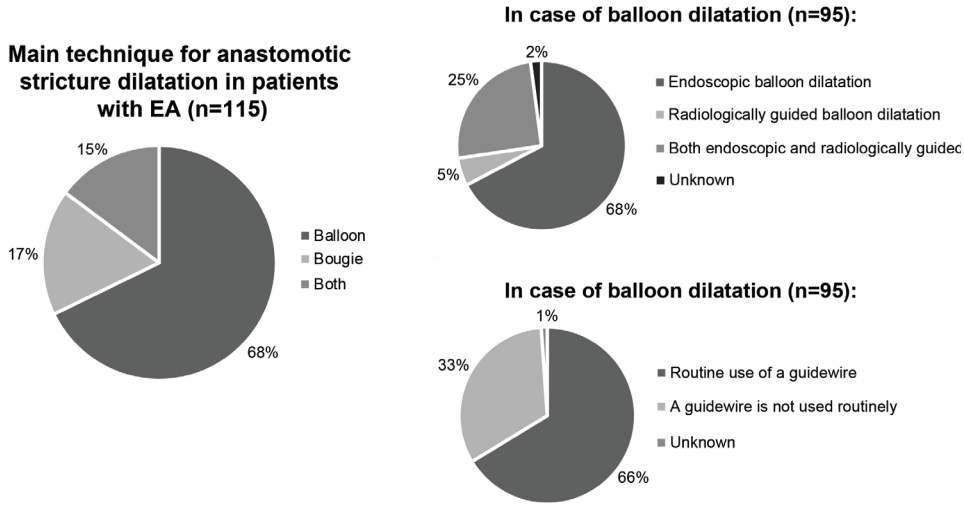

Figure 4. The mainly used techniques to manage esophageal anastomotic strictures in patients with esophageal atresia (EA).

None of the centers had a well-designed protocol to determine which diameter of the dilatator should be used nor to which diameter should be dilated. Eleven centers had set choices, but with specifications like “based on the age of the patient”, “if resistance is felt” and “we progress depending on the situation”.

Recurrent and refractory strictures

Most centers had different adjuvant treatment options available for recurrent and refractory anastomotic strictures. Local injection with steroids was available in 77 centers, topical application of mitomycin in 66, esophageal stenting in 41, and incisional therapy in 30 centers. In 10 centers, other treatment options were available to treat refractory strictures: four centers would prescribe anti-reflux medication or advise fundoplication surgery; four centers would reoperate and perform a resection with a new anastomosis; one center would prescribe budesonide oral gel; and one center would inject vitamin B into the stenosis. Indwelling balloon catheter is a method described in literature[15] but none of the participating centers in this survey mentioned to practice this option. Overall, the majority preferred local injection of steroids (56 centers, 47.1%) or topical application of mitomycin C (37 centers, 31.1%) as first-line adjuvant treatment for a refractory stricture.

DISCUSSION

The aim of this survey study was to provide insight in the differences in endoscopic dilatation methods used for stricture formation after EA repair worldwide. The results show a great

variation in the way dilatation procedures are performed. Overall, the preferred technique was balloon dilatation with a selective approach; i.e., performed only in symptomatic patients.

According to this survey, pediatric gastroenterologists perform the majority of the endoscopies, followed by pediatric surgeons. The literature contains no studies comparing the success rates of dilatation of anastomotic strictures by different specialists. Generally, it is acknowledged that these procedures are the safest and most effective when performed by a skilled and experienced operator.[2] In this age of patient-centered care, one could raise the question of whether there is a place for adult specialists in the treatment of pediatric patients with rare diseases. In this regard, it is our opinion that dilatation of anastomotic strictures in children with EA should be executed by a pediatric gastroenterologist or pediatric surgeon with experience in the management of this population. Smaller centers, where a pediatric gastroenterologist or pediatric surgeon is not available, should refer these children to a nearby expert center. The fact that almost 90% of the centers in this survey were academic centers indicates that this may already be common practice.

The majority of the surveyed centers preferred a selective approach; i.e., dilate an anastomotic stricture only in symptomatic patients. The idea behind this "wait and see" approach is to reduce the number of dilatations, and consequently the exposure to anesthesia and possible complications of a dilatation. Anesthetic exposure at young age is associated with gross motor problems, learning disabilities, behavioral problems, and developmental disorders.[16–19] On the other hand, proponents of routine dilatations advocate that complex strictures – and therefore long-term functional problems – can be prevented by preserving a minimum diameter.

Two retrospective studies have compared selective dilatations with routine dilatations.[20, 21] Selective dilatations were associated with significantly fewer dilatations and a significantly shorter hospital stay than routine dilatations. Occurrences of dysphagia, respiratory complaints, and bolus obstruction did not significantly differ between the two approaches.

The ESPGHAN-NASPGHAN guideline recommends close follow-up during the first 2 years of life, with special attention to the first introduction of solid food. This holds as well for patients with a long gap EA and/or postoperative anastomotic leakage, which are risk factors for stricture development.[1] However, since there is no evidence supporting the more invasive strategy of routine dilatations, the expert opinion in this guideline states that the presence of an anastomotic stricture should be excluded and treated in symptomatic children only.[2]

Two-third of the centers preferred balloon dilatation to bougienage to manage anastomotic strictures in patients with EA. Where bougienage as therapy for esophageal strictures has been reported for almost 200 years, balloon dilatation – introduced in 1981 – is relatively

new.[22, 23] As mentioned earlier, the main difference between the techniques is the type of forces applied to the stricture. Balloon dilatators create radial forces and allow for a consistent treatment when the balloon is insufflated according to a standardized protocol. Bougies exert axial forces, which the operator can adjust as he or she considers necessary.

Literature comparing the two techniques is scarce and with divergent results. Some retrospective studies in both children and adults with a variety of esophageal strictures reported no differences in safety, effectiveness, and complications.[11, 24, 25] Other studies in children found favorable results for balloon dilatation. For example, significantly fewer dilatations required[5] and significantly fewer technical failures, defined as no passage possible through the stenosis.[6] On the other hand, a study in 47 children with congenital esophageal stenosis found a significantly lower perforation rate for bougienage than for balloon dilatation.[7] Two randomized controlled trials in adults with dysphagia due to benign esophageal strictures found no differences between the two techniques except less discomfort during balloon dilatation ($p<0.05$) – which in adults usually is performed in awake or lightly sedated state.[8, 9] Prospective comparative studies in children are lacking.

The most used balloon dilatator in the surveyed centers was the CRE balloon dilatator from Boston Scientific™. All balloon dilatators reported in the survey were through-the-scope dilatators, enabling direct vision during the procedure when being used with a medium-sized scope like for example the Olympus Q180, which has an instrumental channel with a diameter of 2.8 mm. These dilatators are designed to pass the scope without the use of a guidewire. A guidewire is still included; however, in the CRE and Rigiflex dilatators, so they can also be used in combination with a small-sized scope (for example the Olympus GIF-XP190 with an instrumental channel with a diameter of 2.2 mm), separately through the nose or mouth. The CRE and Hercules dilatators are '3-stage dilatators', designed to produce three distinct diameters based on the pressure caused by insufflation. The VACS dilatator is actually designed for heart surgery; its smallest diameter is 4 mm. This makes this dilatator very suitable for severe strictures with a small lumen.

The Savary-Gilliard dilatator (Cook Medical®) was used most frequently for bougienage. This is a wire-guided bougie dilatator with a long tapered tip and a radiopaque marking at the base of the taper. Other wire-guided bougies are American Dilatation System, Tucker, and Rehbein dilatators. In contrast to Savary-Gilliard dilatators, American Dilatation System dilatators have a shorter taper but total radiopacity. Tucker and Rehbein dilatators are small silicone bougies with a tapered end at each side, and can only be used in gastrostomized cases. Hurst and Maloney dilatators are the only bougies that do not accommodate a guidewire. These tungsten-filled dilators are helped by gravity. Hurst dilatators have a blunt tip; Maloney dilatators have a more tapered tip.

The literature contains no studies comparing the different types of dilatators.[26] Currently available studies on dilatation management hardly – or not at all – report the type of dilatator. One could argue that the type of dilatator does not matter as long as it is manipulated by an experienced operator. Nevertheless, it would be good to standardize the application and technical details of both methods in a guideline, especially for rare conditions like anastomotic strictures in children with EA.

Regarding the technical details, it appeared that 29 out of the 95 centers that preferred balloon dilatation used a adiologically guided approach, in line with the finding that most of the centers used water, natrium chloride, or contrast fluid to insufflate the balloon. The manufacturers of the CRE and Maxforce dilatators recommend to insufflate the balloon with water. For the Hercules and VACS dilatators, the manufacturers instruct hydrostatic pressure, which can be either water, saline or contrast fluid. Insufflation with air is advised for the Rigiflex dilatator. We could not find an instruction manual for the Ultra-Thin Diamond dilatator.

As we know from basic physics, gases are easier to compress than are fluids. Hydrostatic pressure is safest: in case of a balloon rupture, air would create a catastrophic burst.[27] Although evidence on this issue is lacking, we advise to only insufflate balloons with fluids (i.e., water, natrium chloride or contrast) and to use a dilatation system that supports hydrostatic pressure.

The insufflation procedure has been standardized in a protocol in half of the participating centers, albeit with a wide range of the dilatation duration, from 5 to 300 seconds. Although a small randomized controlled trial in 20 adults suggested that insufflation for 10 seconds is as effective as insufflation for 2 minutes,[28] we still argue – on the bases of our experience – for a standardized duration of one minute per dilatation to a certain diameter. Standardization provides the opportunity to evaluate the efficacy of this duration, and adjust the duration if necessary.

The optimum diameter for dilatation is difficult to determine, as is also apparent in this survey. None of the centers had a protocol in place to make this decision. Combining the results of this survey with the literature, we conclude that currently the most common method to determine the diameter of the healthy esophagus is the “rule of thumb”. This means that the diameter of the thumb equals the diameter of the esophagus. A recent study found a strong correlation between body weight and the diameter of the esophagus.[29] This is a recent finding which needs further investigation; for now, we support application of the “rule of thumb”.

With regard to recurrent strictures, most of the participating centers preferred local steroid injection or topical mitomycin application as first-line adjuvant treatment. Although promising results have been published for both methods, evidence in children remains scarce.[2, 30–33]

We propose to leave the application of adjuvant treatments to expert centers only, which can decide on the roper treatment based on the patient's characteristics, the stricture and the operator's experience. In addition, centralizing the management of refractory strictures would increase patient numbers, thereby raising the possibilities for comparative research. It has already been acknowledged that centralization and introducing minimal volume standards for referral centers can lead to an improvement in outcome.[34, 35] In a recent consensus conference of the European Reference Network on Rare Inherited and Congenital Anomalies (ERNICA), a minimum caseload of five new patients with EA per year was defined as a requirement of an expert center.[36]. Based on our expert opinion, we therefore propose that a center should perform minimally 10 dilatations in patients with EA per year, and otherwise refer their patients to an expert center. Although 10 dilatations per year is still a low frequency, at least this will avoid incidental dilatation procedures. This volume based strategy should of course be evaluated to see if caseload influences the outcomes and complication rates, especially for recurrent strictures.

To our knowledge, this is the first international survey on dilatation management in anastomotic strictures after EA repair. An earlier EUPSA survey addressed the surgical treatment of EA in general, but did not pay attention to the management of strictures.[37] One of the strengths of our survey is the large response: more than 100 responses of more than 100 centers worldwide. Therefore, this survey represents international treatment strategies.

Some limitations should be addressed. The absence of a response rate could potentially lead to a bias in the results. We excluded almost half of the responses because they were incomplete or empty, which may have led to selection bias. Although the responses covered six continents, Asia and Africa were less represented. The latter makes sense; since they are not involved in any of the medical networks we have sent the survey to, we have not actively approached countries in these continents. As a result, fewer third-world countries were included in this survey. We deliberately did not survey the outcomes of the dilatations, i.e., success rate or complications as the outcomes may have been biased by the presence of non-expert centers.Future research, based on the uniform approach we propose in this study, could elaborate on this.

CONCLUSION

In conclusion, this survey confirms the current lack of consensus on the management of anastomotic strictures after EA repair. It emphasizes the importance of harmonizing the approach towards stricture and dilatation management in patients with EA, for which we present several suggestions (see Figure 5).

As a member of international networks on rare digestive diseases, we strive for optimal patient care for rare inherited and congenital diseases. A systematic and standardized approach is important to improve the clinical standards and patients outcomes, especially in rare diseases where first-level evidence is hard to obtain. In this paper, we extensively discuss the two main dilatation techniques: balloon dilatation and bougienage. The current lack of consent about the choice of dilatation strategy makes it even more important to standardize these two techniques, since this would enable a prospective observational study and possibly a randomized controlled trial in the future.

ACKNOWLEDGEMENTS

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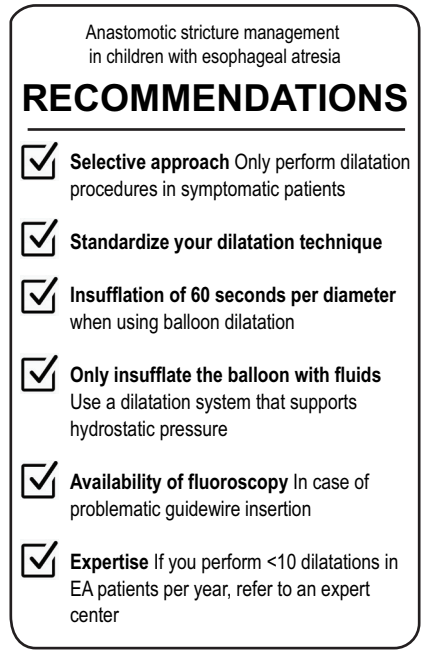

Figure 5. Recommendations for the management of anastomotic strictures in patients with esophageal atresia.

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Supplementary File 1. Survey esophageal dilatation in patients with esophageal atresia

Part A. First, we have some general questions.

1. To which of the following organization are you a member?

☐ EUPSA
☐ ESPGHAN
☐ NASPGHAN
☐ AUSPGHAN
☐ Other, _____

2. What is the name of the center and department you are currently working at?

_____

3. How many physicians are working in your department?

_____

4. What is the medical specialty of the physicians performing endoscopies in your center? Multiple answers possible.

☐ Adult gastroenterologist
☐ Adult general surgeon
☐ Pediatric gastroenterologist
☐ Pediatric surgeon
☐ Other, _____

5. Do residents or fellows receive training in your center?

☐ Yes
☐ No

6. Do residents or fellows perform endoscopies?

☐ Yes
☐ No

Part B. Questions 7-11 concern all patients, with or without esophageal atresia.

7. During a typical month, approximately how many *pediatric endoscopies* (both upper and lower) are performed for any reason (both diagnostic and therapeutic)?

☐ <10
☐ 10-30
☐ 31-50
☐ 51-70
☐ >70

8. During a typical month, approximately how many *pediatric upper endoscopies* are performed for any reason (both diagnostic and therapeutic)?

☐ <10
☐ 10-30
☐ 31-50
☐ 51-70
☐ >70

9. During a typical month, approximately how many *therapeutic pediatric upper endoscopies* are performed for any reason?

☐ <5
☐ 5-10
☐ 11-15
☐ 16-20
☐ >20

10. During a typical month, approximately how many esophageal dilation procedures are performed for any reason?

☐ <5
☐ 5-10
☐ 11-15
☐ 16-20
☐ >20

11. Which is the technique mainly used to manage an esophageal strictures, namely balloon or bougie dilation?

☐ Balloon
☐ Bougie
☐ Both

Part C. The rest of the questionnaire only concerns patients with esophageal atresia.

12. Overall, how many patients with esophageal atresia are currently under follow up in your center?

☐ <20
☐ 20-40
☐ 41-60
☐ 61-80
☐ 81-100
☐ >100

13. Approximately, how many new cases of esophageal atresia are born or referred to your center per year?

☐ ≤5
☐ 6-10
☐ 11-20
☐ >20

14. Approximately, of the total number of new patients with esophageal atresia, what percentage are long gap esophageal atresia?

☐ <5%
☐ 5-10%
☐ >10%

15. Approximately, how many dilation session are performed per month for anastomotic strictures in EA patients?

☐ <3
☐ 3-5
☐ 6-7
☐ 8-10
☐ >10

16. Which is the technique mainly used to manage esophageal anastomotic strictures in EA patients? Choose one option.

☐ Balloon
☐ Bougie
☐ Both

17. If balloon dilatation is the preferred technique, which is the mainly used approach, endoscopic or radiologic?

☐ Endoscopic balloon dilation
☐ Radiologically guided balloon dilation
☐ Both

18. Is a guidewire used routinely?

☐ Yes
☐ No

19. Which balloon do you use for the dilatation? *In the online survey photos were included.*

☐ Rigiflex balloon dilators
☐ Controlled Radial Expension (CRE) balloon dilators
☐ Maxforce balloon dilator
☐ VACS balloon dilator
☐ Ultra-thin Diamond balloon dilator
☐ Gruentzig-type balloon catheters (Schneider, Medi-tech)
☐ I don't know
☐ Other, please specify _____

20. With which material do you insufflate the balloon?

☐ Wateror natrium chloride
☐ Contrast fluid
☐ Air
☐ Other, please specify _____

21. Is the time of insufflation of the balloon recorded in a standardized protocol?

☐ Yes , _____ seconds
☐ No

22. Which bougie do you use for the dilatation? *In the online survey photos were included.*

☐ Hurst (blunt-tipped) dilators (non-guidewired)
☐ Maloney (tapered) dilators (non-wire-guided)
☐ Tucker (rubber) dilators
☐ Jackson (silk-woven) dilators
☐ Savary Gillard dilators
☐ American Dilatation System (Bard)
☐ Emerson Teflon dilators
☐ Rehbein (Rush) dilators
☐ I don't know

☐ Other, please specify _____

23. Is the chosen diameter of the bougie determined by a standardized protocol?

☐ Yes, please specify _____

☐ No

24. Approximately, how many cases per year experience complications after dilation (e.g. perforation, hemorrhage)?

☐ <3

☐ 4-5

☐ 6-7

☐ 7-10

☐ >10

25. Which is the preferred approach used to manage esophageal anastomotic strictures in EA patients?

☐ Routine dilation (to prevent symptoms)

☐ Selective dilations (only in symptomatic patients)

26. In case of recurrent and refractory esophageal anastomotic strictures, which adjunctive treatments are available at your center? Multiple answers possible.

☐ Local injection of steroids

☐ Topical application of mitomycin C

☐ Esophageal stenting

☐ Incisional therapy

☐ Other, please specify _____

27. Which is the preferred first-line adjunctive treatment in use at your center?

☐ Local injection of steroids

☐ Topical application of mitomycin C

☐ Esophageal stenting

☐ Incisional therapy

☐ Other, please specify _____

28. Approximately, how many cases per year undergo surgery due to failure of conservative management for anastomotic strictures?

☐ 0

☐ 1-3

☐ 4-5

☐ >5

Part D. The last questions give us an overview of the number of patients treated in each hospital. You can answer all questions approximately. If you don't know, please fill in 'unknown'.

29. How many EA patients underwent endoscopic assessment/treatment in your center in 2017?

_____

30. How many EA patients were treated for long gap EA in your center in 2017?

_____

31. How many EA patients underwent esophageal dilatation due to an anastomotic stricture in your center in 2017?

_____

32. How many EA patients experienced complications after dilatation in your center in 2017?

_____

33. How many EA patients experienced recurrent anastomotic strictures (≥3 dilations needed) in your center in 2017?

_____

34. How many EA patients experienced recurrent anastomotic strictures (≥5 dilations needed) in your center in 2017?

_____

35. How many EA patients underwent surgery due to a refractory anastomotic stricture in your center in 2017?

CHAPTER 5

The effect of intralesional steroid injections on esophageal strictures and the child as whole: a case series

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ABSTRACT

Background

The most frequent complication after esophageal atresia repair remains anastomotic stricture formation. The initial treatment is endoscopic dilatation. Intralesional steroid injection (ISI) might be an effective adjuvant treatment in case of recurrent strictures. In this series we present our initial experience with this intervention.

Methods

Data on primary surgery, stricture treatment, postoperative complications, outcome and growth were retrospectively collected from electronic patient records. Findings were analyzed by descriptive statistics and mixed model analysis.

Results

Between 2014 and 2017, ISI was performed for severe recurrent anastomotic strictures in six patients (median age at injection 12.4 (2.1-34.7) months) after a median of 6 (2-20) dilatations. In five patients ISI was successful and the stenosis was cleared. No postoperative complications were reported, especially none related to acute adrenal suppression. Comparing the year before with the year after ISI, a significant positive change for weight ($r=0.70$, $p=0.003$) was calculated versus a negative change for height ($r=-0.87$, $p=0.003$).

Conclusions

We found ISI to be an effective adjuvant treatment to recurrent anastomotic stricture dilatation after esophageal atresia repair, without postoperative complications or symptoms of adrenal suppression. It remains important, however, to monitor growth effects. Further evaluation is required in a large prospective study.

INTRODUCTION

Esophageal atresia (EA) is a rare congenital malformation which occurs in approximately 1 per 3500 live births.[1,2] Due to improved treatments, survival rates have increased to over 90%.[2–5] Nevertheless, in up to 60% of cases, anastomotic strictures occur postoperatively, mostly in the first year of life.[6–10] The initial treatment consists of dilatation, either by balloon dilatation or bougienage.[11]

If an anastomotic stricture requires three or more dilatation procedures, it is defined as a recurrent stricture. Recurrent strictures necessitate multiple dilatations under general anesthesia. It therefore forms a large burden for both patients and their parents. In a recent guideline on the management of esophageal strictures, the European Society for Pediatric Gastroenterology, Hepatology and Nutrition (ESPGHAN) reports a number of possible treatments in addition to dilatation.[11] One of these treatments is the injection of intralesional steroids.

Since the introduction in 1969 of intralesional steroid injections as an adjunct to stricture dilatation, only a few case reports or case series have been published on this treatment for esophageal strictures. Generally, they describe other populations than children with EA.[12–17] Recent studies on the use of this intervention in children are all retrospective and mostly include children with caustic strictures.[18–20] There is little evidence yet, but the first results seem promising. Only four randomized controlled trials have been published on this topic, all in adults with other underlying diagnoses than EA.[21,23–24] Beneficial effects of the steroid injections were found in three of these studies, including reduction of the number of dilatation procedures,[23] longer intervals between dilatation procedures,[23] improvement of luminal diameters,[21] and relief of dysphagia.[24] Recent results on intralesional steroid injections in children with EA are lacking, as is a randomized controlled trial in this population.

In this article we present our experience with intralesional steroid injections in six patients with recurrent anastomotic strictures after correction of esophageal atresia, including patient outcomes related to the effectiveness and safety of this treatment.

PATIENTS AND METHODS

Study design and patient selection

We reviewed the electronic patient records of children born with EA between the 1st of January 2014 and the 31st of August 2017, who have been treated with intralesional steroid injections to manage an anastomotic stricture. The indication for injection is not standardized in our hospital and was in all cases determined by the treating physician. All patients have been followed for at least one year after injection, with regular checkup visits every 1 to 3

months. The Erasmus MC Medical Ethics Committee approved this retrospective study (MEC-2018-1462).

Procedure

In all patients, a flexible endoscope (N180 or GIF-160, Olympus, Tokyo, Japan) was introduced to inspect the anastomosis. An endoscopic needle (DVI-23-MH or LVDI-23-240, Cook Medical, Bloomington, USA) was pre-filled with 1 mL triamcinolone acetonide (TAC, Kenacort 40 mg/mL (equals 50 mg prednisone); Bristol-Myers Squibb BV, Utrecht, the Netherlands) and passed through the endoscope (see Figure 1). Under direct vision, in each of four quadrants of the circular stricture maximally 0.25 mL was then injected (see Supplementary File 1; video). After retracting the injection needle from the working channel and re-establishing good visualization of the anastomotic stricture, balloon or semi-rigid dilatation was performed.

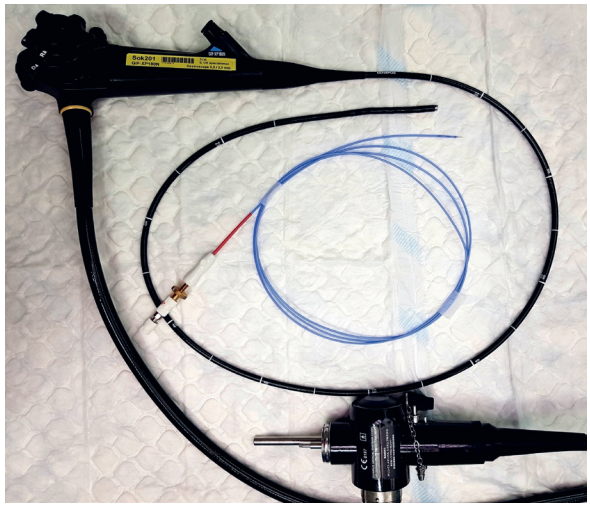

Figure 1. Overview of the instruments needed to inject an esophageal stricture. A flexible endoscope and an endoscopic needle.

Data collection

General information and information about the surgery and previous stricture treatment was obtained by chart review. The presence of anastomotic tension was extracted from the operation report. The duration of effect of the Kenacort injection is several days to weeks. Clinical symptoms of iatrogenic Cushing and subsequently adrenal suppression were scored in the first year of follow up. Cushingoid symptoms were defined as significant growth retardation in combination with weight gain and Cushingoid habitus. Adrenal suppression symptoms were defined as severe fatigue, muscle weakness, anorexia, weight loss, hypoglycemia, hypotension and vomiting.[25] Hematologic changes like anemia, neutropenia and lymphocytosis could also indicate adrenal suppression but unfortunately blood results were only available for one patient at 1 week after the injection. Additionally, absolute

height and weight data and SD scores were collected for the period from 365 days before the injection (T0) and the period until 365 days after the injection. If multiple injections had been applied, the first injection moment was defined as T0. Height and weight data were compared with previously established growth data of 126 patients with EA.[26] All SD scores were corrected for prematurity. By using SD scores we automatically corrected the results for the influence of EA on the growth curves in all patients with EA.

Statistical analysis

Overall, analysis was by descriptive statistics. Linear mixed model analysis was used to evaluate the trajectory of the SD scores of weight and height over time, before and after the injection. The independent variables in the linear mixed models were time since study entry (365 days before T0) and time since injection (T0). These two independent variables were time-varying. A random intercept and a random slope of time since study entry were included in the linear mixed models to account for the within-subject correlations. All data was analyzed using SPSS V.24.0 (IBM, Chicago, Illinois, USA), with a two-sided significance level of 0.05.

RESULTS

Patient demographics

The series included six patients (one boy) out of 60 patients born with EA in this period, with a median gestational age of 39.2 weeks (range 31.9-41.1) and a median birth weight of 2865 grams (range 1275-3750). Two patients were considered small for gestational age with a birthweight <10th centile of Dutch reference curves.[27] Postoperative complications after primary surgery were anastomotic leakage in four patients, chest effusion with signs of systemic infection in three patients and pneumothorax in two patients. Patient demographics are described in Table 1.

Previous dilatation procedures and injection of steroids

Table 2 shows the specifications of the dilatation procedure and the intralesional steroid injection. Median age at time of the steroid injection was 12.4 months (range 2.1-34.7). The first injection was performed after a median of 6 dilatations (range 2-20). Patient 1 already had a medical history of multiple dilatation procedures with eventually a surgical resection of the stricture with a new end-to-end anastomosis. Kenacort was injected during the 8th and 9th dilatation procedures after this new anastomosis.

Four dilatation procedures were planned based on clinical symptoms of dysphagia, four procedures were planned in advance. An esophagram to confirm the presence of a stenosis prior to dilatation was performed only once. Five patients were treated with balloon dilatation. Patient 3 had been briefly resuscitated during the initiation of the anesthetic

Table 1. Patient characteristics. SGA = small for gestational age, EA = esophageal atresia, TEF = tracheoesophageal fistula, N/A = not applicable

	Gender	Gestational age (weeks + days)	Birth weight (grams)	SGA	Type of EA	Type of surgery	Anastomotic tension?	Postoperative complications	VACTERL	Major anomalies
1	female	31+6	1275	<p3	D	primary thoracotomy	yes	recurrent TEF	no	anorectal malformation (cloaca with common channel and double vagina)
2	female	41+0	3660	p10-p90	C	converted to thoracotomy	yes	leakage of the anastomosis, mediastinitis	no	palatoschisis
3	female	41+1	3750	p10-p90	C	thoracoscopy	no	leakage of the anastomosis, pneumothorax	no	N/A
4	female	31+6	1520	p10-p90	C	primary thoracotomy	yes	N/A	no	N/A
5	male	40+6	3300	p10-p90	C	thoracoscopy	no	anastomotic leakage, mediastinitis, pneumothorax	yes	renal agenesis right, anal stenosis
6	female	37+4	2430	p5-p10	C	thoracoscopy	yes	anastomotic leakage, mediastinitis	no	duodenal atresia, renal agenesis right, tracheomalacia

Table 2. Specifications of dilatations and injections. mo = months, TAC = triamcinolone acetide

	Age at time of injection (mo)	Time of injection (no. of dilatations)	Indication for new dilatation	Esophagram prior to dilatation	Type of dilatation	Volume of injection (mg TAC)	No. of subsequent dilatations after injection	Postoperative complications	Clinical symptoms of adrenal suppression
1	1) 34.13 2) 34.60	1) 8th 2) 9th	1, 2) planned 2 weeks after previous dilatation	no	1) bougie, 22Fr 2) bougie, 23Fr	1) 40 2) 32	5x and multiple esophageal stents	no	no
2	10.70	20th	planned 2 weeks after previous dilatation	no	balloon, 8mm	40	0x	no	no
3	1) 2.07 2) 2.80	1) 4th 2) 5th	1) symptoms of dysphagia 2) planned 2 weeks after previous dilatation	no	1) balloon, 10mm 2) balloon, 10mm	1) 40 2) 40	0x	no	no
4	6.17	3rd	symptoms of dysphagia	no	balloon, 12mm	40	1x (7 months later)	no	no
5	14.07	2nd	symptoms of dysphagia	yes	balloon, 12mm	40	1x (9 months later)	no	no
6	34.70	16th	symptoms of dysphagia	no	balloon, 18mm	40	0x	no	no

procedures. Resuscitation had been necessitated by laryngospasm due to tracheomalacia and was not related to the procedure or the injection.

Outcomes

Intralesional steroid injection treatment was successful in five out of the six patients (83.3%). Two of them received one injection without the need for any further dilatations. One patient received two injections after which she needed no extra dilatations. The second injection had already been planned to be given 2 weeks after the first injection and not based on clinical symptoms of a stenosis. Two patients needed one additional dilatation after the injection, but respectively seven and 9 months later. In one patient the treatment was not effective. This patient underwent five additional dilatations and needed multiple esophageal stents before the stenosis was ultimately cleared.

Postoperative complications such as perforation, hemorrhage, or Candida esophagitis had not been reported after intralesional steroid injection treatment. None of the patients showed any clinical symptoms indicating adrenal suppression (see Table 2). None had developed symptoms of a Cushingoid syndrome.

Height and weight followed the growth curves of the general EA population (see Figure 2).With mixed model analysis we calculated the trend in SD scores since the entry of the study (365 days before T0) and since the injection (T0). We found a significantly positive change in trend in SD scores for weight after injection with a coefficient of 0.70 (p=0.003), which means that the rate of change in weight was 0.70 SD per year higher after the injection than before the injection. A significantly negative change in trend in SD scores was shown for height, with a coefficient of-0.87 (p=0.003, see Supplementary Figure 1).

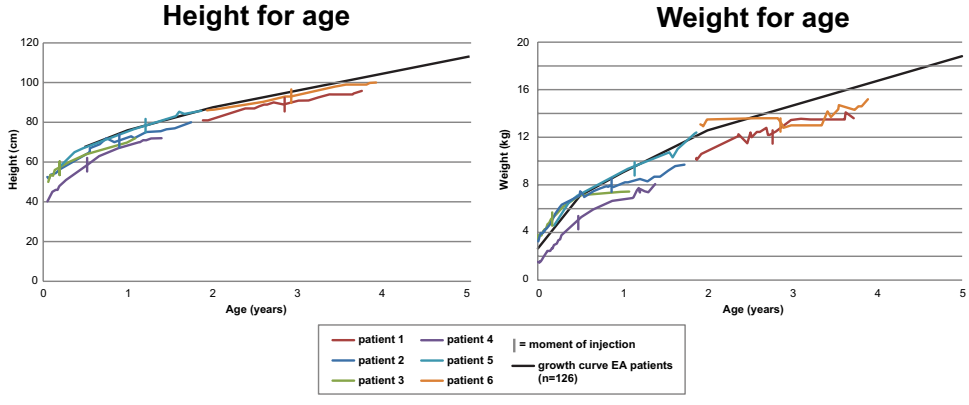

Figure 2. Growth curves on height and weight per patient from 365 days before the injection (T0) until 365 days after the injection.

DISCUSSION

With this case series we aimed to give an overview of our initial experience with intralesional steroid injections in patients who developed an anastomotic stricture after correction of EA. In total, six patients were treated with one or two intralesional steroid injections. In five patients the treatment seemed successful as the stenosis was cleared. Intralesional steroid injections also seemed to be a safe treatment, since no postoperative complications or clinical signs of adrenal suppression were observed.

The exact mechanism by which intralesional steroids enhance the efficacy of dilatation has not been elucidated yet. Every dilatation gives rise to a new scar. An anastomotic stricture is actually a hypertrophic lesion developing after scar formation. It is thought that TAC inhibits collagen formation, enhances collagen breakdown, decreases the fibrotic healing that occurs after dilatation and prevents crosslinking of collagen that causes contractions in scar tissue.[28,29] Therefore, it is hypothesized that TAC prevents the regeneration of hypertrophic tissue after the dilatation procedure.

Although literature is scarce and most available literature is outdated, our results are in line with the reported findings.[12–17] Two retrospective studies have addressed the use of intralesional steroid injections in esophageal strictures in children. One found a positive effect in 32 children with a short-segment caustic stricture (<3 cm); that is, a significantly lower number of dilatations and longer intervals between dilatations.[19] Except for one patient with a transient cushingoid phenotype, no postoperative complications were reported. The other study concerned 15 patients with a long segment (>5 cm) stricture after corrosive ingestion, who received intralesional steroid injections after at least five previous dilatations.[20] No significant difference in treatment effectiveness was found between adjuvant steroid injections and the control group. Four patients suffered from an esophageal perforation, but in all four this occurred at dilatation sessions other than the ones when the steroids were injected. Potentially, the effectiveness of the treatment depends on the length of the stricture, making intralesional steroid injections only effective in short segment strictures. Unfortunately, we could not verify this theory, because the stricture lengths in our patients had not been reported. We may assume perhaps that the stricture lengths were short (<1 cm) because in EA all strictures are anastomotic strictures.

In general, the most frequently reported complications of an esophageal dilatation are perforation, hemorrhage and bacteremia.[30] Potential complications of esophageal steroid injections include adrenal suppression, perforation, intramural infection, candida infection, mediastinitis and pleural effusion.[31]

A recent case series described the effects of intralesional TAC injections in six children (mean age 4.3 years) with a subglottic stenosis.[32] It appeared that ACTH and cortisol levels initially decreased but normalized within a few months after the therapy. This is the only report on a comparison of blood levels of ACTH and cortisol before, during and after multiple TAC injections in children, albeit not children with EA. As mentioned earlier, in children iatrogenic Cushing could also express itself in growth retention. Growth retention has not been described in previous studies that used intralesional steroid injections. Still, one correspondence letter in 1989 described a significant delay in linear growth in a boy with a history of tracheoesophageal fistula who received multiple intermittent steroid injections for over an 18-month period.[33]

Regarding post-operative complications in our series, we specifically looked at signs of acute adrenal suppression in clinical symptoms and growth curves. Clinical symptoms had not been reported but caution is required in view of the retrospective study design. No ACTH or cortisol levels were available for all six patients. Considering the growth curves, there was a significant negative change in trend for height between the year before injection and the year after injection, but not for weight. In other words, after the injection children were gaining more weight, although the height gain was reduced compared to the normal population. Although one could argue that this is a typical side effect of steroids, we noticed these changes several months after the injection. This pleads against iatrogenic Cushing as a side effect of intralesional steroid injections in which case you would expect a significant weight gain immediately after the injection.

One patient (patient 3) stood out with a severe decrease in SD scores for both weight and height. Ever since birth she had suffered from severe gastroesophageal reflux, always had an inflated abdomen and vomited a lot after every feeding. Thirteen months after the two steroid injections a duodenal web was discovered. After surgical correction of the duodenal web she immediately started to gain weight and gradually resumed normal growth. However, she did not return to her original percentile yet. In this specific case, it is hard to define if the growth retention was caused by the steroid injections or by the impossibility to feed because of the duodenal web.

In all cases it is important to monitor height and weight over a longer period of time, since most patients with an esophageal stricture already show a declined growth curve preoperatively due to poor feeding. After release of the stricture it takes time for the child to recover, to learn to drink (again) and to resume normal growth curves. Nevertheless, it should be kept in mind that treatment with steroids can affect children's growth.

Some limitations of this study should be addressed. First, the number of patients included in this series is very small. Next, all patients received the steroid injection at different time points.

The indication for injection was in all cases made by the treating physician and based on the clinical symptoms of the patient. It was nog standardized or recorded in a protocol. Third, many data were missing and the available data were not collected by a standardized protocol. No biochemical measures of adrenal function were monitored. This makes it difficult to make a definite statement about possible complications like adrenal suppression. Conducting a well-designed prospective study is challenging because, fortunately, EA in combination with the incidence of recurrent severe anastomotic strictures is rare.

CONCLUSIONS

We found intralesional steroid injections to be an effective additional treatment to dilatation in the management of anastomotic strictures in children after EA repair. We observed no postoperative complications or clinical signs of adrenal suppression although it remains important to monitor possible growth retardation. A definite statement about this risk would require further investigation on a larger scale with a systematic long-term followup. It remains important to determine the effectiveness and safety of intralesional steroid injections in the management of anastomotic strictures in children after correction of EA in a randomized controlled trial.

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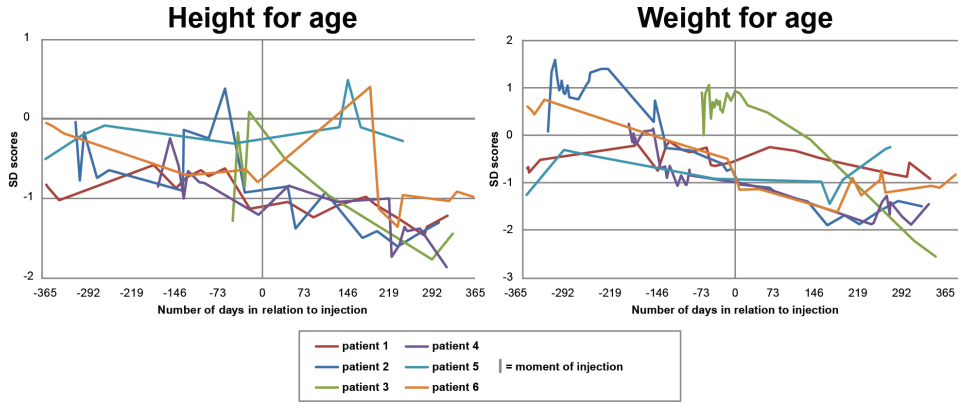
Supplementary material

Supplementary File 1. Video showing the procedure of an esophageal injection. Scan the QR code to find the video online on the journal website.



Supplementary Figure 1. SD scores (corrected for prematurity) on height and weight per patient from 365 days before the injection (T0) until 365 days after the injection.

CHAPTER 6

Intralesional steroid injections to prevent refractory strictures in patients with esophageal atresia: study protocol for an international, multicenter randomized controlled trial (STEPS-EA trial)

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ABSTRACT

Introduction

Anastomotic stricture formation is the most common postoperative complication after esophageal atresia (EA) repair. The standard of care is endoscopic dilatation. A possible adjuvant treatment is intralesional steroid injection, which is thought to inhibit scar tissue formation and thereby to prevent stricture recurrence. We hypothesize that this intervention could prevent refractory strictures and reduce the total number of dilatations needed in these children.

Methods and analysis

This is an international multicenter randomized controlled trial. Children with EA type C (n=110) will be randomized into intralesional steroid injection followed by balloon dilatation or dilatation only. Randomization and intervention will take place when a third dilatation is performed. The indication for dilatation will be confirmed with an esophagram. One radiologist – blinded for randomization – will review all esophagrams. The primary outcome parameter is the total number of dilatations needed with <28 days interval, which will be analyzed with a linear-by-linear $\chi 2$ association test. Secondary outcome parameters include the level of dysphagia, the luminal esophageal diameter and stricture length (measured on the esophagrams), the influence of co-medication on stricture formation, systemic effects of intralesional steroids (cortisol levels, length and weight) and the cost-effectiveness. Patients will undergo a second esophagram; length and weight will be measured repeatedly; a scalp hair sample will be collected; and three questionnaires will be administered. The follow-up period will be 6 months, with evaluation at 2-3 weeks, 3 and 6 months after the intervention.

Ethics and dissemination

Patients will be included after written parental informed consent. The risks and burden associated with this trial are minimal. The institutional review board of the Erasmus Medical Centre approved this protocol (MEC-2018–1586/NL65364.078.18). The results of the trial will be published in a peer-reviewed scientific journal and will be presented at international conferences.

Trial registration numbers

2018-002863-24 and NTR7726/NL7484.

INTRODUCTION

Esophageal atresia (EA) is a congenital malformation which can present with or without a tracheoesophageal fistula, with a European prevalence of 2.43 cases per 10,000 births.[1, 2] With better treatments, survival rates have increased to over 90%.[1, 3, 4] Still, anastomotic stricture formation remains the most frequent postoperative complication in up to 60% of cases.[5] Especially refractory strictures form a great burden for both patients and their parents. The incidence of refractory strictures is poorly reported due to the variety in definitions used in literature. Regarded as a consensus among experts, the European Society for Pediatric Gastroenterology Hepatology and Nutrition (ESPGHAN) has set the following definition for a refractory esophageal stricture: an anatomical restriction without endoscopic inflammation that results in dysphagia after a minimum of five dilatation procedures at maximally 4-week intervals.[6, 7]

Recently, the Dutch Consortium for Esophageal Atresia (DCEA) conducted a retrospective multicenter study in the Netherlands to assess risk factors for stricture formation in children with EA. The study population consisted of 436 children born with EA between 1999 and 2013 with an end-to-end esophageal anastomosis. Thirty-two (7.3%) of them required ≥5 dilatations within an interval of 28 days.[5]

The initial treatment of an anastomotic stricture consists of endoscopic dilatation, either balloon dilatation or semirigid dilatation.[6] Consensus on the preferred technique has not yet been established. A refractory stricture requires multiple dilatations under general anesthesia, for which the child needs to be hospitalized. This adds significantly to the burden of the disease. It is therefore important to minimize the occurrence of refractory strictures and with that the need for dilatations.

In a recent ESPGHAN guideline, various adjuvant treatments are mentioned, for example, intralesional or systemic steroids, topical mitomycin C, esophageal stents and surgical resection. Our trial will focus on intralesional steroid injections since we as well as the other centers involved in this trial have had successful results with this treatment in several patients.[8]

The literature on intralesional steroid injection in children with EA is scarce, but promising results have been described in both children and adults with all types of esophageal strictures (see Table 1). Most studies are outdated case reports or series.[9-14] Four relatively recent randomized controlled trials (RCTs) on this topic included only adults with underlying diagnoses other than EA, like caustic strictures after acid ingestion, peptic strictures and anastomotic strictures after esophagectomy with gastric tube reconstruction.[15-18] Reported beneficial effects were reduction of dilatation procedures,[16] longer intervals between dilatation procedures,[17] improvement of luminal diameter[15] and relief of dysphagia.[18]

Table 1. Summary of literature on clinical findings on intralesional steroid injections for esophageal strictures, including retrospective cohort studies in children <12 years (range 0-14 years).

Author, year	Type of study	Characteristics	Main outcomes
Camargo, 2003[15]	Double-blind RCT	14 adult patients, corrosive strictures	· No significant difference in dilatation frequency and dysphagia. · Significant improvement in obtained diameter (p<0.05). · No adverse events reported.
Ramage, 2005[16]	Double-blind RCT	30 adult patients, peptic strictures	· Less patients required repeat dilatation in the steroid group (13% vs. 60%, p=0.0209). · Shorter time to repeat dilatation in control group (p=0.01). · No adverse events reported.
Hirdes, 2013[17]	Double-blind RCT	60 adult patients, anastomotic strictures after esophagectomy with gastric tube reconstruction	· No significant decrease in frequency of repeat dilatation or prolongation of dysphagia-free period. · Four patients developed Candida oesophagitis.
Pereira-Lima, 2015[18]	Double-blind RCT	19 adult patients, anastomotic strictures after esophagectomy with gastric tube reconstruction	· Significant improvement on dysphagia at 1 and 6 months (p=0.021, p=0.009). · No perforation, haemorrhage of oesophageal candidiasis. No other adverse events reported.
Kochar, 2002[19]	Prospective	71 patients (13-78 year), all kinds of strictures	· Periodic dilatation index decreased significantly after injection (p<0.001). · No adverse events reported.
Nijhawan, 2016[20]	Prospective	11 adult patients, corrosive strictures	· Significant improvement of maximum dilatation (p<0.001) and number of dilatations per month (p<0.001). · No adverse events reported.
Divarci, 2016[21]	Retrospective	32 children (mean age 3.6 year), corrosive strictures	· Mean number of dilatation sessions was decreased (p=0.003). · Mean frequency of dilatations in weeks extended (p<0.001). · Only a positive effect in short segment strictures (<3 cm, 92% of patients dysphagia-free). · No serious adverse events reported. One transient cushingoid phenotype, but no real adrenal suppression.
Cakmak, 2016[22]	Retrospective	38 children (median age 1.5 year), EA (n=19) and corrosive strictures (n=19)	· No significant difference in treatment effectiveness between steroid injection and others (p>0.05). · Intralesional steroid injections only performed in patients with long (>5 cm) and corrosive strictures & ≥5 dilatations. · Four patients with oesophageal perforation, at other dilatation sessions than the intralesional steroid injection.

In children, only retrospective studies have been performed, which mostly included caustic strictures.[19, 20] Divarci et al analyzed data of 32 children with corrosive strictures with a mean age of 3.6 years (±2.5 years); after the intervention, the number of dilatations had significantly decreased and the intervals between dilatations had extended.[19] Cakmak et al included 38 children with either EA or corrosive strictures with a median age of 1.5 years (range 0-14 years)[20] but did not find a significant difference in treatment effectiveness. None of the above-mentioned studies reported any systemic effects of the local intralesional steroid injections.

All studies used triamcinolone acetonide (TAC). The exact mechanism by which TAC enhances the efficacy of dilatation is unclear. It has been proven very effective in the treatment of hypertrophic scars of the skin and keloid. A recurring anastomotic stricture can be seen as a hypertrophic lesion. The injected TAC inhibits collagen formation, enhances collagen breakdown, decreases fibrotic healing that occurs after dilatation and prevents cross-linking of collagen that causes contractions in scar tissue.[21, 22]

The recurrent dilatations and readmissions impose a substantial burden on the healthcare system. To date, there is no evidence on the cost-effectiveness of intralesional steroid injections. However, in the current era of evidence-based and cost-effective medicine, proof of cost-effectiveness is highly relevant.

The primary objective was to evaluate whether intralesional steroid injections combined with endoscopic dilatation can prevent refractory strictures in children with EA and recurrent esophageal stenosis, and thus can minimize the number of dilatations needed with a 28 days interval between the dilatations.

The secondary objectives were:

- To compare the level of dysphagia and the child's eating behavior between the two groups.
- To compare the effect of intralesional steroid injections on the luminal diameter and the stricture length between the two groups.
- To evaluate a possible influence of co-medication (eg, antacids) on stricture formation.
- To analyze the possible systemic effects of a one-time intralesional steroid injection.
- To analyze the cost-effectiveness of the use of intralesional steroid injections to prevent refractory strictures.

METHODS AND ANALYSIS

The STEPS-EA trial is an international, multicenter, single-blinded RCT with a 1:1 randomization to injection with 10 mg/mL TAC (Kenacort-A 10) prior to balloon dilatation and balloon

dilatation without any injection. The participating centers are tertiary (academic) hospitals that collaborate within the European Reference Network on Inherited and Congenital Abnormalities (ERNICA, ern-ernica.eu) and that routinely provide care for children with EA.

Patient and public involvement

Parents of patients were involved in the end stage of the design of the trial. We presented our plans for the trial to a DCEA meeting in which also representatives of the patients' association Vereniging voor Ouderen en Kinderen met een Slokdarmafsluiting (VOKS) took part. They were invited to comment on the study design, intervention or time required to participate in this trial. Consensus was reached on the final design during this meeting. The patients' association will not be involved in the recruitment and conduct of the trial. We will involve them in dissemination, however, by presenting the trial results at a members' day or in their monthly newsletter. As the VOKS is a member of the European Federation of Esophageal Atresia and Tracheoesophageal fistula Support Groups E.V., patients and parents throughout Europe will be informed about the results.

Participants

EA can be a very heterogeneous disease. About 90% of the children with EA has type C.[5, 23] In order to make the two treatment groups as equal as possible, only children with EA type C who underwent surgery with primary anastomosis within the first days of life and who developed a recurrent esophageal stricture will be eligible. Children will be included if they are ≥3 months old at the time of the intervention and in need of a third dilatation. Written parental informed consent will be obtained by the local principal investigator (PI) or another member of the local research team. Exclusion criteria are lack of parental consent or an impossibility – known from previous dilatations – to use an endoscope with a large enough diameter working channel to pass the endoscopic injector.

Sample size calculation

The power calculation is based on a linear-by-linear $\chi 2$ association test comparing the total number of dilatations required within the study period (all strictures) and within a 28-days interval (refractory strictures) between the two treatment groups. The total number of dilatations will be categorized into the categories 3, 4, 5, 6, 7-10 and >10 dilatations, and a separate category for patients who are not dysphagia-free at the end of the follow-up period.

For this power calculation, we used data of the original dataset of our retrospective study in the Netherlands.[5] We selected patients from this dataset who underwent at least three dilatations with a 28-day interval (n=53). The retrospective study's observed numbers of patients and the relative frequencies for each category are listed in Table 2. We assumed that the use of intralesional steroid injections combined with endoscopic dilatation will reduce the total number of dilatations by 50%. Note that this 50% reduction applies only to dilatations

after the third dilatation, and therefore no change in the number of dilatations is assumed for the first three dilatations. This assumption leads to a different distribution for the number of dilatations within the categories, which is shown as the assumed relative frequencies for the steroid group in the final column of Table 2. The details of the calculation are provided in the Supplementary material.

In a simulation model, the required sample size to obtain a power of 80% (with a two-sided significance level of 0.05) was calculated as 52 patients per group, thus 104 in total. To account for the effects of dropout and missing data, we aimed to include a total of 110 patients.

Recruitment

Patients will be recruited from hospitals in various European countries. Up until now, hospitals in Denmark, United Kingdom, Finland, France, Italy and Sweden have agreed to participate. During the inclusion period, it will remain possible for other centers to join. To achieve adequate participant enrolment, we have minimalized the exclusion criteria. Collaboration within ERNICA should make patient accrual achievable despite the rarity of the disease.

Randomization, blinding and treatment allocation

Randomization will be conducted via ALEA (FormsVision B.V./ALEA Clinica B.V.), a validated software program. To achieve equal distribution of the intervention among the participating sites, block randomization stratified per center will be carried out. The software was prepared by an independent statistician who is not otherwise involved in the study. After inclusion, the local PI will enter the patient in ALEA and will thereupon receive an email stating the allocated treatment.

Randomization will be blinded for the radiologist who will review all esophagrams. The control group will not receive sham treatment. The steroid that will be used, Kenacort-A 10 (see further), is a white suspension, which complicates creating a placebo. Adding excipients to normal saline is undesirable, considering the unknown effect on the healing process of the stricture. Moreover, it is deemed undesirable to inject an infant with a fluid with an unknown effect.

Table 2. Assumed relative frequencies of the number of dilatations in the control and steroid groups

Number of dilatations within 28 days interval	Observed number of patients (n=407)[5]	Relative frequencies control group	Assumed relative frequencies steroid group
3 dilatations	4	0.075	0.142
4 dilatations	7	0.132	0.302
5 dilatations	9	0.170	0.170
6 dilatations	7	0.132	0.160
7-10 dilatations	16	0.302	0.132
>10 dilatations	10	0.189	0.094
Total (all numbers of dilatations combined)	53	1.000	1.000

Investigational product

The intervention will be a one-time endoscopic injection of 0.25 mL Kenacort-A 10 (Bristol-Myers Squibb BV, Utrecht, the Netherlands) in each quadrant of the stricture prior to the third endoscopic dilatation. Thus, in total, 1 mL (10 mg/mL TAC, equals 12.5 mg prednisone) will be injected. During the study period, none of the patients will receive a second injection.

Kenacort-A 10 will be prepared, labelled and distributed by the trial pharmacy of the coordinating hospital. It will be delivered to the local pharmacies by courier, and the local pharmacy will deliver it to the operation room when needed. After the trial, a specific procedure for destruction of the remaining drugs is not needed; they can be disposed of locally.

Patient timeline

Figure 1 presents a flowchart for this study and the study procedures. The treating physician will decide on a third dilatation on the basis of the clinical signs of dysphagia and the findings on the esophagram (thoracic X-ray with contrast, anterior-posterior and lateral). Clinical signs of dysphagia are defined as the inability to be fed age appropriately. Findings on the esophagram indicating a stricture are defined as a significant narrowing of the lumen, seen as a waist in the contrast on the X-ray. The treating physician can be a pediatric surgeon or a pediatric gastroenterologist, depending on the local agreements in the different countries. After parental informed consent, the patient will be included and randomized to one of the study arms.

Prior to the balloon dilatation, an endoscopic needle (DVI-23-MH varices injector or equivalent) is prefilled with 1 mL Kenacort-A 10 and passed through the endoscope. Under direct vision, 0.25 mL will be injected in each of the four quadrants of the circular stricture. After the injection needle has been retracted from the working channel and good visualization has been re-established, balloon dilatation will be performed up to the desired diameter. The balloon will remain insufflated for 1 min.

During a follow-up period of 6 months, patients will not receive any (additional) steroid injections, only balloon dilatations if needed. Meaning, patients in the steroid group will only receive one injection, and patients in the control group will receive no injections at all. After the study period has ended, treatment is again free of choice.

Two to 3 weeks after the dilatation procedure, a second esophagram will be made in children in both study groups. All esophagrams will be reviewed by one specialized pediatric radiologist of the coordinating hospital, who will determine the esophageal diameter and stricture length. Earlier studies have proven that these measurements can be obtained from an oesophagram.[24] The parents will be informed about the results and the normal standard of care will be continued.

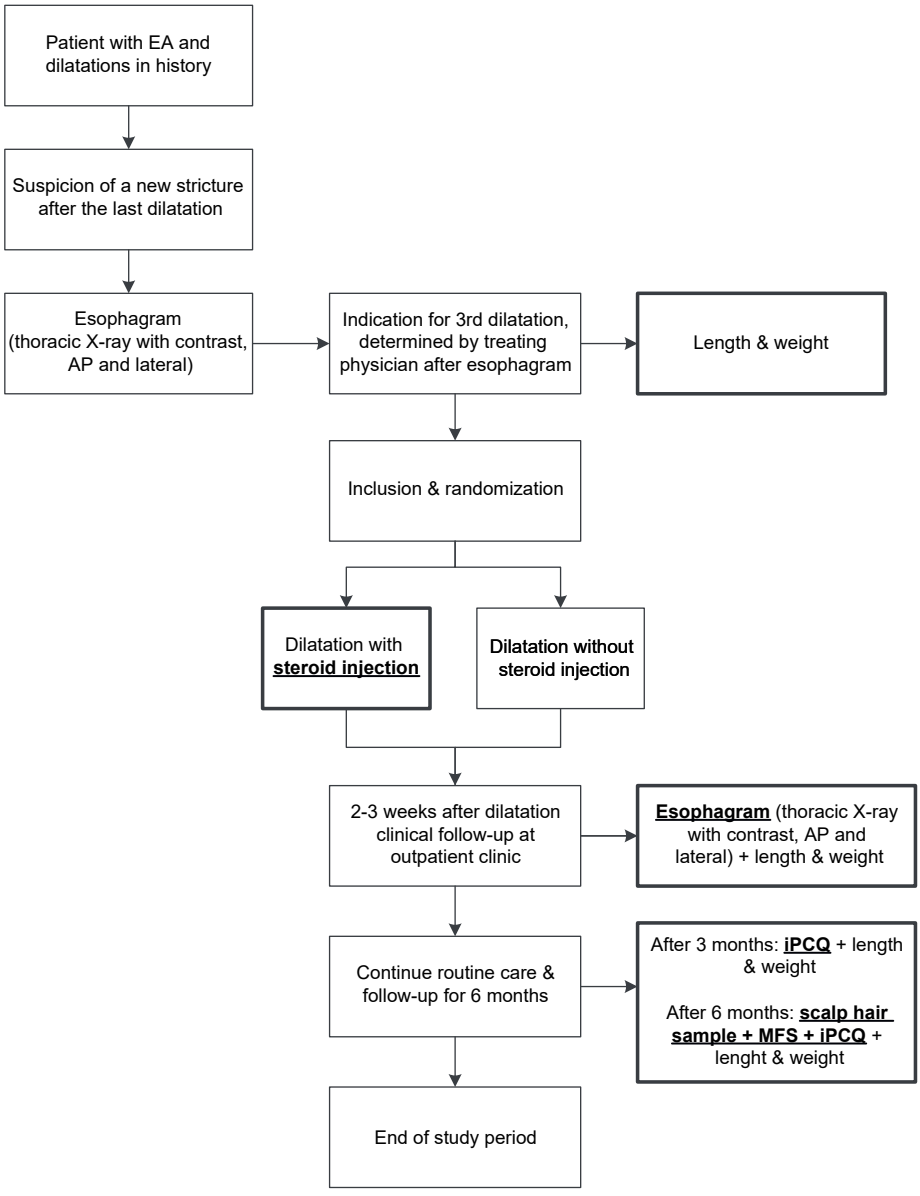

Figure 1. Flowchart of the study design. Bold with underline indicates study procedures; the rest is standard of care. AP = anterior–posterior, iPCQ = iMTA Productivity Cost Questionnaire, MFS = Montreal Feeding Scale, EA = esophageal atresia

Each patient will be followed up for 6 months after the third dilatation, with recording of possible side effects, complications and additional dilatations. Length and weight will be measured at 2-3 weeks, 3 and 6 months after the third dilatation. In the context of the cost-

effectiveness analysis, the parents will be asked to fill out a modified version of the iMTA Productivity Cost Questionnaire (iPCQ)[25] at 3 months after the third dilatation.

At evaluation at 6 months, we will collect a scalp hair sample from the child to determine long-term cortisone and cortisol levels.[26, 27] The hair locks will be stored for final batch analysis. Lastly, the parents will be asked to fill out the Montreal Feeding Scale (MFS)[28-30] and again the iPCQ. After this, the study period will end.

Outcome parameters

The primary outcome parameter is the total number of dilatations required per patient with a 28-day interval between the dilatations during the study period of 6 months, which is defined as the period from the day of the third dilatation until 6 months later.

The secondary outcome parameters are as follows:

1. Total number of dilatations within the study period, regardless of the interval.
2. Interval (in weeks) between the start of the study and the last dilatation procedure within the study period.
3. Scores on the MFS.
4. The change in maximal luminal diameter after the third dilatation relative to the diameter before the third dilatation: relative change in luminal diameter=(maximal diameter after-maximal diameter before)/maximal diameter before. The diameter will be measured at the narrowest point of the esophagus.
5. The change in the length of the esophageal stricture after the third dilatation relative to the length before the third dilatation: relative change in stricture length=(stricture length after-stricture length before)/stricture length before. The length will be measured between the two points where the esophageal diameter starts narrowing.
6. The use of comedication (e.g. antacids) during the study period.
7. The mean hair cortisol levels in the first 3 months after the third dilatation. Cortisol levels will be adjusted for age and sex.
8. Delta length SD scores (SDSs) and delta weight SDS between the third dilatation (intervention) and 3 and 6 months after the third dilatation.
9. Total costs of the treatment, including medical and non-medical costs.
10. Incremental costs per refractory stricture prevented and incremental costs per additional dysphagia-free patient.

Data collection

All participating centers are familiar with the procedure of injecting Kenacort-A 10 in the lesion via an injection needle through the endoscope. However, to guarantee equality of the intervention, the relevant practitioners in all centers will be trained by the PI of the trial. Radiological inter-observer variability will be avoided by having all esophagrams reviewed by one radiologist.

The internationally validated MFS[28-30] will be used to measure dysphagia. The MFS has often been used in previous research in children with EA. The scalp hair sample will be taken from the posterior vertex; cortisol levels will be determined with the liquid chromatography-tandem mass spectrometry method for quantification of steroids.[26]

The cost-effectiveness analysis will follow established methods for economic evaluations and costing studies in healthcare.[31, 32] Both medical and non-medical costs will be collected. Medical costs will include costs of surgeries (dilatations), steroid injections, hospital days (on the ward or the intensive care unit), medication (such as analgesics) and diagnostic radiography. Costs of healthcare provided by others than the participating centers (such as other hospitals or general practitioners) will be ignored in this study, as these are unlikely to be affected by the intervention. Non-medical costs will include costs of special diets, costs related to hospital visits and productivity losses related to both paid and unpaid works. The non-medical costs will be measured using the iPCQ,[25] supplemented with additional questions on costs of special diets and costs related to the child's hospitalization. The original iPCQ questions are validated in English. The complete questionnaire including the additional questions will be translated to the languages required for this trial using the forward-backward translation method, as will the MFS for the languages it has not been validated for.

Statistical analysis

Since a standardized treatment protocol will be adopted in all centers, statistical adjustment for center effects is considered unnecessary.

The primary outcome parameter will be analyzed with a linear-by-linear χ^2 association test. The total number of dilatations required with a 28-day interval (i.e. refractory strictures) within the study period of 6 months per patient will be categorized and compared between treatment groups. In case of death during the follow-up period, the outcome will be set to the highest (i.e. most severe) category. In case of drop-out during the follow-up period due to other causes (e.g. emigration and withdrawal), the subject will be excluded from the study.

The analyses for the secondary study parameters are as follows:

1. The total number of dilatations required within the study period (regardless of the interval, i.e. all strictures) will be categorized and compared between treatment groups with a linear-by-linear χ^2 association test.
2. The interval (in weeks) until the patient is dysphagia-free will be compared between groups with the log-rank test and with Cox proportional hazards regression, with adjustment for treatment group and factors such as age, sex, diagnostic information and described risk factors for stricture formation like anastomotic leaking and thoracoscopic repair.[5] Patients who do not become dysphagia-free during follow-up are treated as censored at the end of the follow-up period.

3. The scores on the MFS (reflecting the level of dysphagia and the eating behavior) will be compared between study groups with a Mann-Whitney test.
4. The relative change in the esophageal diameter and hat in the length of the esophageal stricture will be compared between study groups with an analysis of covariance model. The dependent variables in this model will be the log-transformed esophageal diameter and length after the third dilatation, and the independent variables will be the treatment group and the log-transformed esophageal diameter and length before third dilatation.
5. The effect of comedication on the primary study outcome will be assessed using a stratified Mann-Whitney test with stratification for the treatment group.
6. The mean cortisol level over the first 3 months after the third dilatation will be compared between study groups with a linear regression model with adjustment for age and sex. No missing data are expected for the independent variables in the analysis of covariance models and the Cox proportional hazards regression models. In case of missing data for any of the outcomes, a complete case analysis (i.e. exclusion of the subjects who dropped out during the follow-up period) will be performed for the corresponding outcome.

Cost-effectiveness analysis

All medical and non-medical costs will be summated for each individual patient. Regarding the patient outcomes, the number of refractory strictures prevented and the number of dysphagia-free patients will be considered. Incremental cost-effectiveness ratios will be calculated, expressed as incremental costs per refractory stricture prevented and incremental costs per additional dysphagia-free patient. Analysis of uncertainty will be illustrated through cost-effectiveness planes (via bootstrapping).

As this RCT is done in an international setting, it must be recognized that the results may differ between countries because healthcare systems, treatment patterns and prices may vary. Therefore, country-level information will be collected. Data (especially resource quantities and cost prices) will be collected in all countries, and results will also be reported for each country separately. Next, a pooled summary calculation of the intervention's cost-effectiveness will be made, converting all costs into a common currency base (i.e. euros).

Adverse events and auditing

Adverse events will be handled according to the guidelines of the institutional review board (IRB) of the Erasmus Medical Centre. All adverse events will be registered during the study. Serious adverse events will be reported to the sponsor immediately and registered appropriately within 24 hours.

All participating sites will be audited once a year with monitoring of patient recruitment, source data verification, drug accountability and sample storage. The auditor will be independent and not involved in the study.

Benefits and risk assessment

The risks and burden associated with this study are minimal. Potential complications of esophageal steroid injections include adrenal suppression, perforation, intramural infection, *Candida* infection, mediastinitis and pleural effusion.[33] However, in previous studies, no adverse events have been reported in relation to the steroid injections (see Table 1). Additionally, Kenacort-A 10 is a slow-release medicine and therefore a gradual exposure. This implies that the likelihood of acute exposure to a high dose of steroids will be minimal.

The burden of filling out the questionnaires and taking a hair sample are negligible. Filling out the MFS and the iPCQ will take maximally 30 minutes. All participants will undergo one extra esophagram after the third dilatation procedure. The potential reduction in the number of anesthetic procedures needed for dilatations outweighs the burden and the radiation exposure of this esophagram. Potential benefits of intralesional steroid injections are fewer dilatation procedures needed, with concomitant fewer anesthetic procedures and hospital admissions and less risk of perforation.

Data management

All data will be handled confidentially and anonymously using OpenClinica V.3.12.2 (OpenClinica LLC, USA) for data collection. The questionnaires will be conducted through an online survey using LimeSurvey (LimeSurvey GmbH, Germany) and GemsTracker (Erasmus MC, Rotterdam, the Netherlands). All patient data will be coded using a subject identification code list. The local PI safeguards the key to the code; the sponsor will have access to these codes. The local PI will only have access to the data of patients of their own center; the sponsor will have access to the final trial dataset. All has been stated in a clinical trial site agreement signed by all participating sites.

ETHICS ANS DISSEMINATION

This study protocol was approved by the IRB of the Erasmus Medical Centre (MEC-2018-1586/NL65364.078.18). In case of any modifications of the protocol, a formal amendment will be submitted to the IRB. Approved changes will be communicated to all relevant parties according to the rules of the IRB. The protocol has currently been submitted for local ethical approval in Amsterdam (the Netherlands), Odense (Denmark), Copenhagen (Denmark), Stockholm (Sweden), Rome (Italy) and Padua (Italy). Nijmegen (the Netherlands) and Helsinki (Finland) have already obtained local ethical approval and joined the study. To guarantee

the respect of ethical rules and standard of care in all participating centers, the protocol was reviewed by the two chairs of the work package on EA within ERNICA. The informed consent and assent process of this trial is in line with the Good Clinical Practice guideline.[34]

Furthermore, we involved external experts in the form of a data safety monitoring board (DSMB). These experts are a pediatric surgeon, a pharmacologist and a statistician. All experts are independent of the sponsor and therefore competing interests will be avoided. The DSMB will monitor the safety of the study subjects and data. The board will meet at least three times: within 1 year of recruitment commencing, at the time of the planned interim analyses at 50% (n=55) of enrolment and at the conclusion of the trial.

The results of this trial will be published in an international peer-reviewed scientific journal, within 1 year after the end of the follow-up period of the last included patient. In addition, we aimed to present the results at several international conferences to inform healthcare professionals worldwide.

TRIAL STATUS

The study has started in Rotterdam (the Netherlands) in February 2019 after ethical approval had been obtained. The first patient is included. Nijmegen (the Netherlands) has joined the study in October 2019 and Helsinki (Finland) in December 2019. At this moment, Amsterdam (the Netherlands), Odense (Denmark), Copenhagen (Denmark), Stockholm (Sweden), Rome (Italy) and Padua (Italy) are still waiting for local ethical approval. We expect to start the study in these sites the latest in 2020. Considering the rarity of the disease, we expect to complete the inclusions and finish data collection for this study in 5 years.

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Supplementary material

To calculate the distribution of the number of dilatations in the steroid group, we assumed the following:

- The first 3 dilatations are unaffected by the intervention; thus the 4 patients with 3 dilatations in the observed data would still have 3 dilatations with steroid treatment.
- The 7 patients with 4 dilatations in the observed data would, if steroid treatment were applied, be equally divided over the categories 3 dilatations and 4 dilatations (3.5 patients in each category).
- The 9 patients with 5 dilatations in the observed data would, if steroid treatment were applied, have 4 dilatations.
- The 7 patients with 6 dilatations in the observed data would, if steroid treatment were applied, be equally divided over the categories 4 dilatations and 5 dilatations (3.5 patients in each category).
- The 16 patients with 7-10 dilatations in the observed data would, if steroid treatment were applied, be divided over the categories 5, 6 and 7-10 dilatations (patients with 7 dilatations would have 5 dilatations, patients with 8 dilatations would have 5 or 6 dilatations, patients with 9 dilatations would have 6 dilatations and patients with 10 dilatations would have 6 or 7 dilatations).
- The 10 patients with >10 dilatations in the observed data would, if steroid treatment were applied, be divided over the categories 7-10 and >10 dilatations (5 patients in each category).

These assumptions lead to the predicted numbers of patients shown in Supplementary Table 1. The relative frequency distribution in the steroid group is then calculated by dividing the predicted numbers of patients by the total number of 53 patients.

Supplementary Table 1. Assumed relative frequencies of the number of dilatations in the control and steroid groups, including the calculated predicted number of patients with steroid treatment. *See Supplementary Table 2 for the exact number of dilatations within these categories.

Number of dilatations within 28 days interval	Observed number of patients (n=407)[1]	Predicted number of patients with steroid treatment	Relative frequencies control group	Assumed relative frequencies steroid group
3 dilatations	4	7.5 (4+3.5)	0.075	0.142
4 dilatations	7	16 (3.5+9+3.5)	0.132	0.302
5 dilatations	9	9 (3.5+2+3.5)	0.170	0.170
6 dilatations	7	8.5 (3.5+3+2)	0.132	0.160
7-10 dilatations*	16	7 (2+5)	0.302	0.132
>10 dilatations*	10	5 (5)	0.189	0.094
Total (all numbers of dilatations combined)	53	53	1.000	1.000

Supplementary Table 2. Exact number of dilatations as extracted from the original dataset of the retrospective study in the Netherlands.[1]

Number of dilatations within 28 days interval	Observed number of patients (n=407)[1]
3 dilatations	4
4 dilatations	7
5 dilatations	9
6 dilatations	7
7 dilatations	2
8 dilatations	7
9 dilatations	3
10 dilatations	4
12 dilatations	2
13 dilatations	2
15 dilatations	1
18 dilatations	2
24 dilatations	1
30 dilatations	1
34 dilatations	1
Total (all numbers of dilatations combined)	53

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CHAPTER 7

Patient-driven health care recommendations for adults with esophageal atresia and their families

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ABSTRACT

Background

Adults with esophageal atresia (EA) require a multidisciplinary follow-up approach, taking into account gastroesophageal problems, respiratory problems and psychosocial wellbeing. Too little is known about the full scope of these individuals' healthcare needs. We aimed to map all medical and psychosocial needs of adults with EA and their family members, and to formulate healthcare recommendations for daily practice.

Methods

A qualitative study was performed, using data from recorded semi-structured interviews with two focus groups, one consisting of adult patients with EA (n=15) and one of their family members (n=13). After verbatim transcription and computerized thematic analysis, results were organized according to the International Classification of Functioning, Disability and Health. Ethical approval had been obtained.

Results

Healthcare needs were described through 74 codes, classified into 20 themes. Most important findings for patients included the impact of gastrointestinal and pulmonary problems on daily life, long- term emotional distress of patients and parents and the need of a standardized multidisciplinary follow- up program during both child- and adulthood.

Conclusion

The focus groups revealed numerous physical and mental health problems, as well as so- cial difficulties, that require attention from different healthcare providers. We have formulated several healthcare recommendations that physicians may use in long-term follow-up.

INTRODUCTION

Advancements in treatment strategies have led to increased survival rates of newborns with esophageal atresia (EA). Subsequently, more children with this rare congenital malformation nowadays reach adulthood. Many adults with EA, however, still experience sequelae: gastrointestinal symptoms such as dysphagia or gastroesophageal reflux (GER);[1, 2] respiratory problems such as wheezing, coughing or lung function abnormalities; or impaired exercise capacity due to pulmonary problems.[3] Therefore, we have ex tended our hospital's longitudinal multidisciplinary follow-up program for these children with transition to adult healthcare.[4]

An important element of follow-up is psychosocial wellbeing. In the past, the quality of life (QoL) of adults with EA has been assessed with different questionnaires. Overall, a normal health-related QoL was reported on the general SF-36 questionnaire.[5-7] However, when focusing on gastrointestinal symptoms with the Gastrointestinal Quality of Life Index, or on pulmonary complaints with the Respiratory Symptoms-Related Quality of Life Index, impaired QoL was reported.[7-10] The currently available literature does not sufficiently describe the healthcare needs of adults with EA. Questionnaires are either too broad or too specific, and do not address the specific problems encountered in daily life. Moreover, certain aspects have never been studied adequately such as mental problems or the impact on relationships. Recently started endoscopic surveillance programs for Barrett's esophagus and esophageal cancer have brought new burdens, [11] adult relationships could raise concerns about heredity, and parent-child relationships could be affected by events in the past. The impact of having a child with EA on parents has only been described for school-aged children.[12] The long-term effects of EA on mental health and family relationships have never been published.

It is recommended that patients with EA are followed up by physicians with expertise in EA care.[13] Thus far, too little is known about the full scope of healthcare needs of EA adults. Patient experience data are considered meaningful for healthcare improvement in terms of safety and effectiveness.[14] In focus groups, thoughts and feelings can be elicited by promoting self-disclosure among its participants.[15] The aim of this study was to map all medical and psychosocial healthcare needs of adult EA patients and their family members, resulting in the formulation of recommendations to be used in daily practice.

PATIENTS AND METHODS

This is a qualitative study using in-depth data from semi-structured focus group interviews addressing the worries, needs and preferences of EA adults and their family members. Approval from the institutional ethics review board had been obtained (MEC-2019–0160).

The COREQ (COnsolidated criteria for Reporting Qualitative studies) checklist was used as a reporting framework.[16]

Participants

Two focus groups were composed, aiming at 10-15 participants as recommended;[15] one consisting of adult patients with EA, the other of their family members and/or partners. Patients were randomly selected from all adults with EA currently participating in our follow-up program,[11] using an online tool (www.randomizer.org).[17] Patients were invited to participate through a personal letter, and were asked to invite their partner or a family member of their choice as well. Inclusion criteria were age ≥18 years, born with EA or a partner/family member of a patient with EA and Dutch-speaking. Written informed consent for interview recording was obtained from all participants.

Data collection

An expert team was formed for this study, consisting of a gastroenterologist (MS) and a pulmonologist (LK), both specialized in treating adults with EA; a pediatrician (SG), who is the coordinator of the standardized longitudinal follow-up program for children with congenital anomalies in our hospital and involved in the transition of children with EA to adult healthcare; a neuropsychologist (AR) with experience in moderating focus group interviews; an adult patient with EA; a representative of the Dutch patient association (JF) and a researcher (CtK). Prior to the focus group interviews, the expert team met to determine the interview topics, based on literature research and clinical experience.

Both focus group interviews were conducted on June 20th 2019, within the framework of a national symposium for adults with EA. The interviews were moderated by a male neuropsychologist (AR). After a brief introduction, the topics were introduced and with open questions participants were stimulated to discuss their worries and needs (see Supplementary File 1). The interviews were recorded audio-visually an transcribed verbatim. Additionally, participants were asked to fill out a questionnaire on baseline characteristics and, if applicable, physical complaints.

Data analysis

Transcripts were imported into the qualitative software ATLAS.ti 8.3.20 (Scientific Software Development GmbH, Berlin, Germany). Following the steps of thematic analysis,[18] the transcripts were reviewed and coded by two members of the research team (CtK and DL) independently and systematically. Initial codes covered the basic element of a text fragment, and were modified or merged during the analysis. Codes from both transcripts were combined into overarching themes. All codes and themes were reviewed by a third investigator (AR) and discussed until consensus was reached. The expert team reviewed the themes and supplemented clinically relevant subjects where necessary.

Next, the themes were structured according to the International Classification of Functioning, Disability and Health (ICF),[19] which describes five health-related domains: body functions and structures, activity, participation, personal factors and environmental factors. Results are described in a qualitative manner and illustrated by quotes extracted from the interviews (see Table 1). Quotes were translated from Dutch to English by forward-backward translation by a native speaker to validate consistency of the translation.

Educational levels were classified according to the International Standard Classification of Education (ISCED).[20] Descriptive data for the baseline characteristics were generated using SPSS V.24.0 (IBM, Chicago, Illinois, USA).

RESULTS

Participants

In June 2019, our follow-up program contained 195 adults with EA, of whom 55 had been randomly selected. Three patients were excluded because of intellectual disability. Therefore, 52 were invited for this study. Thirteen invitees did not respond, and 24 invitees refused because of lack of time or transportation. Thus, fifteen patients consented to participate. Eleven of them brought a family member; one brought two family members. In total, 28 participants were included: 15 patients and 13 family members (10 parents, 2 children and 1 partner). Based on the distribution of the baseline characteristics and physical complaints, we considered this sample representative for the EA population (see Table 2 and Supplementary Table 1).

The interview with the patient group lasted 62 minutes; that with the family member group 67 minutes. Thematic analysis identified 74 codes which could be classified into 20 themes (see Table 3). Eventually, no new codes could be identified, suggesting data saturation.

Patient perspectives

Physical and psychosocial problems

Childhood was characterized by frequent hospitalizations due to pulmonary infections or upper endoscopies for esophageal dilatations or stuck food boluses. The main problem in adulthood was dysphagia. All patients could eat solid food but had to drink water with every meal. Other major physical complaints were pain, postprandial bloating, coughing, impaired lung capacity and poor exercise capacity. Anti-reflux surgery at childhood because of GER has led to new forms of discomfort such as an inability to vomit. Coughing caused a poor night's sleep for some patients. Some patients are still afraid to visit the hospital or to undergo medical procedures.

Table 1. Extracted quotes from the focus group interviews. ICF = International Classification of Functioning, Disability and Health, GP = general practitioner

ICF classification	Theme	Quote
Body functions and structures	Physical problems	*"My mother was raised very protected. As a child, she was told that it was better not to exercise. Nowadays, she has a worse exercise capacity than her peers."* – daughter, 43 years old
	Mental health problems	*"My father is what I would call traumatized. Whenever I talk about it, he starts crying."* – patient, 32 years old
Activity & participation	Obstacles in daily life	*"Going to a restaurant is different for me than for others. I have to be careful with my choices."* – patient, 54 years old
	Social difficulties	*"With my first girlfriend, I pretended to have already eaten at home. I did not want to offend her parents by not being able to eat their food."* – patient, 21 years old
	Limitations in employment	*"One time, there was not enough time to eat, after which I started vomiting. Since then, they give me the time I need."* – patient, 21 years old
Personal factors	Feeling guilty	*"I'm an only child because I needed a lot of care. My father would have liked more children. Now that I have two healthy sons of my own, I feel like he has finally gotten the sons he always wished for."* – patient, 37 years old
Environmental factors	Specialized healthcare	*"Our GP told us that we should call the hospital if we had questions. He did not want anything to do with it."* – mother, 62 years old
	Follow-up and transition to adult healthcare	*"The transition to the adult hospital was awful. I could no longer stay with my child all day."* – mother, 64 years old
	Impact on family relationships	*"You go through a very intensive period together. For us, we grew as a couple. Whatever will come, we can handle it."* – father, 55 years old

Worries about the future

Pregnancy was stressful for patients due to concerns about heredity and the unborn baby's health. Endoscopic screening of the esophagus raised concerns about Barrett's esophagus. Some patients had not visited the hospital for 40 years. Older patients never had a proper explanation about their condition, and were happy to be finally informed.

Obstacles in daily life

Eating had the greatest impact on patients' everyday life. They needed more time to finish a meal than their peers, and always had to consider the type of food they ate. This made certain activities challenging, e.g. restaurant visits, a quick meal on the street or buffet meals. Some patients could not eat or drink anything in the evening, because this led to severe heartburn overnight. Some older patients were limited in their daily activities due to a poor exercise capacity. As a child, they were advised not to go outside in the winter or play sports, due to increased susceptibility to respiratory infections.

Social difficulties

Patients received negative comments about EA-related situations, e.g. when a stuck food bolus needed to be pushed through. Patients with coughing complaints were often unfairly criticized for smoking. Some older patients had large scars, whereas most of the younger patients had hardly visible scars. A possible coping strategy at school age was giving a talk about EA to their classmates.

The extent to which patients were open about having EA varied. In partner relationships, some patients had not informed their partner until having children was discussed. In friendships, some patients did not want to bother friends with their story. In general, patients kept their explanation short when informing people.

Table 2. Baseline characteristics of the focus group participants. Data are presented as median (range) or n (%). One patient and two family members have not filled out a questionnaire. EA = esophageal atresia, ISCED = International Standard Classification of Education, VACTERL = vertebral, anorectal, cardiac, tracheoesophageal, renal or limb anomalies. [A] According to Gross classification.[2] [B] Birth weight <10th centile.[3] [C] According to Solomon criteria.[4] * Five patients were 20-30 years old, six patients were 30-40 years old, three patients were 50-60 years old and one patient was 71 years old.

	Patients (n=15)	**Family members (n=13)**
Age (years)	32.6 (20.8-71.0) *	63.5 (22.7-67.1)
Male	10 (66.7)	5 (38.5)
Relationship to EA patient		
Parent of EA patient		10 (76.9)
Child of EA patient		2 (15.4)
Partner of EA patient		1 (7.7)
Educational level		
Low (ISCED 0-2)	4 (28.6)	4 (36.4)
Middle (ISCED 3-4)	2 (14.3)	3 (27.3)
High (ISCED 5-8)	8 (57.1)	4 (36.4)
Type of EA [A]		
Type A	1 (7.7)	
Type C	11 (84.6)	
Type E	1 (7.7)	
Gestational age in weeks	38.72 (32.0-43.0)	
Birth weight in grams	3100 (1465-3600)	
Preterm birth	4 (26.7)	
Small for gestational age [B]	4 (26.7)	
Staged repair	2 (13.3)	
VACTERL association [C]	2 (13.3)	
Dysphagia score[1]		
Grade 0	11 (78.6)	
Grade 1	3 (21.4)	

Table 3. Overview of identified themes and corresponding codes. ICF = International Classification of Functioning, Disability and Health, EA = esophageal atresia, GP = general practitioner

ICF classification	Themes	Codes
Body functions and structures	Physical problems (past)	Dilatations, eating disorder, food getting stuck, fundoplication, tracheoesophageal fistula, variable course of treatment, chocking incidents
	Physical problems (present)	Barrett's esophagus, vomiting, comorbidities, exercise capacity, food getting stuck, no complaints at all, general health, pulmonary problems, pain when eating, frequent hospital visits
	Mental problems	Anxiety of parents, anxiety of patients, worries, memories, optimism, home birth
	Worries	Heredity, reassurance
Activity & participation	Obstacles in daily life	Alcohol, exercise capacity, pulmonary problems, gastroesophageal reflux
	Sleeping	Sleeping
	Eating	Frustrations about eating, restaurant visit
	Illness	Pulmonary problems, frequent hospital visits
	Exercise	Sports
	Appearance	Scars, looks
	Social contacts	Insecurities of environment
	Maintaining relationships	Impact on relationship and family, relationships
Personal factors	Coping	Coping, resilience
	Feeling a patient	Feeling a patients, wish for medical identification
	Guilt	Feeling guilty as a child, feeling guilty as a parent
Environmental factors	Expertise in healthcare	Relationship with treating physician, need for specialized care, frustrations about healthcare, satisfied about healthcare, guidance in regional hospital, lack of expertise in regional hospital, positive experience with GP, negative experience with GP, late diagnosis, home birth, explanation by parents
	Support	Somebody to talk to, contact with fellow peers, pedagogical healthcare, support network, trauma processing
	Screening, follow-up and transition	Prenatal screening, follow-up childhood, transition to adult healthcare, surveillance program
	Family relationships	Parents involved during adulthood, parent-child bonding, parents as caregivers
	Interaction with environment	Frustrations about work, telling people about EA, getting attention from environment

At work, the main problem was the mealtime. Patients needed more time to finish their lunch than co-workers. Some did not get enough time from their boss; others found it hard to take the time they needed, feeling guilty for letting their work pile up.

Effects on personal life

Overall, patients became more resilient. Some still had trouble letting people get emotionally close due to hurtful comments or experiences from their youth. Others were full of fighting spirit, and would not give up easily. Patients felt guilty towards their siblings and parents as they grew up, for being born with EA and receiving so much attention.

Patients clearly stated that they no longer feel like a patient, or even found it annoying to be called a patient. Some started feeling like a patient again when they received the endoscopic surveillance invitation. Patients wished for medical identification to carry with them in case of emergency, e.g. a choking incident or a stuck food bolus.

Transition to adult healthcare

Patients remembered the pedagogical staff guiding them through unpleasant procedures or preparing them for surgery. Younger patients often had multiple check-ups, but older patients had not received any follow-up after their first year of life. They were surprised being invited for a check-up after several decades. Most patients did not know what EA entailed until this check-up. Patients appreciated the possibility of follow-up. It resulted in a better understanding, for example their pulmonary complaints finally fell into place.

Patients notice that general practitioners (GPs) lack medical expertise on EA. They expressed the desirability of designating one coordinating physician. Usually, the gastroenterologist was in charge, with which patients were satisfied.

Family perspectives

Psychosocial problems

All parents could vividly describe memories from the first years of their child's life. Parental anxiety was widespread during the perinatal period and remained over the years. Some parents had been told that the child would not survive. Parents were especially worried during pulmonary infections or choking incidents. Most parents still suffered from emotional problems, some parents even were severely traumatized by such experiences.

Social difficulties

Parents received negative comments as well, e.g. when they gave their child medication in public. Finding a babysitter was difficult for parents. People were afraid to look after their child and to administer medicines, for example.

Effects on personal life

Some parents found their child more sensitive or emotional than peers. Parents felt guilty towards their other children because it was difficult to divide attention. They also felt guilty towards themselves for neglecting their own health sometimes when their child was sick.

Specialized healthcare

Most parents had negative experiences with giving birth at regional hospitals due to the lack of medical expertise. At the academic hospital they felt reassured and got a satisfying explanation about the diagnosis, surgery and what to expect. Most common frustrations during hospitalizations were limited visiting ours or postponement of surgery. Another frustration was the lack of knowledge of the GP, who for example did not take the pulmonary problems of their child seriously. Parents could call a direct phone number from the hospital if they had any questions.

Parents emphasized the importance of receiving timely and proper information. When their newborn was transported to an academic hospital, often the mother stayed behind and remained uninformed about the condition or prognosis of her child for several days.

Parents experienced insufficient support from the hospital in the first period. They missed a professional to talk to. Although they turned to other sources of support – such as keeping a journal, or talking to family, friends or other parents on the ward – this was felt not enough compensation. For parents of younger patients, transition to adult healthcare was a major step.

Impact on family relationships

For parents, especially the first years were hard. Early parent-child bonding was difficult. Due to travel distances and lack of transportation, parents sometimes could not visit their child for several weeks. After a long and intensive hospital period, discharge home was an enormous transition. Regular maternity care that new parents usually receive was no longer available. Later, parents struggled between their roles as parent and caregiver because they had to give their child medication, tube feeding, or parenteral feeding.

The sick child represented also a large burden on the relationship between parents; sometimes resulting in divorce, sometimes strengthening the relationship. Parents felt it was difficult to remain strict in the child's upbringing. Parents had a hard time letting go of the child when it left home at adult age.

DISCUSSION

This study is the first to provide an overview of the medical and psychosocial health status of adults with EA and their family members. The patient-driven data from focus group interviews with patients and family members of different ages gives insight in the impact of the disease over time, which may help to optimize medical care and psychological guidance.

Body functions and structures

Various symptoms have been described in adolescents and adults with EA, such as regurgitation, heartburn, aspiration and dysphagia.[21] In our study, dysphagia was the main complaint. Dysphagia is often caused by delayed esophageal clearance due to disturbed motility.[22] Surprisingly, GER was not addressed as a problem. A likely explanation is that all patients in this study are followed by a gastroenterologist, and have been given anti-reflux medication if needed. From our own experience, we know that patients often will not bring up GER complaints themselves as they do not experience these as symptoms.[11] Hence, it is important to actively ask patients about this during follow-up.

In line with the literature, patients reported pulmonary complaints and poor exercise capacity. Previous studies described significantly more respiratory symptoms and infections in EA adults compared to controls ($p \leq 0.002$). Pulmonary function tests showed both obstructive, restrictive and combined lung disease.[21, 23, 24] Unfortunately, recent data on large cohorts is lacking. Nevertheless, considering the patients' experiences revealed in our study, we recommend that every adult with EA should be referred to a pulmonologist specialized in EA to optimize lung condition.

The poor exercise capacity of older patients (>50 years old) was striking. We know from previous studies that children with EA are at risk for decreased exercise tolerance,[25-28] possibly influenced by diminished physical activity as a child, which may be partially due to parental anxiety. A standardized follow-up program – which was not yet available during the childhood of these older patients – will allow intervention at an early stage. Adults with EA suffer from impaired performance capacity as well.[29] Still, pulmonary rehabilitation may improve exercise tolerance.[30] This emphasizes the importance of extending the multidisciplinary care approach for EA into adulthood.

Despite increasing pedagogical guidance in the last decades, hospital anxiety remained present even among younger patients. The relationship between preoperative anxiety and postoperative anxiety and sleeping problems in children is well known,[31] but the long-term effects of undergoing multiple procedures – like in EA – have not been studied.

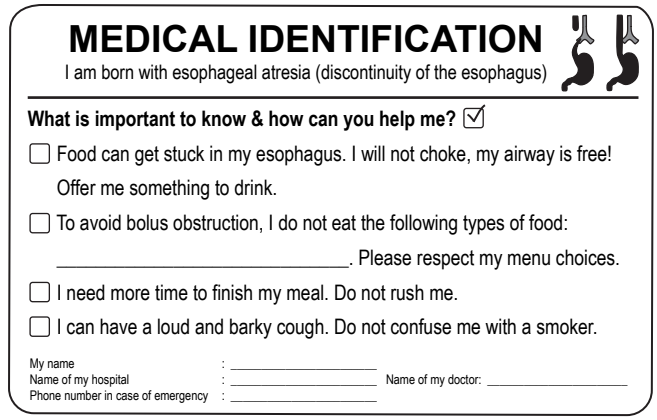
Likewise, in adults with EA anxiety associated with the endoscopic surveillance program should be considered. Given the relatively new nature of this program, this has not yet been investigated. In other surveillance programs, endoscopies were reported as burdensome, with elevated anxiety levels beforehand.[32] Participating in a follow-up program might cause problems in getting mortgages or insurance since it emphasizes the chronicity of EA. The attitudes of banks and insurance companies in this respect are still unknown.

Patients worried more around the fertile age and throughout pregnancy. Currently, non-syndromic EA is considered to have a multifactorial cause[33] with a recurrence risk of 2-4% for offspring.[34] However, many pathophysiological mechanisms still remain unclear. In our hospital, a geneticist is involved in the transition to adult healthcare. We strongly suggest personalized genetic counselling when there is an active child wish, preferably before pregnancy.

Activities and participation

Although all patients functioned autonomously, certain food- related activities remained difficult. Coping strategies (e.g. drinking water) prevent major limitations in daily life. EA could potentially influence one' working career. Sick leave, longer lunch breaks or taking days off for hospital visits may lead to potential career limitations.[35] Proper explanation and educational material such as brochures can be supportive in explaining EA to other people. Herein lies an imported role for healthcare providers and patient associations.

It is noteworthy that some patients found it offensive to be labelled as patients because they feel healthy. Healthcare providers should keep this in mind when addressing this population. Interestingly, patients expressed a wish for medical identification. A credit card-sized pass (see Figure 1) that can be adjusted, filled out and printed by each individual patient might fulfill this need.


MEDICAL IDENTIFICATION

I am born with esophageal atresia (discontinuity of the esophagus)

What is important to know & how can you help me? ☑

☐ Food can get stuck in my esophagus. I will not choke, my airway is free! Offer me something to drink.

☐ To avoid bolus obstruction, I do not eat the following types of food: _____. Please respect my menu choices.

☐ I need more time to finish my meal. Do not rush me.

☐ I can have a loud and barky cough. Do not confuse me with a smoker.

My name : _____

Name of my hospital : _____ Name of my doctor: _____

Phone number in case of emergency : _____

Figure 1. Example of a medical identification for patients with esophageal atresia. Patients can adjust, fill out and print this card themselves.

Personal and environmental factors

Lack of medical expertise is the main frustration for both patients and parents. Parents must be informed as soon as possible about their newborn's condition, even when the baby is not born at an academic hospital. Standardized follow-up programs might better ensure that patients and parents adequately understand all aspects and consequences of EA. This should be verified – and if necessary clarified – at transition to adult healthcare.

The follow-up for different specialties should by coordinated by one designated physician. A recent patient-led survey study found that half of the surveyed EA adults had no current healthcare provider.[36] In our opinion, the gastroenterologist would be best qualified as coordinating physician for adult patients. It is his responsibility to inform the GP about what EA implies and how GPs can anticipate to specific problems that patients can encounter.

Being separated from their child due to travel distances or lack of transportation was traumatizing for parents. Previous research acknowledged that hospitalization of a newborn can disturb the parent-infant relationship and attachment.[37] Today, in our hospital parents can stay with their child around-the-clock, in line with the family-centered care strategy that has been associated with improved outcomes.[38] We suggest all centers to offer this in order to promote parent-child bonding.

Although the results of this study are not sufficient to draw a conclusion on posttraumatic stress disorder (PTSD), parents showed multiple symptoms: re-experiencing traumatic events, avoiding certain situations and getting overly emotional.[39] A study among parents of school-aged children with EA reported PTSD in more than half of the parents, and increased levels of anxiety.[40] Similar results were found for parents of children with other congenital anomalies.[41] Feeling guilty – as parents in our study described – could be a possible risk factor for long-term PTSD.[42] One could wonder if enough attention is paid to trauma stressors during hospitalization.[43] It is recommended to provide sufficient information about support resources at discharge, including contact details of patient associations and primary care providers.[44] Also, professional psychological support may be offered to parents during initial hospitalization and follow-up, with awareness of the strain parents might experience because of their dual role as parent and caregiver.

Strengths and limitations

To our knowledge, this is the first qualitative study addressing the needs and worries of both adults with EA and their family members. Nowadays, patient-centered care and patients' perspectives become more and more important. Patient-driven data provides new insights that quantitative research cannot provide, such as persistent hospital anxiety or how to address these patients. The widespread age of the participants can be considered as both a strength and limitation. It represents the population on one hand, but complicates the

interpretation of the results on the other hand. Moreover, using the ICF classification to measure health and disability enabled us to identify the consequences for daily life. Despite the small sample size, data saturation suggests sufficient quality of the data. However, given the nature of this study, a quantitative analysis of the data was not possible.

Some limitations should be addressed. First, since patients were selected from our follow-up program in a tertiary hospital with a response rate of 29% (15 out of 52 patients), results may be influenced by a selection bias of well-informed and assertive participants. Second, although the focus groups were characterized by a safe atmosphere, some patients could have been reluctant to share particular feelings or concerns. Third, topics about childhood relied on memories, which could be less accurate for parents of older patients. Next to this, the Netherlands is a small and high-income country with well-organized healthcare. In contrast to many countries, home birth is common. Last, qualitative research is explorative and does not aim to represent the entire population. These are all facts that should be taken into account when extrapolating our results worldwide.

CONCLUSIONS

This qualitative study gives a unique insight into the health-care needs of adults with EA and their families. The focus groups revealed numerous physical and mental health problems and social difficulties, that require attention from different healthcare providers. Our findings therefore emphasize the importance of a structured, long-term, multidisciplinary follow-up program for these patients. We have formulated several healthcare recommendations that physicians may use (see Table 4).

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We thank all participants for their time and effort during the focus group interviews. We thank the Dutch patients association VOKS (Vereniging voor Ouderen en Kinderen met een Slokdarmafsluiting) for supporting this study and their input in the manuscript. We thank Evelien Huizinga for her contribution in the expert team as an adult patient with esophageal atresia. Finally, we thank Ko Hagoort, who provided editorial advice. This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Table 4. Healthcare recommendations to be used in daily practice when addressing adults with EA or their parents during childhood. EA = esophageal atresia, GER = gastroesophageal reflux, GP = general practitioner, PTSD = post-traumatic stress disorder

	Problem	Screen for	Provide
Adult patients	Gastrointestinal problems	Dysphagia, coping strategies, pain or discomfort during/ after the meal, GER	Inform about EA related problems Explain esophageal motility rather than stenosis Prescribe anti-reflux medication if necessary
		Risk for Barrett's esophagus and esophageal cancer	Inform about the risk and surveillance possibilities Refer to gastroenterologist for endoscopic surveillance program
	Pulmonary problems	Coughing, pulmonary infections, lung capacity, asthma	Inform about EA related problems Refer to a specialized pulmonologist (computed tomography scan, lung function tests)
		Exercise capacity	Start pulmonary rehabilitation, advise about sports/exercise
	Mental health problems	Anxiety for the hospital or medical procedures	Refer to psychologist if necessary Point out possibility of peer support through patients association
		Pregnancy-related worries	Refer to a clinical geneticist
	Social participation	Problems with telling people about having EA	Help patients find the right explanation Provide information material Provide medical identification (see Figure 1)
		Problem with emotions of connecting to people	Refer to psychologist if necessary Point out possibility of peer support through patients association
	Economic participation	Problems longer lunch breaks or taking a personal day, concerns about career	Help patients find the right explanation Provide information material Provide medical identification (see Figure 1)
		Concerns about mortgage or insurance	Provide information material Contact with patients association
	Specialized healthcare	Coordination of multidisciplinary follow-up	Appoint one coordinating physician, preferably the gastroenterologist
		Lack of knowledge GP	Inform healthcare provides about EA and EA related problems through folders, symposia or scientific journals.

Parents	Mental health problems	PTSD, feeling guilty	Be aware of mental complaints during hospitalization and follow-up Offer professional support during hospitalizations Refer to psychologist if suspicion of PTSD Point out possibility of peer support through patients association
		Disturbed parent-child interaction	Possibility of staying with their child around-the-clock
	Social participation	Reactions/fears from environment, finding a babysitter, getting comments when giving medication	Offer help of a social worker Point out possibility of peer support through patients association
	Specialized healthcare	Understanding diagnosis and prognosis	Explain condition as soon as suspected
		Coordination of multidisciplinary follow-up	Appoint one coordinating physician, preferably the pediatric surgeon
		Lack of knowledge GP or regional hospital	Inform healthcare provides about EA and EA related problems through folders, symposia or scientific journals.

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Supplementary File 1. Topics for the focus group interviews

Topics were presented to the focus group in a random order, following the natural course of the conversation. With open questions, the participants will be stimulated to discuss these topics.

1. Eating and drinking
- Experiences with eating in general
- Duration of each meal
- Restaurants and dinner parties, options on the menu, choices
- Coughing, choking, moments of stress during a meal
- Pain during or after a meal, bloated feeling
- Constipation, dumping syndrome, other physical complaints after a meal

2. Physical problems in general
- Sleeping and sleep problems due to pulmonary problems or gastroesophageal reflux
- Scars, reactions and comments from the environment, support from the hospital to cope with this
- Asymmetric thorax, scoliosis, other visible birth defects
- Exercise, sports, condition, overweight or underweight
- Motor skills, clumsiness
- Abdominal surgery in history, adhesions, bladder or anorectal problems, other VACTERL problems
- Course of complaints over time, new complaints with age or improvement
- Medication, coordination of medication (general practitioner or hospital)

3. Psychological burden
- Being in the hospital as a child, traumatic events, traumatic memories.
- Psychological support for the parents and the patients, support in general, support after discharge
- Preferences of support of patients and parents, during and after hospitalization
- Burden for the parents, how did patients experience this
- Involvement of siblings, partners, children, grandparents and other family members, burden for them
- Feelings of guilt
- Sleeping and sleep problems due to worrying

4. Perception as a patient
- Explanation of OA, having to explain to others what OA implies

- Differences between different specialties, functions of different medical specialists, presence of a coordinating physician, understanding the relationship between different symptoms.
- Coordinating physician, need for such a person
- Role of general practitioner in daily life and in treatment of OA, knowledge of general practitioner of OA related problems
- Do you feel like a patient?
- Sadness, anxiety or concerns of parents. What did patients notice of this?
- Hospitalizations: memories and association with hospital

5. Social life

- Response of environment, for example to barky cough
- Puberty, first boyfriends/girlfriends
- Telling friends or partner about having OA: how do you tell this, difficulties, what do you tell them, how do they respond
- Restaurants, diners, parties
- Exercising, hobbies

6. Work and future

- Finding a job, medical inspection, limitations in career opportunities, sick leave
- Understanding of boss and colleagues: sick leave, hospital visits, extended lunch break, time to take medication, fatigue
- Getting insurance, or mortgage
- Wish for children, questions about heredity or fertility (due to abdominal surgery in case of VACTERL association)

EA = esophageal atresia
VACTERL = vertebral, anorectal, cardiac, tracheoesophageal, renal or limb anomalies

Supplementary Table 1

Supplementary Table 1. Physical complaints of participating patients over the last weeks. Questionnaire was filled out in June 2019. One patient did not fill out a questionnaire. Some patients did not answer all questions.

Complaint	Patients (n=14)
Nausea	
Never	8
Rarely	3
Often	2
Missing	*1*
Vomiting	
Never	11
Rarely	1
Often	0
Missing	*2*
Pain when swallowing	
Never	9
Rarely	1
Often	2
Missing	*2*
Burping	
Never	6
Rarely	3
Often	3
Missing	*2*
Regurgitation	
Never	6
Rarely	7
Often	0
Missing	*1*
Food getting stuck in the esophagus	
Never	3
Rarely	6
Often	4
Missing	*1*
Choking	
Never	5
Rarely	6
Often	1
Missing	*2*
Gastroesophageal reflux	
Never	8
Rarely	3
Often	2
Missing	*1*
Chest pain	
Never	9
Rarely	2
Often	1
Missing	*2*

Coughing	
Never	4
Rarely	4
Often	5
Missing	*1*
Respiratory infections	
Never	7
Rarely	3
Often	2
Missing	*2*

CHAPTER 8

Recommendations for endoscopic surveillance after esophageal atresia repair in adults

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ABSTRACT

Background

Endoscopic surveillance of adults with esophageal atresia is advocated, but the optimal surveillance strategy remains uncertain. This study aimed to provide recommendations on appropriate starting age and intervals of endoscopic surveillance in adults with esophageal atresia.

Methods

Participants underwent standardized upper endoscopies with biopsies. Surveillance intervals of 3–5 years were applied, depending on age and histopathological results. Patient's age and time to development of (pre)malignant lesions were calculated.

Results

A total of 271 patients with esophageal atresia (55% male; median age at baseline endoscopy 26.7 (range 15.6-68.5) years; colon interposition n=17) were included. Barrett's esophagus was found in 19 (7%) patients (median age 32.3 (17.8-56.0) years at diagnosis). Youngest patient with a clinically relevant Barrett's esophagus was 20.9 years. Follow-up endoscopies were performed in 108 patients (40%; median follow-up time 4.6 years). During surveillance, four patients developed Barrett's esophagus but no dysplasia or cancer was found. One 45-year-old woman with a colon interposition developed an adenoma with high-grade dysplasia which was radically removed. Two new cases of esophageal carcinoma were diagnosed in patients (55 and 66 years old) who were not under surveillance. One of them had been curatively treated for esophageal carcinoma 13 years ago.

Conclusions

This study shows that endoscopic screening of patients with esophageal atresia, including those with a colon interposition, can be started at 20 years of age. Up to the age of 40 years a surveillance interval of 10 years appeared to be safe. Endoscopic surveillance may also be warranted for patients after curative esophageal cancer treatment.

INTRODUCTION

The high prevalence of gastroesophageal reflux (GER) and esophageal stasis of food and saliva in patients born with esophageal atresia (EA) has raised concerns about a possible increased risk of Barrett's esophagus (BE) and esophageal carcinoma.[1] BE is a premalignant lesion in which the squamous epithelium of the distal esophagus is replaced by gastric columnar epithelium containing goblet cells, which can progress into esophageal adenocarcinoma (EAC).[2] Multiple cases of esophageal carcinoma, both EAC and esophageal squamous cell carcinoma (ESCC), have been described in patients with EA at a relatively young age.[1] Given these concerns, endoscopic surveillance has been recommended in the European Society for Paediatric Gastroenterology Hepatology and Nutrition (ESPGHAN)-North American Society for Pediatric Gastroenterology, Hepatology and Nutrition (NASPGHAN) guideline.[3] These recommendations are based on expert opinions, since outcomes of standardized endoscopic surveillance programs in patients with EA are lacking.

In 2013, a prospective screening and surveillance program with upper endoscopies for adults with EA was initiated in our hospital. The initial screening of 151 patients revealed a 4-times higher prevalence of BE compared to the general population (6.6% vs. 1.6%) and at a much younger age (median age 34 years vs. 60 years).[4] This is in line with previous literature, in which a prevalence up to 12.5% has been reported.[1]

However, the optimal surveillance strategy remains unknown. The 5-year survival of esophageal carcinoma is known to improve to 90-95% when detected at an early stage.[5] Since no data exists on neoplastic progression times for both EAC and ESCC, relatively short surveillance intervals may be warranted to be on the safe site. On the other hand, endoscopic surveillance may cause an unnecessary burden – both physically and psychologically – for those not at risk, impact on endoscopic resources, and add substantial costs to the health care system. So far, no specific risk factors have been identified in this population, and longitudinal cohort studies on the yield of surveillance in this population are lacking.

The aim of this study was to assess the yield of an endoscopic surveillance program in adult patients with EA, and to assess patients' age and time of development of a (pre)malignant disease. These data may provide guidance in recommendations with regard to starting age and chosen interval of endoscopic surveillance programs for adults with EA.

MATERIALS AND METHODS

Study population

Details about the design of our screening and surveillance program have been described previously.[4] In short, since 2013 all patients with EAwho are enrolled in the longitudinal follow-up program for children born with congenital anomalies in our hospital,[6] are being routinely transferred to the Gastroenterology Department for further follow-up after the age of 17 years. From 2019 onwards,we have been expanding our cohort with patients from other Dutch university hospitals. Data were collected until January 2021. Endoscopies were performed both in our center and in general hospitals. Over 95% of the endoscopies were performed under conscious sedation (midazolam and fentanyl). The Institutional Review Board waived the need for formal ethical approval (MEC-2015-093).

Data collection

The following data were retrieved from the electronic patient records: date of birth, gender, type of EA according to Gross,[7] type of primary surgery, and relevant medical history including dilatations and prior diagnosis of gastroesophageal reflux disease (GERD) and/or BE. GERD was defined as the need for fundoplication surgery, pathological reflux established by pH monitoring, or signs of reflux esophagitis at a previous upper endoscopy.[8] Data on the presence of gastrointestinal symptoms, and use of medication, tobacco and alcohol were collected. Dysphagia was graded according to Mellow and Pinkas.[9] GER complaints were defined as chest pain, pyrosis, or regurgitation.

Endoscopies were performed according to a standardized protocol with white light and – in case of suspicion of BE – with narrow-band imaging.[4] Esophagitis and BE were graded according to the Los Angeles and Prague criteria, respectively.[10,11] At very endoscopy, four random biopsies were taken from the esophagus right above the gastroesophageal unction or above the proximal anastomosis (in case of bowel interposition). In case of BE, biopsies were taken according to the Seattle protocol.[12] In patients of 25 years and older, Lugol staining was applied to detect early squamous lesions.[13] All biopsies were reviewed by a gastrointestinal pathologist experienced with BE. Presence of esophagitis (including the number of eosinophils per high-power field), metaplasia and dysplasia were scored. Following the American College of Gastroenterology (ACG) guidelines, BE was defined as columnar metaplasia with the presence of goblet cells.[2] Short segment BE was defined as <3 cm, and long segment BE as ≥3 cm. Endoscopic and histologic findings were classified according to the most severe abnormality found.

In case of BE, surveillance intervals of the ACG guidelines were followed.2 In absence of BE, an interval of 5 years was applied for patients up to 30 years old, and of 3 years for patients ≥30 years old.[4]

Statistical analysis

Data are presented as number (%) or median (interquartile range). The yield of surveillance between the baseline endoscopies and follow-up endoscopies was determined by descriptive statistics. Baseline endoscopy was defined as the first endoscopy within the surveillance program. The patient's age at development of a premalignant lesion as well as the time to development of a premalignant lesion were calculated. The number of patient-years was the sum of the follow-up time between the baseline endoscopy and the last surveillance endoscopy of all patients who underwent at least one follow-up endoscopy. Derived from previous research,[14] the progression rate of BE development was calculated by dividing the proportion of progressive cases by the median follow-up time.

Clinical characteristics of patients without metaplasia were compared with patients with columnar metaplasia and with patients with BE (columnar metaplasia with presence of goblet cells), using Mann–Whitney U tests. Multivariable logistic mixed regression analysis was used to identify potential predictors of metaplasia. Columnar metaplasia and BE were selected as dependent variables, whereas age, history of GERD, gender, hiatus hernia, (prior) smoking, and (prior) use of alcohol were selected as independent variables. Cases with missing covariate values were excluded. Results were summarized as odds ratios (OR) and 95% confidence intervals (CI). Statistical analyses were performed using SPSS V.25.0 (IBM, Chicago, IL, USA) and R version 4.0.3 (R Foundation for Statistical Computing, Vienna, Austria), with a two-tailed significance level of $P<0.05$.

RESULTS

Patient characteristics

A total of 271 patients (55% male) participated in our surveillance program. Baseline characteristics are depicted in Table 1. The median age of these 271 patients was 26.7 (19.1-38.4) years at baseline endoscopy, ranging from 15.6 to 68.5 years. Of them, 96 patients underwent one follow-up endoscopy at a median age of 31.3 (24.1-40.3) years, after a median interval of 4.4 years. Twelve patients underwent a total of two follow-up endoscopies, with the last endoscopy at a median age of 30.9 (24.4-42.3) years, after a median interval of 2.7 years.

Yield of surveillance endoscopy

In total, 391 endoscopies have been performed in 271 patients since the start of the surveillance program. Endoscopic esophagitis was observed in 24 patients (9%). Columnar-lined esophagus was found in 73 patients (27%), with a circumferential extent of 0-5 cm, and a maximum extent of 0-6 cm. A hiatus hernia was present in 183 (68%) patients, with a length ranging from 1 to 10 cm. An inlet patch (ectopic gastric mucosa) was observed in 17 (6%) patients. No dysplastic squamous lesions were found.

Of the 73 patients with columnar-lined esophagus, histopathology revealed columnar metaplasia in 38 patients (14% of total cohort). Nineteen (50%) of them were male, and patients had a median age of 28.3 (22.8-33.6) years. BE was present in 19 patients (7% of total cohort), of whom 14 (74%) were male, and patients had a median age of 36.9 (24.9-51.8) years. No cases of dysplasia were found.

Table 1. Patient characteristics of 271 participants of surveillance program. Data are presented as n (%) or median (interquartile range, IQR). EA = esophageal atresia. [A] Livaditis myotomy (n=7). [B] Livaditis myotomy (n=7), ten Cate procedure (n=1), Rehbein procedure (n=1). [C] gastric pull up (n=2), colon interposition (n=17), jejunal interposition (n=3), ileocaecal interposition (n=1). [D] Nissen fundoplication (n=49), Thal fundoplication (n=2), unspecified (n=3). [E] Defined as chest pain, pyrosis, or regurgitation.

	n (%)
Male/female	150 (55.4)/121 (44.5)
Type of EA[24]	
Type A	26 (9.6)
Type B	1 (0.4)
Type C	213 (78.6)
Type D	5 (1.8)
Type E	5 (1.8)
Unknown	21 (7.7)
Type of surgery	
Primary anastomosis [A]	212 (78.2)
Delayed primary anastomosis [B]	21 (7.7)
Esophageal replacement [C]	23 (8.5)
Resection fistula	5 (1.8)
Unknown	10 (3.7)
History of ≥1 dilatation of esophageal stenosis	144 (53.1)
History of fundoplication [D]	54 (19.9)
Age in years, at time of first surveillance endoscopy; median (IQR)	26.7 (19.1-38.4)
Body mass index (kg/m^2); median (IQR)	22.4 (20.1-24.7)
Antireflux medication	
Yes, daily	37 (13.7)
Yes, when needed	7 (2.6)
No	213 (78.6)
Unknown	14 (5.2)
Tobacco smoking	
Yes	42 (15.5)
Former smoker, quit >2 years	39 (14.4)
No	179 (66.1)
Unknown	11 (4.1)
Alcohol consumption	
Yes, ≤7 units/week	156 (57.6)
Yes, >8 units/week	24 (8.9)
No	75 (27.7)
Unknown	16 (5.9)
Dysphagia score[25]	
Grade 0	199 (73.4)
Grade 1	53 (19.6)
Grade 2	2 (0.7)
Grade 3	2 (0.7)
Unknown	15 (5.5)
Gastroesophageal reflux complaints [E]	130 (48.0)

Of the remaining 16 patients with columnar-lined esophagus, histopathology showed either normal mucosa (n=1) or active esophagitis (presence of neutrophil granulocytes, n=13), or no biopsies were taken (n=2). Biopsies were lacking in 22 patients during surveillance endoscopy, due to discomfort of the patient or because of a protocol violation. Endoscopic and histologic findings are shown in Table 2.

Progression of lesions

Out of the 271 patients, 108 (40%) underwent at least one follow-up endoscopy. Table 3 shows progression of findings between the baseline and the last surveillance endoscopy. Of the 71 patients with normal mucosa at baseline, nine (13%) patients developed columnar-lined esophagus during surveillance. In one of them, histopathology confirmed BE. Additionally, BE was diagnosed in 2 out of 17 patients with columnar metaplasia at baseline. No progression to dysplasia or cancer was found in any of these patients. A total of 84 out of 95 patients (88%) did not show any progression between the endoscopies.

Time to premalignant development

Median follow-up time between the baseline endoscopy and the last surveillance endoscopy was 4.6 years, ranging from 2.0 to 7.8 years, resulting in a total of 495 patient-years. Out of the 19 patients with BE (see Table 4), two had a history of BE before the start of the surveillance program and 15 were diagnosed at baseline. The median age at first diagnosis of BE was 32.3 (24.4-47.7) years. The youngest age at which a clinically relevant BE (≥1 cm or presence of dysplasia, requiring surveillance[2]) was diagnosed was 20.9 years. This was a female without GER or dysphagia complaints.

Progression to BE within the surveillance program occurred in four patients (4% of the patients with ≥1 follow-up endoscopy) at a median age of 39.0 (24.4-54.1) years. Clinical details are depicted in Table 4. These four patients equal one case of progression to BE per 124 patient-years. Given the median follow-up time of 4.6 years, the progression rate of BE development was 0.8% per year.

Predictors of metaplasia

Compared to patients without metaplasia, patients with BE more often had an history of GERD (p=0.028), were older (p=0.015) and more often had a hiatus hernia (p=0.028) at time of diagnosis. Patients with columnar metaplasia more often had a hiatus hernia (p=0.035) compared to patients without metaplasia at time of diagnosis. See Table 5.

Multivariable logistic regression analysis did not show any significant associations between columnar metaplasia and the included variables (age, history of GERD, gender, hiatus hernia, (prior) smoking, and (prior) use of alcohol). Due to the limited number of BE cases, regression analysis was only possible for two variables (age and history of GERD). Both were associated

with an increased risk of BE development (OR 1.07, 95% CI 1.01-1.12 and OR 4.16, 95% CI 1.24-13.91, respectively). See Table 6.

Table 2. Endoscopic and histologic results from the last surveillance endoscopy in adults born with esophageal atresia (n=271). FU = follow-up, IQR = interquartile range, GERD = gastroesophageal reflux disease.[A] According to the Los Angeles classification.[10] [B] Endoscopy performed in general hospital, grade was missing in endoscopy report. [C] Short segment <3cm (n=63), long segment ≥3 cm (n=10). Circumferential extent ranges 0-5 cm, maximum extent ranges 0-6 cm. [D] Median length 2 (range 1-10) cm. [E] Macroscopic findings: normal mucosa (n=18), esophagitis (n=2) and gastric epithelium above the gastroesophageal junction (n=2).

		n (%)
Follow-up	Number of FU visits	
	1	163 (60.1)
	2	96 (35.4)
	3	12 (4.4)
	Age in years at last FU visit; median (IQR)	29.4 (22.5-38.9)
	<20 years	44 (16.2)
	20-30 years	93 (34.3)
	30-40 years	70 (25.8)
	40-50 years	40 (14.8)
	>50 years	24 (8.9)
	History of GERD	122 (45.0)
	History of Barrett's esophagus	10 (3.7)
Endoscopic findings	Normal esophagus	174 (64.2)
	Endoscopic esophagitis [A]	
	Grade A	21 (7.7)
	Grade B	2 (0.7)
	Grade unknown [B]	1 (0.4)
	Extension of gastric epithelium above the gastroesophageal junction [C]	
	With esophagitis	19 (7.0)
	Without esophagitis	54 (19.9)
	Secondary findings	
	Hiatus hernia [D]	183 (67.5)
	Inlet patch	17 (6.3)
Histologic findings	Normal mucosa	82 (30.3)
	Esophagitis	
	Mild	86 (31.7)
	Moderate	14 (5.2)
	Erosive	3 (1.1)
	Ulcerative	2 (0.7)
	Eosinophilic	5 (1.8)
	Columnar metaplasia	
	With esophagitis	25 (9.2)
	Without esophagitis	13 (4.8)
	Barrett's esophagus	
	With esophagitis	15 (5.5)
	Without esophagitis	4 (1.5)
	No biopsy taken [E]	22 (8.1)

Table 3. Progression in terms of endoscopic (A, upper table) and histologic (B, lower table) results at first surveillance endoscopy (baseline) compared with the last performed surveillance endoscopy (follow-up) (n=108). Gray color indicates patens with the same endoscopic or histologic result at both baseline and follow-up. GEJ = gastroesophageal junction, BE = Barrett's esophagus. [A] This is a macroscopic finding, which means that the histology could show either columnar metaplasia or Barrett's esophagus. [B] This includes both normal mucosa and esophagitis. [C] The clinical details of the four patients that developed BE during surveillance are depicted in Table 4.

	Baseline	No. of patients	Surveillance						
			Normal	Esophagitis	Gastric epithelium above GEJ [A]		Stable	Progression	Unknown
A	Normal	71	57	5	9		57 (80%)	14 (20%)	
	Esophagitis	11	6	1	4		7 (64%)	4 (36%)	
	Gastric epithelium above GEJ [A]	26	-	1	25		24 (100%)	-	
			Normal [B]	**Columnar metaplasia**	**BE [C]**	**No biopsy**			
B	Normal [B]	81	64	7	1	9	64 (79%)	8 (10%)	9 (11%)
	Columnar metaplasia	17	-	11	2	4	11 (65%)	2 (12%)	4 (24%)
	BE	9	-	-	9	-	9 (100%)	-	
	No biopsy	1	-	-	1	-	-	-	1 (100%)

Table 4. Summary of the patients with BE (n=19). BMI = body mass index, GER = gastroesophageal reflux, FU = follow-up. [A] These patients were already diagnosed with BE before the start of the surveillance program, respectively at 45.3 and 56.0 years old. [B] These patients underwent two follow-up surveillance endoscopies. Interval with previous endoscopy respectively 5.3 and 3.0 years, both showing columnar metaplasia.

Case	Age at diagnosis	Gender	BMI	Dysphagia	GER complaints	Alcohol	Antireflux medication	Prague criteria	FU visit	Baseline result	FU interval (years)
1	17.8	Male	20.1	Grade 0	No	Yes, ≤7 units/week	No	C0M0.5	Baseline		
2	20.9	Female	Unknown	Grade 0	No	Yes, ≤7 units/week	No	C0M2	Baseline		
3	22.0	Male	21.6	Grade 0	Yes	No	Daily	C2M0	Baseline		
4	23.4	Male	21.5	Grade 0	No	Yes, ≤7 units/week	No	C2M6	Baseline		
5	24.2	Male	22.6	Grade 0	No	Yes, >8 units/week	No	C0M0.5	FU 1	Normal	5.9
6	24.5	Female	18.2	Grade 0	Yes	No	No	C0M2	FU 2	Columnar metaplasia	6.3 [B]
7	25.3	Female	24.5	Grade 0	No	Yes, ≤7 units/week	No	C1M2	Baseline		
8	30.6	Male	16.2	Grade 0	No	No	Daily	C0M0.5	Baseline		
9	31.8	Male	Unknown	Grade 0	No	Yes, ≤7 units/week	No	C1M1	Baseline		
10	32.3	Male	Unknown	Grade 1	Yes	Yes, ≤7 units/week	No	C2M3	Baseline		
11	38.6	Female	38.8	Grade 1	Yes	Yes, ≤7 units/week	Daily	C0M2	Baseline		
12	39.3	Female	23.4	Grade 1	Yes	Yes, ≤7 units/week	Daily	C5M5	Baseline		
13	44.9	Male	23.4	Grade 0	Yes	Yes, ≤7 units/week	Daily	C0M2	Baseline		
14	50.2	Male	26.6	Grade 0	No	Yes, ≤7 units/week	Daily	C0M0.5	Baseline		
15	52.7 [A]	Male	17.6	Grade 1	Yes	Unknown	Daily	C1M3	Baseline		
16	53.5	Male	23.1	Grade 0	Yes	No	Daily	C1M1	FU 1	No biopsy	3.3
17	54.3	Male	23.6	Grade 0	Yes	No	Daily	C0M1	Baseline		
18	54.6	Male	Unknown	Grade 1	No	No	Daily	C0M0.5	FU 2	Columnar metaplasia	7.2 [B]
19	58.3 [A]	Male	24.7	Unknown	No	Yes, ≤7 units/week	No	C3M3	Baseline		

Table 5. Clinical characteristics at time of the last surveillance endoscopy in adults born with esophageal atresia (EA), participating in our screening and surveillance program. Data are presented as n (%) or median (interquartile range). Asterisk indicates significance (p<0.05). [A] Patients without metaplasia versus patients with columnar metaplasia (including Barrett's esophagus). [B] Patients without metaplasia versus patients with Barrett's esophagus. In case no biopsies were taken during the last surveillance endoscopy, patients were excluded from this analysis (n=22). [C] According to the Gross classification[24]. GERD = gastroesophageal reflux disease, BE = Barrett's esophagus, BMI = body mass index, GER = gastroesophageal reflux

	No metaplasia (n=192)	**Columnar metaplasia (including BE) (n=57)**		**Barrett's esophagus (n=19)**	
			p-value [A]		*p-value* [B]
Male	109 (56.8)	33 (57.9)	0.502	14 (73.7)	0.222
EA type A [C]	15 (7.8)	4 (7.0)	0.552	2 (10.5)	0.655
Esophageal replacement	14 (7.3)	1 (1.8)	0.103	1 (5.3)	1.000
History of ≥1 dilatation of esophageal stenosis	101 (52.6)	37 (64.9)	0.275	12 (63.2)	0.508
History of fundoplication surgery	35 (18.2)	15 (26.3)	0.191	6 (31.6)	0.219
History of GERD	87 (45.3)	29 (50.9)	0.546	14 (73.7)	**0.028 ***
(Prior) smoking	58 (30.2)	17 (29.8)	1.000	9 (47.4)	0.197
(Prior) use of alcohol	138 (71.9)	38 (66.7)	0.240	14 (73.7)	1.000
At time of last diagnosis of no metaplasia, or first diagnosis of columnar metaplasia/BE					
Age in years	29.3 (22.2-39.0)	28.2 (22.9-38.8)	0.694	36.9 (24.5-53.5)	**0.015 ***
BMI (kg/m$^{2)}$	22.7 (20.4-24.8)	22.4 (20.1-24.7)	0.821	22.9 (20.9-24.5)	0.998
Antireflux medication	40 (20.8)	17 (29.8)	0.170	6 (31.6)	0.457
Dysphagia	91 (47.4)	28 (49.1)	0.857	10 (52.6)	0.769
GER complaints	89 (46.4)	27 (47.4)	0.757	9 (47.4)	1.000
Hiatus hernia	129 (67.2)	47 (82.5)	**0.035 ***	17 (89.5)	**0.028 ***

Table 6. Results of multivariable logistic regression analysis. BE = Barrett's esophagus, OR = odds ratio, CI = confidence interval, GERD = gastroesophageal reflux disease

	Columnar metaplasia (including BE) (n=87)		**Barrett's esophagus (n=30)**	
	OR	*95% CI*	*OR*	*95% CI*
Age	1.02	0.99-1.05	1.07	1.01-1.12
History of GERD	1.26	0.72-2.20	4.16	1.24-13.91
Male gender	0.83	0.43-1.58		
Hiatus hernia	1.75	0.88-3.46		
(Prior) smoking	1.03	0.54-1.99		
(Prior) use of alcohol	0.85	0.48-1.51		

Esophageal carcinoma

Since the start of our surveillance program, two new cases of esophageal carcinoma were diagnosed in patients with EA at our hospital. One patient was a 68-year-old female with ESCC, who was not included in our endoscopic surveillance program. She is currently being treated with chemoradiotherapy. The other case was a tumor-recurrence in one of the previously described ESCC patients.[4] He was treated with curative intent by chemoradiotherapy for an unresectable ESCC at the age of 42 years. After oncological discharge – and start of our surveillance program in 2013 – he was invited for endoscopic surveillance, but refused. At the age of 55 years, he returned with complaints of dysphagia, and endoscopy revealed a recurrent ESCC. He is currently scheduled for a laryngopharyngoesophagectomy with esophageal replacement by a jejunal interposition. This is the second patient with EA in our hospital in whom the esophageal cancer has reoccurred more than 10 years after curative oncological treatment.[4]

Besides these two patients with esophageal carcinoma, a tubular adenoma with high-grade dysplasia was detected in a 45-year-old woman with a colon interposition, which was radically removed by endoscopy. She will undergo a surveillance endoscopy in 3 years. This is the second patient who developed a neoplasm of the colon interposition. Earlier, we described a 48-year-old male with an adenocarcinoma in his colon interposition.[4]

DISCUSSION

This is the first prospective cohort study reporting on the yield of standardized endoscopic surveillance in adult patients with EA. We confirmed a 5-fold higher prevalence of BE in these patients compared to the general population (7% vs. 1.3-1.6%).[15] Four (4%) new cases of short segment BE were found during follow-up. Overall, patients had a median age of 32.3 years at first diagnosis of BE, and the youngest patient with a clinically relevant BE was diagnosed at the age of 20.9 years. No dysplasia nor cancer was found in any of the patients

who participated in the surveillance program. In 1 of the 17 patients with a colon interposition a tubular adenoma with highgrade dysplasia was found.

Based on the data we recommend endoscopic surveillance of all adults with EA, including those with a colon interposition. It seems safe to start surveillance at the age of 20 years and to extent the interval to 10 years up to the age of 40 years, since the youngest patient with a clinically relevant BE was 20.9 years old and no dysplasia nor malignances were detected in patients younger than 40 years. In addition, in patients who have developed esophageal cancer and have been treated with curative intent endoscopic surveillance of the remaining esophagus may be warranted.

Endoscopic screening for BE and esophageal carcinoma is not recommended for the general population.[2, 16] Screening in the general population can be considered in males with >5 years and/or weekly GER symptoms plus ≥2 predetermined risk factors, such as age >50 years, Caucasian race, presence of central obesity (waist circumference >102 cm or waist-hip ratio >0.9), history of smoking, or a family history of BE or EAC.[2] However, given the current literature showing an increased risk of EAC and ESCC in adults with EA and the fact that these patients often do not recognize GER symptoms or complaints of dysphagia, the ESPGHAN-NASPGHAN guideline recommends endoscopic surveillance in adults with EA every 5-10 years, starting at time of transition into adulthood without any other screening criteria.[3]

Over the years, different endoscopic surveillance strategies have been proposed for adults with EA. The optimal age to start endoscopic screening remains a topic of discussion. Rintala et al. recommended upper endoscopy at the age of 15, 30, 40, 50, and 60 years.[17] In case of erosive esophagitis, columnar metaplasia, stricture formation, recurrent tracheoesophageal fistula or severe GER symptoms and/or need for chronic GER medication, the surveillance interval should be decreased to 5 years. In case of BE, they advised to repeat endoscopy after 1 year. In a study from the USA, it was suggested to screen all patients at the age of 10 years.[18] In case of erosive esophagitis, endoscopy needed to be repeated after 3-4 months of antireflux therapy. In case of columnar metaplasia or BE, endoscopy should be repeated after 3-5 years. No recommendations were made for patients with a negative endoscopy at the age of 10 years. A French research group recommended endoscopic screening before transition to adult health care.[19] In case of columnar metaplasia or BE, surveillance should be performed every 3 years. Otherwise, endoscopy should be repeated every 5-10 years. The abovementioned recommendations are all expert opinions based on retrospective or small studies in patients with EA at a relatively young age.

Some studies recommend to start endoscopic screening already during childhood,18 other advices vary between 15 and 30 years as starting age.[17, 19-21] In our pediatric follow-up program, children do not undergo surveillance endoscopies in childhood since this would require

general anesthesia. Therefore, we scheduled the first surveillance endoscopy at the age of transition to adulthood, namely 17-18 years of age. When evaluating the 391 endoscopies in this study, the youngest patient diagnosed with a clinically relevant premalignant lesion was 20.9 years old. Therefore, we propose to start endoscopic screening adults with EA from the age of 20 years onwards (see Figure 1). However, one can consider to maintain the transition to an adult gastroenterologist at the age of 17-18 years, in order to keep these patients in follow-up and provide them a contact person in case they do develop symptoms.

Currently, the interval in our surveillance program is 3 or 5 years, depending on the patient's age. In the present study, of the 98 patients without BE at baseline (normal mucosa n=81, columnar metaplasia n=17), only three progressed to BE (short segment) during surveillance. A quarter of the patients in our surveillance program are 30-40 years old, and no cases of neoplasm were found in this age category. Based on these findings, combined with the determined progression rate to BE development of 0.8% per year, we consider it justified to extent the surveillance interval to 10 years for patients up to 40 years old in case no BE has been diagnosed. After the age of 40 years, we still suggest intervals of 5 years due to the observed increased incidence of ESCC in adults with EA from the age of 40 years.[4] Over time, it should be evaluated whether surveillance intervals may also be extended for patients ≥40 years old. Furthermore, we recommend to perform chromoendoscopy with Lugol's staining in patients ≥30 years and in patients who have been previously curatively treated for esophageal cancer, to detect dysplasia and early ESCC. Currently, we take biopsies at every endoscopy from all lesions and at random just proximal of the gastroesophageal junction. The latter is done based on the fact that most of the ESCC were located in the distal esophagus. In case of BE, surveillance remains according to the ACG guidelines, regardless of age.

So far, we have found two patients with EA with a (pre)neoplasm in the colon interposition. One case was a moderately differentiated adenocarcinoma (ypT2N1M0) in a 47-year-old male (previously described[22]), who was not under surveillance. The other case was described in this study and concerned a 45-year-old woman with a tubular adenoma with high-grade dysplasia detected during surveillance. Although number of cases are small, based on the prevalence (5%) and young age of onset, we currently would recommend to include patients with EA and a colon interposition in the endoscopic surveillance program as well.

Same matters for endoscopic surveillance after oncological treatment for esophageal cancer in patients with EA. Due to the fact that these patients evelop esophageal cancer at a relatively young age, they have potentially more life years to gain. Our findings may underline the importance of endoscopic surveillance after the patient is discharged from oncological check-ups.

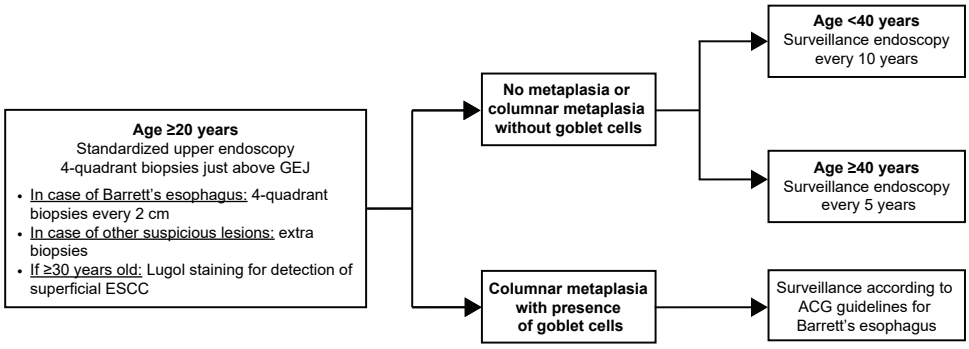

Figure 1. Flowchart of the updated screening and surveillance program for adults patients with esophageal atresia. GEJ = gastroesophageal junction, ESCC = esophageal squamous cell carcinoma, ACG = American College of Gastroenterology[2]

In the past, questions have been raised about the effect of screening on survival rates, taking into account the side effects and the cost-effectiveness.[23] The current median follow-up time of 4.6 years over 495 patient-years is too short to draw any conclusions on long-termsurvival. However, two new ESCCs have developed in patients who did not participate the surveillance program. It is known and confirmed by our results that GER complaints and dysphagia are underreported by patients with EA, due to a different perception of symptoms. Since these complaints have often been present their whole life, they do not recognize them as such. Standardized surveillance of adults with EA therefore may lead to early diagnosis of malignancies in this specific population.

On the other hand, repeated endoscopies may also form a psychological burden for patients, as well as additional costs to the health care system. These two aspects require further research, and would be helpful for future harm-benefit analyses in which potential complications of upper endoscopies should be taken into account as well.

The main strengths of our study are the prospective data collection within the infrastructure of a standardized surveillance program, and the large sample of patients born with this rare congenital anomaly. Yet, some limitations should be addressed. First, our study has a limited median follow-up time of 4.6 years. To be able to draw conclusions on the benefit of screening on survival, longer follow-up is required. Second, in 13 patients no biopsies were taken during follow-up endoscopy. This is explained by the fact that in some general hospital standard biopsies were not taken in case no endoscopic lesions were found. Last, only a few patients developed BE and therefore identification of clinical predictive factors with multivariable analysis was not applicable. This illustrates the importance of expanding the cohort. We pursue collaboration and merge of data within national and international networks, such as the Dutch Consortium of Esophageal Atresia and the European Reference Network for Rare Inherited Congenital Anomalies, in which we both are involved.

CONCLUSION

Our study underlines the importance of standardized endoscopic surveillance for all adults with EA, including those with a bowel interposition. Although the yield of new cases of BE warrants surveillance endoscopies, even if no abnormalities have been found at baseline, our findings justify to start screening at the age of 20 years with a surveillance interval of 10 years up to the age of 40 years (see Figure 1). Patients with EA who have survived esophageal carcinoma may also benefit from endoscopic surveillance of the remnant esophagus.

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QUALITY OF LIFE

CHAPTER 9

Longitudinal health status and quality of life after esophageal atresia repair

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ABSTRACT

Objectives

To longitudinally evaluate self-reported and proxy-reported health status (HS) and quality of life (QoL) of school-aged children born with esophageal atresia (EA).

Methods

We obtained Pediatric Quality of Life Inventory (HS) and DUX-25 (QoL) questionnaires from children born with EA between 1999 and 2011 at 8 and/or 12 years old. Children completed self-reports during neuropsychological assessments in a prospective longitudinal follow-up program. Parents filled out proxy-reports at home. Total and subscale scores were evaluated longitudinally and compared with sex-specific reference norms.

Results

In total, 110 participants (62% boys) were included. Self-reported HS improved significantly between 8 and 12 years for both boys (mean difference (md) 4.35, effect size (ES) 0.54, p=0.009) and girls (md 3.26, ES 0.63, p=0.004). Proxy-reported HS tended to improve over time, while self-reported and proxy-reported QoL tended to decline. Self-reported HS at 8 years was below normal for both boys (md-5.44, ES-0.35, p<0.001) and girls (md-7.61, ES-0.32, p<0.001). Girls' self-reported QoL was below normal at 8 (md-5.00, ES-0.18, p=0.019) and 12 years (md-10.50, ES-0.26, p=0.001). Parents reported normal HS at both ages, whereas they rated the QoL of their daughters below normal at 12 years (md-10.00, ES -0.16, p=0.022). All above results are total scores.

Conclusions

Self-reported and proxy-reported HS of children with EA improved between 8 and 12 years, while their QoL tended to decline. We recommend to consider HS and QoL as two separate concepts and to measure both simultaneously and longitudinally when evaluating the burden of disease.

INTRODUCTION

Improved treatment strategies have led to lower mortality rates of newborns with esophageal atresia (EA).[1] Long-term morbidities remain a topic of attention in these children. Children with EA not only suffer from esophageal dysmotility and gastroesophageal reflux,[2,3] but school-aged children are also known to have an impaired motor function, reduced exercise capacity, and airflow obstruction.[4-6] Moreover, those with low birth weight and those who underwent fundoplication surgery had growth problems at an older age.[7] As the standard of care, every child born with EA is prospectively included in a multidisciplinary standardized follow-up program since 1999.[8] Health status (HS) and quality of life (QoL) are two separate concepts, which are equally important and can be distinguished according to the World Health Organization (WHO) criteria.[9, 10] HS (also known as functioning, disability and health (FDH)) focuses on potential limitations and well-being in terms of physical, mental, and social functioning.[9] QoL describes an individual's perception of their position in life in relation to their expectations, and focuses on being bothered by potential limitations.[10] Both are important when evaluating the burden of disease during follow-up.

Two reviews have summarized the literature regarding self-reported and proxy-reported HS and QoL in patients born with EA.[11, 12] Although conflicting results have been published, literature in general suggests lower scores in children born with EA compared with the general population.[13-15] Differing questionnaires, study designs, and study populations preclude comparison of different studies. The Pediatric Quality of Life Inventory (PedsQL) questionnaire has been used most frequently. The conceptual content of several questionnaires according to the WHO criteria was reviewed by Fayed et al. These authors concluded that the PedsQL's content represented FDH (i.e. HS) instead of QoL.[16] The DUX-25 (Dutch-Child-AZL-TNO-Quality-of-Life) questionnaire measures a patient's appreciation of their daily functioning and therefore represents QoL.[17] HS and QoL have never been studied simultaneously or longitudinally in children born with EA.

Based on our observations that associated long-term morbidities in children born with EA persist but tend to diminish over time,[4,6,7] we hypothesized that HS would improve over time as well. Next, as EA can be considered a chronic health condition, QoL might decline over time as children become more aware of being different from peers. In this study, we longitudinally evaluated self-reported and proxy-reported HS and QoL of children with EA at the ages of 8 and/or 12 years. Moreover, we compared the results with recently collected norm data.

METHODS

Study population

We retrospectively evaluated available data from children born with EA between January 1999 and December 2011 who had joined our prospective longitudinal follow-up program for children with congenital anomalies.[18] Data were collected until April 2020. The Institutional Review Board declared that the Medical Research Involving Human Subjects Act was not applicable for this study (MEC-2017-185).

Data collection

During neuropsychological assessments, children routinely filled out two age-appropriate, internationally validated questionnaires (PedsQL 4.0 and DUX-25) on paper.[17,19] Proxy-reports were filled out by one of the parents at home before the outpatient clinic visit; we used paper-and-pencil versions until 2012 and an online survey thereafter. Results of the PedsQL were taken to represent HS and results of the DUX-25 were taken to represent QoL, since the latter takes the child's perspective into consideration. See Supplementary Material 1 for a detailed description of each questionnaire and of the different domains covered.

We collected the following data from electronic patient records: sex, gestational age, birth weight, type of EA according to Gross,[20] type of primary surgery, duration of anesthetic exposure within the first 24 months of life, other anomalies, educational level of the child, and highest maternal educational level (MEL). EA was considered a long gap in any case of staged repair, that is when primary anastomosis was not feasible at first attempt. Small for gestational age was defined as birth weight <10th percentile.[21] Duration of anesthetic exposure was defined as start of anesthetic procedures until departure from the theatre. Anomalies were classified as major if they required surgical intervention or frequent hospital visits. VACTERL (vertebral, anorectal, cardiac, tracheoesophageal, renal, limb anomalies) associationwas defined according to Solomon.[22] MEL was classified according to the International Standard Classification of Education (ISCED).[23]

Statistical analysis

Data are presented as number (%) or as median (interquartile range). Since most data were not distributed normally (see Supplementary Material 2), nonparametric tests were used. Demographic variables were compared between participants and non-participants – those who did not fill out either of the questionnaires – using a Fisher exact test or a Mann-Whitney test. Items of both questionnaires were answered on a 5-point Likert scale, reversed, and linearly transferred to a 0-100 scale. Subscales and total scores were computed by the mean, with a maximum of 50% missing items per subscale.

For the longitudinal assessment of PedsQL and DUX-25 scores, we only included children or parents who had filled out the questionnaires at both 8 and 12 years. Scores differences

between 8 and 12 years were determined with a Wilcoxon signed-rank test. For the cross-sectional comparison of the PedsQL and DUX-25 scores with norm data, we included all available self-reports and proxy-reports at 8 and/or 12 years. Scores were compared with recent age and sex-specific norm data from healthy Dutch children (see Supplementary Material 1) using a Mann-Whitney test, the raw data of which were requested from the authors. Effect sizes (ES) were calculated by converting z scores (r=z/√n).[24]

To assess the influence of potential factors associated with HS and QoL, we performed multivariable linear regression analyses. Total PedsQL and DUX-25 scores were selected as dependent variables, whereas sex, MEL, VACTERL association, and staged repair were selected as independent variables. Results were summarized as regression coefficients (B), 95% confidence intervals (CI), and p-values.

Statistical analyses were performed using SPSS V.24.0 (IBM, Chicago, Illinois, USA), with a significance level of P<0.05. Given the explorative nature of this study, we did not correct for multiple comparisons.

RESULTS

Patient characteristics

Between 1999 and 2011, 180 children born with EA were treated in our hospital. Twenty-two (13%) children had died within the first year, and 12 (7%) had a severe intellectual disability. Of the remaining 146 eligible children, 110 (75%) had been assessed at 8 and/or 12 years (Figure 1 details the number of participants for each part of the data analysis). Participants more frequently had EA type C and fewer associated anomalies than non-participants (see Table 1). At 8 years, 102 (93%) children attended regular education, which proportion is in line with the Dutch educational system.[25]

Longitudinal evaluation of self-reported and proxy-reported health status and quality of life

Of the 110 participants, 45 children (31% of eligible) and 44 parents (30% of eligible) filled out the PedsQL at both 8 and 12 years. Total scores of the self-reported PedsQL improved between 8 and 12 years for both boys (n=24, ES 0.54, p=0.009) and girls (n=21, ES 0.63, p=0.004). For boys, physical functioning (ES 0.47, p=0.021), emotional functioning (ES 0.53, p=0.010), and psychosocial health (ES 0.54, p=0.009) improved. For girls, physical functioning (ES 0.58, p=0.008), social functioning (ES 0.54, p=0.014), and psychosocial health (ES 0.52, p=0.018) improved. Proxy-reported total and subscale PedsQL scores did not change significantly over time (total n=44), except for girls' school functioning (n=21, ES 0.46, p=0.035).

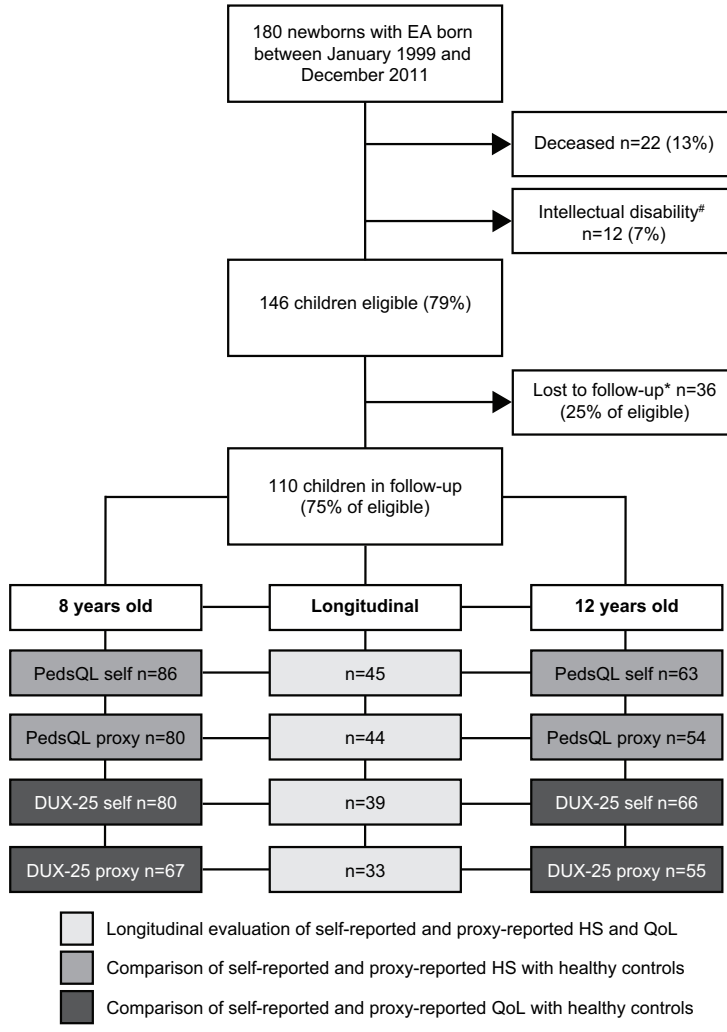

Figure 1. Flowchart of included patients. EA = esophageal atresia, HS = health status, QoL = quality of life. # Down syndrome n=5, Opitz syndrome n=1, Goldenhar syndrome n=1, Mandibulofaciale Dysostosis Guion Almeida type n=1, other n=4. * emigration n=6, organization reasons n=12, refusal n=9, follow up elsewhere n=5, untraceable n=4.

Of the 110 participants, 39 children (27% of eligible) and 33 parents (23% of eligible) filled out the DUX-25 at both 8 and 12 years. Total scores of the self-reported DUX-25 did not change significantly over time (total n=39); however, for 20 boys, close social functioning declined (ES-0.70, p=0.002), while far social functioning improved (ES 0.51, p=0.02). For 19 girls, physical functioning declined (ES-0.66, p=0.004). Proxy-reported total DUX-25 scores did not change significantly over time (total n=33). Parents reported that physical functioning declined over time for girls (n=15, ES-0.59, p=0.022).

Table 1. Demographic variables of participating and non-participating children with esophageal atresia (EA). Data are presented as n (%) or median (range). Asterisk indicates significance (p<0.05). ISCED = International classification of education, VACTERL = vertebral, anorectal, cardiac, tracheoesophageal, renal or limb anomalies. [A] Birth weight <10th percentile.[19] [B] According to Gross classification.[18] [C] Surgery abroad. [D] According to Solomon criteria.[20]

	Participants (n=110)	Non-participants (n=36)	p-value
Male	68 (61.8)	23 (63.9)	1.000
Gestational age in weeks	38.0 (28.0-42.3)	38.4 (28.6-42.0)	0.302
Birth weight in grams	2863 (1080-3996)	2745 (750-3775)	0.053
Preterm birth	34 (32.1)	14 (38.9)	0.299
Small for gestational age [A]	41 (37.3)	4 (11.1)	0.401
Type of EA [B]			
Type A	7 (6.4)	6 (16.7)	0.090
Type B	1 (0.9)	1 (2.8)	0.439
Type C	97 (88.2)	25 (69.4)	**0.006 ***
Type D	0 (0)	1 (2.8)	0.250
Type E	3 (2.7)	3 (8.3)	0.165
Unknown [C]	2 (1.8)	0 (0)	
Staged repair	12 (10.9)	10 (27.8)	**0.029 ***
Associated problems			
VACTERL association [D]	11 (10.0)	10 (27.8)	**0.013 ***
Major anomalies	43 (39.1)	16 (44.4)	0.696
Minor anomalies	32 (29.1)	18 (50.0)	**0.027 ***
Type of primary surgery			
Thoracotomy	76 (69.1)	30 (83.3)	0.132
Thoracoscopy	31 (28.2)	6 (16.7)	0.191
Converted	2 (1.8)	0 (0)	1.000
Unknown	1 (0.9)	0 (0)	
Duration of anesthetic exposure, in the first 24 months of life, in hours	6.7 (2.1-56.8)	8.1 (2.4-40.8)	0.315
Maternal educational level			
Low (ISCED 0-2)	16 (14.5)	7 (19.4)	0.258
Middle (ISCED 3-4)	44 (40.0)	11 (30.6)	1.000
High (ISCED 5-8)	47 (42.7)	10 (27.8)	0.521
Unknown	3 (2.7)	8 (22.2)	

Figure 2 illustrates the longitudinal course between 8 and 12 years for both HS and QoL. See Supplementary Material 3 for details.

Comparison of self-reported and proxy-reported health status with norm values

At 8 years, 86 children reported lower total PedsQL scores (p<0.001) compared with the healthy population, reflected on all subscales except for social functioning for 52 boys and emotional functioning for 34 girls. At 12 years, the total scores of 63 children did not differ significantly from those of the reference group. On a subscale level, boys reported lower scores for physical functioning and higher scores for emotional functioning. Girls reported lower scores for physical functioning and school functioning.

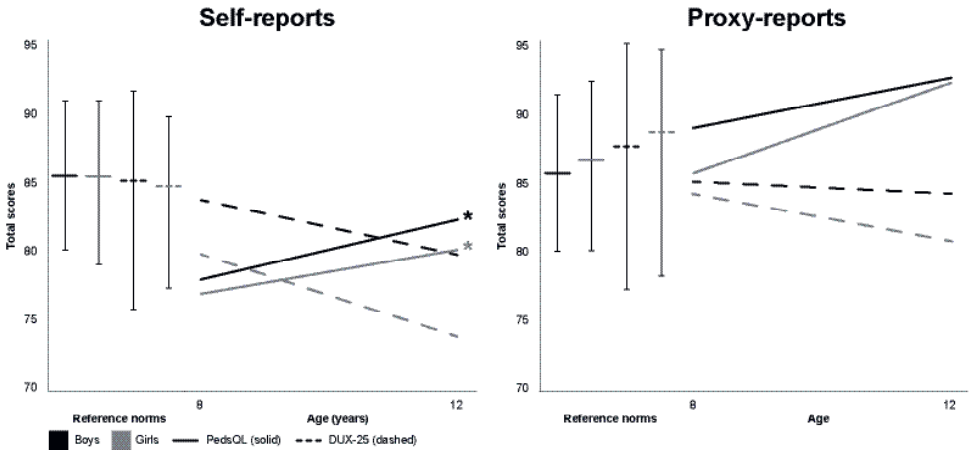

Figure 2. Longitudinal evaluation of median PedsQL and DUX-25 total scores between 8 and 12 years in children with esophageal atresia. Reference norms are shown as median (interquartile range). Asterisk indicates significant change between 8 and 12 years ($p<0.05$). Solid line = PedsQL, dashed line = DUX-25.

At 8 years, 80 parents reported normal total scores. At subscale level, they reported lower scores for school functioning for girls. At 12 years, 54 parents reported normal total scores, and slightly higher scores for emotional functioning for both boys and girls. See Table 2 for a detailed presentation of all total and subscale scores, with the corresponding sample sizes, ESs and P values.

Comparison of self-reported and proxy-reported quality of life with norm values

At 8 years, boys reported total DUX-25 scores equal to those of their peers but lower scores for home functioning. Girls that age reported lower total scores, reflected in home functioning and emotional functioning. At 12 years, boys reported normal total scores but lower scores for home functioning, lower scores for close social and higher scores for far social functioning. Twelve-year-old girls reported lower total scores (n=28, ES-0.26, p=0.001), reflected on all subscales but emotional functioning and far social functioning.

At 8 years, 67 parents reported normal total and subscale DUX-25 scores. At 12 years, they reported normal total and subscale scores for 31 boys. For 24 girls, they reported lower total scores (ES-0.16, p=0.022), reflected in physical functioning and home functioning. See Table 3 for a detailed presentation of all total and subscale scores, with the corresponding sample sizes, ESs and p-values.

Table 2. Scores for PedsQL scales for children with esophageal atresia at 8 and 12 years, compared to sex-specific norm values. [A] Mann-Whitney test. IQR = interquartile range

			Male			**Female**		
			Median (IQR)	*Effect size (r)*	*p-value* [A]	*Median (IQR)*	*Effect size (r)*	*p-value* [A]
Self-reports	**8 years**		**n=52**			**n=34**		
		Physical functioning	81.25 (71.88-89.84)	-0.50	**<0.001**	78.13 (65.63-84.38)	-0.40	**<0.001**
		Emotional functioning	72.50 (60.00-80.00)	-0.22	**0.007**	67.50 (50.00-86.25)	-0.10	0.183
		Social functioning	90.00 (71.25-95.00)	-0.09	0.288	85.00 (70.00-91.25)	-0.21	**0.005**
		School functioning	75.00 (65.00-80.00)	-0.25	**0.003**	75.00 (65.00-85.00)	-0.30	**<0.001**
		Psychosocial health	78.33 (68.33-84.58)	-0.23	**0.006**	76.67 (64.58-83.33)	-0.24	**0.001**
		Total score	80.43 (71.74-84.78)	-0.35	**<0.001**	78.26 (64.96-83.97)	-0.32	**<0.001**
	12 years		**n=35**			**n=28**		
		Physical functioning	84.38 (78.13-96.88)	-0.27	**0.002**	87.50 (75.00-92.97)	-0.22	**0.004**
		Emotional functioning	85.00 (75.00-90.00)	0.21	**0.016**	75.00 (61.25-90.00)	0.01	0.868
		Social functioning	90.00 (85.00-95.00)	0.07	0.399	90.00 (81.25-98.75)	0.00	0.973
		School functioning	80.00 (70.00-85.00)	-0.12	0.167	75.00 (66.25-85.00)	-0.28	**<0.001**
		Psychosocial health	85.00 (75.00-90.00)	0.09	0.335	80.84 (73.33-88.33)	-0.07	0.351
		Total score	83.70 (79.35-92.39)	-0.04	0.624	81.52 (74.73-88.86)	-0.14	0.065
Proxy-reports	**8 years**		**n=47**			**n=33**		
		Physical functioning	93.75 (84.38-100.00)	-0.03	0.654	87.50 (78.13-98.44)	-0.05	0.451
		Emotional functioning	80.00 (60.00-95.00)	0.13	0.089	75.00 (70.00-88.75)	0.10	0.128
		Social functioning	90.00 (66.25-100.00)	-0.02	0.839	90.00 (75.00-100.00)	0.01	0.862
		School functioning	80.00 (65.00-93.60)	0.03	0.674	75.00 (65.00-90.00)	-0.18	**0.007**
		Psychosocial health	81.67 (66.67-93.33)	0.05	0.555	80.00 (70.00-88.33)	-0.02	0.775
		Total score	84.71 (72.42-93.32)	0.00	0.921	83.70 (73.93-89.36)	-0.04	0.599
	12 years		**n=30**			**n=24**		
		Physical functioning	92.19 (81.25-100.00)	-0.03	0.721	96.31 (81.25-100.00)	0.09	0.213
		Emotional functioning	82.50 (62.50-100.00)	0.16	**0.047**	80.00 (66.25-100.00)	0.14	**0.043**
		Social functioning	95.00 (78.75-100.00)	0.11	0.162	97.40 (71.25-100.00)	0.08	0.240
		School functioning	80.00 (60.00-100.00)	0.03	0.721	95.00 (76.25-100.00)	0.07	0.308
		Psychosocial health	81.67 (68.33-96.67)	0.10	0.227	88.33 (71.67-96.25)	0.13	0.065
		Total score	85.77 (76.60-96.74)	0.10	0.210	90.38 (77.25-95.92)	0.11	0.119

Table 3. Scores for DUX-25 scales for children with esophageal atresia at 8 and 12 years, compared to sex-specific norm values. [A] Mann-Whitney test. IQR = interquartile range

			Male			**Female**		
			Median (IQR)	*Effect size (r)*	*p-value* [A]	*Median (IQR)*	*Effect size (r)*	*p-value* [A]
Self-reports	**8 years**		**n=48**			**n=32**		
		Physical functioning	87.50 (75.00-95.83)	-0.08	0.374	81.25 (67.71-91.67)	-0.10	0.184
		Home functioning	90.00 (75.00-98.75)	-0.20	**0.021**	87.50 (76.25-98.75)	-0.19	**0.012**
		Emotional functioning	75.00 (64.29-85.71)	-0.10	0.244	71.43 (64.29-82.14)	-0.16	**0.031**
		Social functioning	82.14 (71.43-89.29)	-0.06	0.494	78.57 (71.43-84.82)	-0.12	0.124
		Close social functioning	83.33 (83.33-100.00)	-0.06	0.491	83.33 (77.08-100.00)	-0.09	0.249
		Far social functioning	75.00 (64.06-85.94)	-0.04	0.600	71.88 (62.50-85.94)	-0.09	0.227
		Total functioning	83.00 (73.00-89.00)	-0.12	0.150	80.00 (70.00-86.75)	-0.18	**0.019**
	12 years		**n=38**			**n=28**		
		Physical functioning	85.42 (70.83-91.67)	-0.15	0.085	72.92 (59.38-79.17)	-0.31	**<0.001**
		Home functioning	90.00 (80.00-95.00)	-0.19	**0.031**	85.00 (80.00-90.00)	-0.26	**0.001**
		Emotional functioning	75.00 (64.29-85.71)	-0.11	0.204	73.21 (64.29-87.50)	-0.15	0.051
		Social functioning	78.57 (71.43-85.71)	-0.08	0.371	75.00 (67.86-84.82)	-0.21	**0.006**
		Close social functioning	75.00 (68.75-81.25)	-0.45	**<0.001**	75.00 (62.50-87.50)	-0.37	**<0.001**
		Far social functioning	91.67 (83.33-100.00)	0.36	**<0.001**	75.00 (70.31-91.67)	0.04	0.569
		Total functioning	82.00 (74.00-89.00)	-0.14	0.102	74.50 (67.00-85.25)	-0.26	**0.001**
Proxy-reports	**8 years**		**n=39**			**n=28**		
		Physical functioning	87.50 (66.67-95.83)	-0.10	0.222	83.33 (62.50-91.67)	-0.12	0.085
		Home functioning	90.00 (75.00-100.00)	-0.08	0.303	90.00 (85.00-100.00)	0.00	0.978
		Emotional functioning	78.57 (67.86-89.29)	-0.11	0.150	82.14 (75.00-89.29)	-0.05	0.455
		Social functioning	82.14 (71.43-85.71)	-0.08	0.303	85.71 (78.57-89.29)	0.03	0.644
		Close social functioning	83.33 (75.00-91.67)	-0.05	0.540	91.67 (77.08-100.00)	0.02	0.827
		Far social functioning	75.00 (68.75-81.25)	-0.08	0.339	81.25 (75.00-100.00)	-0.05	0.474
		Total functioning	83.00 (71.00-92.00)	-0.12	0.142	83.00 (78.25-94.00)	-0.04	0.598
	12 years		**n=31**			**n=24**		
		Physical functioning	79.17 (75.00-91.67)	-0.13	0.119	75.00 (62.50-83.33)	-0.24	**0.001**
		Home functioning	90.00 (75.00-100.00)	-0.06	0.439	85.00 (71.25-95.00)	-0.15	**0.037**
		Emotional functioning	75.00 (71.43-85.71)	-0.07	0.391	82.14 (67.86-84.82)	-0.10	0.147
		Social functioning	78.57 (71.43-92.86)	-0.03	0.695	82.14 (72.32-91.96)	-0.08	0.230
		Close social functioning	83.33 (75.00-100.00)	-0.04	0.632	83.33 (83.33-91.67)	-0.09	0.213
		Far social functioning	75.00 (68.75-87.50)	-0.04	0.656	75.00 (68.75-92.19)	-0.08	0.285
		Total functioning	81.00 (72.00-89.00)	-0.08	0.314	77.00 (69.75-89.75)	-0.16	**0.022**

Potential influences on health status and quality of life

At 8 years, VACTERL association was significantly associated with lower self-reported and proxy-reported total PedsQL scores (p=0.021 and p<0.001, respectively). At 12 years, VACTERL association was associated with lower proxy-reported total PedsQL scores (p=0.044). At 12 years, male sex was associated with higher self-reported total DUX-25 scores (p=0.034), and staged repair was associated with lower self-reported total DUX-25 scores (p=0.038).

DISCUSSION

In this first longitudinal cohort study in school-aged children with EA, self-reported HS improved significantly between 8 and 12 years, whereas self-reported QoL declined – although only significantly regarding close social functioning. The same holds for parental proxy-reported HS and QoL, although none of the differences reached significance (see Figure 1). The longitudinal course was irrespective of sex. At 8 years, self-reported HS was lower than that of healthy peers, which normalized at 12 years. At both ages, girls reported impaired QoL when compared to unique sex-specific reference data. Parents reported normal HS but significantly lower QoL at 12 years for their daughters – but not for their sons.

In the absence of longitudinal research, earlier cross-sectional studies comparing HS of different age groups were in line with our findings. The lowest self-reported and proxy-reported PedsQL scores were found in 8-to-12-year-olds after primary EA repair.[14,26] Proxy-reported physical functioning improved beyond age ten, but psychosocial health and total scores declined.[14]

Previous studies that compared the HS of children with EA with that of healthy controls showed divergent results. The conclusions of Flieder et al.[15] were contradictory with our findings: self-reported total PedsQL scores did not differ from those of the healthy population, while proxy-reported scores were significantly lower (p=0.022); however, they drew these conclusions from the analysis of pooled data of 175 children of all ages. This approach can be questioned given the earlier described variation in HS when children get older. Legrand et al.[13] combined the self-reported and proxy-reported PedsQL scores of 57 teenagers born with EA and found that these were significantly lower than total scores of healthy peers. In another study, self-reported total PedsQL scores of 55 adolescents did not differ from those of healthy controls, whereas their proxy-reported total scores were significantly lower.[27] Using the Child Health Questionnaire (CHQ), teenagers scored significantly better for family activities – but lower for general health perception – compared to the reference population, whereas parents reported lower physical scores for their children.[28]

To our knowledge, genuine QoL (i.e. general well-being not limited to health) had until now never been studied in children born with EA. DUX-25 scores have never been reported in this population. In one study using the KIDSCREEN27, children reported significantly lower scores compared to the reference population for social functioning, while their parents reported significantly higher scores for psychological well-being.[29] Similar to our findings, parents reported better scores than the children themselves, irrespective of age. Three studies in teenagers who had undergone replacement surgery because of long gap EA reported normal scores on the CHQ and the Gastrointestinal Quality of Life Index (GIQLI, a gastrointestinal disease-specific health-related QOL tool).[30-32]

It can be questioned whether generic questionnaires such as the PedsQL and DUX-25 are applicable to children with a specific condition such as EA. Condition-specific questionnaires have been found more sensitive to the clinical characteristics of these children than generic instruments.[33] Dellenmark-Blom et al.[34] have developed and validated a condition-specific health-related QoL instrument for children with EA, the EA-QOL© questionnaire, with which they found for example a noticeable difference in the effect of digestive and respiratory symptoms on QoL between different age groups.[35] Future research should confirm whether condition-specific questionnaires are better able to identify differences between children with EA and healthy controls.

Although EA can be considered a chronic health condition requiring lifelong medical care,[36-38] the improved HS from 8 to 12 years that we found might reflect the improvement of children's clinical condition over time. We know from clinical experience that respiratory tract infections, feeding difficulties, and growth failure diminish when these children grow up. Our current data did not allow in-depth investigation of the relationship between HS and these comorbidities. Nevertheless, participants of our multidisciplinary follow-up program are being offered early and tailor-made interventions if needed. Still, they remain vulnerable due to their chronic health condition, which may hamper regular school visits and thus may explain why school functioning remains below the norm over time (see Supplementary Material 3). School functioning can also be influenced by neurocognitive deficits that children grow into with advancing age. This has been shown for children who survived neonatal critical illness,[39, 40] and is currently being investigated for children with EA.

Despite the improved HS over time, VACTERL association negatively affected the self-perceived HS of children with EA at 8 years and the parent-perceived HS at 8 and 12 years. The fact that most associated anomalies are lifelong conditions, might explain why the self-perceived physical functioning – although improving – is lower compared with healthy children at both 8 and 12 years, for boys and girls.

Peer relationships influence QoL and being equal to age peers becomes increasingly important when growing up.[41] This may explain why self-perceived QoL diminished over time in our study. Another speculation is that competence becomes more important when growing up. Parental stimulation may enhance feelings of self-competence in younger children, especially those with a chronic health condition. Less direct stimulation may occur at an older age, when one faces physical challenges in front of peers rather than of one's parents. Last, this downward tendency could also have been caused by the physiological onset of puberty. Healthy teenagers report lower QoL as they get older,[42,43] and this effect is even more prominent in the 12-year-olds with EA. Also, our results suggest an additional sex-specific disease effect. Even in comparison with sex-specific reference norms, girls in our study showed larger differences in HS and QoL than boys. Potentially girls perceive the effects of EA more severe than boys. More research – preferably with age-matched controls – with sex-specific data would be needed to explore this hypothesis.

Strengths of our study are the relatively large sample of children born with a rare congenital anomaly and the structured data collection within the infrastructure of a standardized longitudinal follow-up program. Some limitations should be addressed. First, the separation of data by sex led to relatively small sample sizes of the subgroups. Second, selection bias cannot be ruled out completely as non-participants had more often undergone staged repair and had more associated problems. To add to this, the longitudinally assessed children had more often undergone a thoracotomy, which used to be standard of care until 2006 at our department as compared to a thoracoscopy. Third, only cross-sectional reference norms for age groups are available. Therefore, we were unable to compare our longitudinal data with a control population. Fourth, we did not evaluate the influence of comorbidities on HS and QoL. Future studies should focus on the correlation between actual outcomes determined by standardized assessments and perceived HS and QoL. Last, we assessed HS and QoL with generic questionnaires in this study. As mentioned earlier, condition-specific questionnaires become more and more important in the evaluation of patients with chronic health conditions. Once the EA-QOL© questionnaire,[34] a condition-specific QoL instrument for children with EA, has been translated and validated in other countries, this would be a valuable addition to the assessment battery of longterm outcome in this population.

CONCLUSION

To conclude, self-reported and proxy-reported HS of children born with EA improved between 8 and 12 years of age, while their QoL declined over this period. Self-reported HS was significantly lower than the reference population at 8 years for both boys and girls, and self-reported QoL was below the norm values at 8 and 12 years for girls. Proxy-reported HS did not differ for both boys and girls, and proxy-reported QoL was impaired at 12 years for girls.

Given the chronic course of EA and the persistence of morbidities until adulthood, it remains important to consistently evaluate these long-term outcomes. We advocate standardized longitudinal follow-up with early tailor-made interventions applied when necessary. Our findings, too, emphasize that HS and QoL should be considered two separate concepts and suggest a sex-specific difference. We recommend measuring HS and QoL simultaneously when evaluating the burden of disease. Future research should further investigate this potential sex-specific effect, and explore the value of the use of condition-specific instruments such as the EA-QOL questionnaire.

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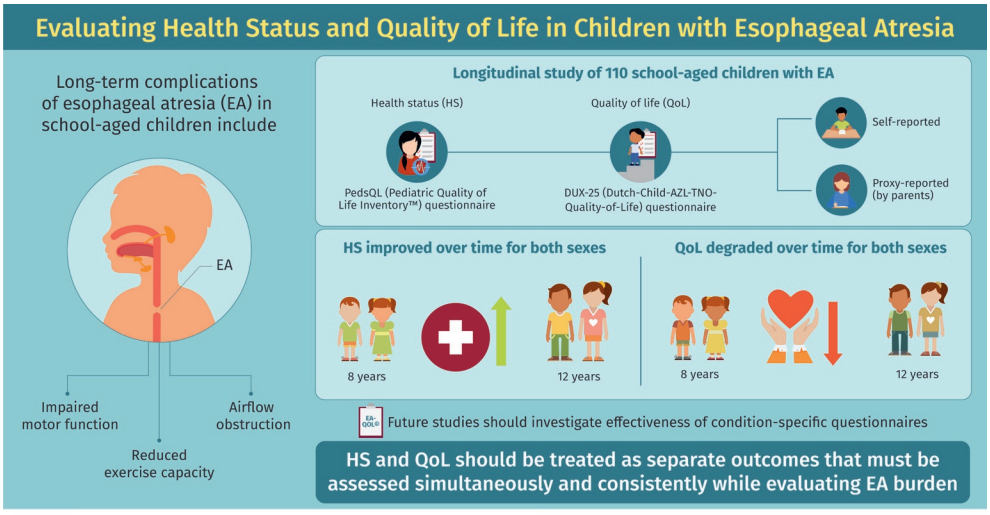
GRAPHICAL ABSTRACT



Longitudinal health status and quality of life after esophageal atresia repair
ten Kate et al. (2021)

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S1. Detailed description of questionnaires

Paediatric Quality of Life Inventory (PedsQL)[1]

Description:

The PedsQL is an instrument for measuring health status in children and adolescents. The items of this questionnaire covers a large part of the International Classification of Functioning, Disability and Health (ICF) domains function, activity, and participation as defined by the World Health Organization (WHO) criteria.[2, 3] Various age-specific versions are available. For this study, we used the questionnaire for children aged 8-12 years (self-report) and their parents (proxy-report). It consists of 23 questions within five domains: physical functioning (8 items), emotional functioning (5 items), social functioning (5 items), school functioning (5 items), and psychosocial health. Each item asks about a problem in the past month: physical functioning includes running, exercise and pain; emotional functioning includes fear, sadness, anger, sleeping and worrying; social functioning includes the interactions with other children; and school functioning includes paying attention in class, memory and missing out on school due to sickness or a hospital visit. Psychosocial health is a composition of emotional, social and school functioning. Items responses are given on a 5-point Likert scale. Total and subscale scores are rescaled to a score between 0 and 100. Higher scores indicate better health status.

Validated:

In 2009, the questionnaire has been validated for Dutch children aged 5-18 years.[4] For this study, we used recently updated and sex-specific norm values (see Supplementary Table 1), since girls generally reported significantly lower PedsQL scores than boys, both in parent proxy-reports and child self-reports. The manuscript in question is currently under review.

Dutch-Child-AZL-TNO-Quality-of-Life (DUX-25)[5]

Description:

The DUX-25 is an instrument for measuring health-related quality of life in children between 5 and 16 years old. It assesses the emotional and cognitive perception of physical, emotional, social, and home functioning. The DUX-25 is derived from the original TNO-AZL Child Quality of Life (TACQOL) and TNO-AZL Preschool Children Quality of Life (TAPQOL) questionnaires.[6, 7] The conceptual content of the TNO-AZL series represents a significant quality of life component according to the WHO criteria.[3, 8] Self-report and proxy-report versions are available. It contains 25 questions within four domains: physical functioning (6 items), emotional functioning (7 items), social functioning (7 items) and home functioning (5 items). Each item evaluates the child's feelings in daily life and is answered on a visual 5-point Likert scale with a happy-to-sad faces scale using smileys. Physical functioning includes condition, appearance, physical length and weight. Emotional functioning includes feelings and thoughts about certain activities. Social functioning can be split into 'close social functioning' and 'far social functioning'. Close social functioning includes the interaction with peers, such as friends and classmates. Far

social functioning includes the interaction with adults or teachers, or how one feels about other children. Home functioning includes the interaction with parents and feeling about activities at home. Total and subscale scores are rescaled to a score between 0-100. Higher scores indicate a better quality of life.

Validated:
Recently, Dutch reference data have been collected and norm values have been calculated for Dutch children aged 8-17 years (see Supplementary Table 2). These are also sex-specific normative data, since we observed significant differences between healthy boys and girls. A manuscript is currently in preparation.

Background data on sex-specific norm values for PedsQL and DUX-25
The recent Dutch norm values for the PedsQL and DUX-25 questionnaires have been collected between April 2015 and June 2017, using a program for online surveys (LimeSurvey GmbH version 2.06lts, Hamburg, Germany). In total 882 parents and 581 children had been recruited through regular primary and secondary schools in the Netherlands. Children with a chronic disease and/or mental disorder (e.g. asthma, autism) were excluded from analysis. In the age group 8-12 years, 239 children (40% boys) participated with a median age of 11.0 years (range 8.0-12.0). In this same age group, 300 parents (11% male) participated.

Supplementary Table S1.1. Sex-specific Dutch norm values for PedsQL scales for 8-to-12-year-old healthy children. SD = standard deviation, IQR = interquartile range

		Median (IQR)	
Self-reports		*Male (n=93)*	*Female (n=146)*
	Physical functioning	93.75 (90.63-100.00)	90.63 (84.38-96.88)
	Emotional functioning	80.00-65.00-85.00)	75.00 (60.00-85.00)
	Social functioning	90.00 (75.00-100.00)	90.00 (80.00-100.00)
	School functioning	80.00 (75.00-90.00)	85.00 (75.00-95.00)
	Psychosocial health	81.67 (75.00-88.33)	83.33 (76.67-90.00)
	Total score	85.87 (80.44-91.30)	85.87 (79.35-91.30)
Proxy-reports		*Male (n=121)*	*Female (n=179)*
	Physical functioning	93.75 (84.38-100.00)	90.63 (84.38-96.88)
	Emotional functioning	75.00 (65.00-82.50)	75.00 (60.00-90.00)
	Social functioning	90.00 (75.00-100.00)	90.00 (75.00-100.00)
	School functioning	75.00 (65.00-90.00)	85.00 (75.00-95.00)
	Psychosocial health	78.33 (71.67-86.67)	81.67 (73.33-90.00)
	Total score	83.70 (77.17-90.22)	84.78 (77.17-91.30)

Supplementary Table S1.2. Sex-specific Dutch norm values for DUX-25 scales for 8-to-12-year-old healthy children. SD = standard deviation, IQR = interquartile range

		Median (IQR)	
Self-reports		*Male (n=92)*	*Female (n=145)*
	Physical functioning	89.58 (79.17-95.83)	87.50 (75.00-95.83)
	Home functioning	95.00 (90.00-100.00)	95.00 (90.00-100.00)
	Emotional functioning	78.57 (67.86-89.29)	82.14 (67.86-89.29)
	Social functioning	82.14 (71.43-89.29)	82.14 (75.00-89.29)
	Social close functioning	91.67 (83.33-100.00)	91.67 (83.33-100.00)
	Social far functioning	75.00 (62.50-87.50)	75.00 (68.75-87.50)
	Total functioning	85.50 (76.00-92.00)	85.00 (77.50-90.00)
Proxy-reports		*Male (n=121)*	*Female (n=179)*
	Physical functioning	91.67 (75.00-100.00)	91.67 (75.00-96.88)
	Home functioning	95.00 (80.00-100.00)	95.00 (80.00-100.00)
	Emotional functioning	82.14 (71.43-92.86)	85.71 (71.43-92.86)
	Social functioning	85.71 (71.43-92.86)	85.71 (75.00-96.43)
	Social close functioning	91.67 (75.00-100.00)	91.67 (75.00-100.00)
	Social far functioning	81.25 (62.50-93.75)	82.25 (68.75-93.75)
	Total functioning	86.00 (74.00-94.50)	87.00 (75.00-94.00)

S2. Tests for normality for PedsQL and DUX-25 scales

Supplementary Table S2.1. Tests for normality for scores for PedsQL scales for children with oesophageal atresia at 8 and 12 years. SD = standard deviation, IQR = interquartile range. [A] Shapiro-Wilk test.

			Mean ± SD	Median (IQR)	p-value [A]	Mean ± SD	Median (IQR)	p-value [A]
Self-reports	**8 years**		*Male (n=52)*			*Female (n=34)*		
		Physical functioning	79.33 ± 14.7	81.25 (71.88-89.84)	**0.003**	75.74 ± 15.0	78.13 (65.63-84.38)	**0.022**
		Emotional functioning	68.75 ± 15.2	72.50 (60.00-80.00)	0.216	68.38 ± 18.2	67.50 (50.00-86.25)	**0.047**
		Social functioning	83.85 ± 14.1	90.00 (71.25-95.00)	**<0.001**	78.53 ± 18.1	85.00 (70.00-91.25)	**0.004**
		School functioning	73.27 ± 14.0	75.00 (65.00-80.00)	0.128	73.38 ± 14.3	75.00 (65.00-85.00)	**0.046**
		Psychosocial health	75.29 ± 11.8	78.33 (68.33-84.58)	0.079	73.43 ± 13.7	76.67 (64.58-83.33)	0.100
		Total score	76.73 ± 11.9	80.43 (71.74-84.78)	**0.005**	74.23 ± 13.3	78.26 (64.96-83.97)	0.053
	12 years		*Male (n=35)*			*Female (n=28)*		
		Physical functioning	85.27 ± 12.7	84.38 (78.13-96.88)	**0.002**	83.26 ± 11.0	87.50 (75.00-92.97)	**0.036**
		Emotional functioning	81.43 ± 15.7	85.00 (75.00-90.00)	**<0.001**	73.93 ± 17.1	75.00 (61.25-90.00)	0.186
		Social functioning	88.86 ± 10.9	90.00 (85.00-95.00)	**<0.001**	86.96 ± 14.0	90.00 (81.25-98.75)	**<0.001**
		School functioning	77.29 ± 12.2	80.00 (70.00-85.00)	**0.013**	74.82 ± 12.7	75.00 (66.25-85.00)	**0.006**
		Psychosocial health	82.53 ± 10.6	85.00 (75.00-90.00)	**0.002**	78.57 ± 12.9	80.84 (73.33-88.33)	**0.012**
		Total score	83.48 ± 10.6	83.70 (79.35-92.39)	**0.011**	80.20 ± 10.8	81.52 (74.73-88.86)	**0.006**
Proxy-reports	**8 years**		*Male (n=48)*			*Female (n=33)*		
		Physical functioning	87.78 ± 14.7	93.75 (84.38-100.00)	**<0.001**	86.61 ± 12.3	87.50 (78.13-98.44)	**0.006**
		Emotional functioning	76.70 ± 19.1	80.00 (60.00-95.00)	**0.004**	77.65 ± 14.0	75.00 (70.00-88.75)	0.156
		Social functioning	82.40 ± 20.3	90.00 (66.25-100.00)	**<0.001**	85.30 ± 14.9	90.00 (75.00-100.00)	**0.001**
		School functioning	77.28 ± 19.1	80.00 (65.00-93.60)	**0.004**	75.76 ± 17.5	75.00 (65.00-90.00)	0.112
		Psychosocial health	78.86 ± 15.6	81.67 (66.67-93.33)	**0.020**	79.57 ± 13.3	80.00 (70.00-88.33)	0.141
		Total score	81.41 ± 14.3	84.71 (72.42-93.32)	**0.004**	81.98 ± 11.7	83.70 (73.93-89.36)	0.053
	12 years		*Male (n=30)*			*Female (n=24)*		
		Physical functioning	87.86 ± 15.2	92.19 (81.25-100.00)	**<0.001**	87.78 ± 18.1	96.31 (81.25-100.00)	**<0.001**
		Emotional functioning	78.83 ± 19.4	82.50 (62.50-100.00)	**0.005**	80.21 ± 18.9	80.00 (66.25-100.00)	**0.008**
		Social functioning	87.67 ± 16.9	95.00 (78.75-100.00)	**<0.001**	85.83 ± 20.1	97.40 (71.25-100.00)	**<0.001**
		School functioning	78.17 ± 19.1	80.00 (60.00-100.00)	**0.011**	84.38 ± 19.0	95.00 (76.25-100.00)	**<0.001**
		Psychosocial health	81.11 ± 17.0	81.67 (68.33-96.67)	**0.008**	83.47 ± 16.9	88.33 (71.67-96.25)	**0.002**
		Total score	83.59 ± 15.7	85.77 (76.60-96.74)	**0.002**	84.65 ± 16.0	90.38 (77.25-95.92)	**0.001**

Supplementary Table S2.2. Tests for normality for scores for DUX-25 scales for children with oesophageal atresia at 8 and 12 years. SD = standard deviation, IQR = interquartile range. [A] Shapiro-Wilk test

			Mean ± SD	Median (IQR)	p-value [A]	Mean ± SD	Median (IQR)	p-value [A]
Self-reports	**8 years**		*Male (n=48)*			*Female (n=32)*		
		Physical functioning	84.03 ± 13.4	87.50 (75.00-95.83)	**<0.001**	79.43 ± 15.8	81.25 (67.71-91.67)	**0.022**
		Home functioning	86.46 ± 12.9	90.00 (75.00-98.75)	**<0.001**	86.88 ± 11.0	87.50 (76.25-98.75)	**0.019**
		Emotional functioning	75.60 ± 13.8	75.00 (64.29-85.71)	0.268	73.10 ± 13.5	71.43 (64.29-82.14)	0.710
		Social functioning	79.61 ± 11.8	82.14 (71.43-89.29)	**0.017**	78.91 ± 12.7	78.57 (71.43-84.82)	0.228
		Close social functioning	86.28 ± 12.1	83.33 (83.33-100.00)	**<0.001**	86.20 ± 12.6	83.33 (77.08-100.00)	**0.002**
		Far social functioning	74.61 ± 14.1	75.00 (64.06-85.94)	0.252	73.44 ± 17.0	71.88 (62.50-85.94)	0.202
		Total functioning	80.92 ± 10.7	83.00 (73.00-89.00)	**0.033**	79.00 ± 9.8	80.00 (70.00-86.75)	0.595
	12 years		*Male (n=38)*			*Female (n=28)*		
		Physical functioning	82.02 ± 13.4	85.42 (70.83-91.67)	**0.026**	70.98 ± 14.2	72.92 (59.38-79.17)	0.243
		Home functioning	87.76 ± 11.1	90.00 (80.00-95.00)	**0.001**	85.71 ± 9.7	85.00 (80.00-90.00)	0.108
		Emotional functioning	75.28 ± 13.3	75.00 (64.29-85.71)	0.712	73.09 ± 13.9	73.21 (64.29-87.50)	0.329
		Social functioning	79.89 ± 9.6	78.57 (71.43-85.71)	0.266	75.77 ± 11.1	75.00 (67.86-84.82)	0.765
		Close social functioning	74.23 ± 11.5	75.00 (68.75-81.25)	0.068	74.70 ± 13.9	75.00 (62.50-87.50)	0.181
		Far social functioning	87.83 ± 11.8	91.67 (83.33-100.00)	**<0.001**	78.35 ± 14.8	75.00 (70.31-91.67)	**0.005**
		Total functioning	80.68 ± 9.8	82.00 (74.00-89.00)	0.537	75.86 ± 9.7	74.50 (67.00-85.25)	0.290
Proxy-reports	**8 years**		*Male (n=39)*			*Female (n=28)*		
		Physical functioning	81.62 ± 16.1	87.50 (66.67-95.83)	**0.002**	79.46 ± 15.8	83.33 (62.50-91.67)	**0.015**
		Home functioning	86.41 ± 13.1	90.00 (75.00-100.00)	**<0.001**	90.18 ± 10.0	90.00 (85.00-100.00)	**0.001**
		Emotional functioning	78.02 ± 13.1	78.57 (67.86-89.29)	0.201	80.99 ± 10.0	82.14 (75.00-89.29)	0.257
		Social functioning	80.22 ± 10.3	82.14 (71.43-85.71)	0.076	85.59 ± 11.1	85.71 (78.57-89.29)	0.125
		Close social functioning	85.04 ± 12.0	83.33 (75.00-91.67)	**0.004**	88.10 ± 10.3	91.67 (77.08-100.00)	**0.004**
		Far social functioning	76.60 ± 12.8	75.00 (68.75-81.25)	0.229	83.71 ± 13.7	81.25 (75.00-100.00)	**0.010**
		Total functioning	81.18 ± 11.2	83.00 (71.00-92.00)	0.083	83.75 ± 9.8	83.00 (78.25-94.00)	0.145
	12 years		*Male (n=31)*			*Female (n=24)*		
		Physical functioning	79.84 ± 17.5	79.17 (75.00-91.67)	**0.005**	73.09 ± 16.9	75.00 (62.50-83.33)	0.169
		Home functioning	86.77 ± 13.6	90.00 (75.00-100.00)	**0.001**	83.96 ± 12.8	85.00 (71.25-95.00)	**0.049**
		Emotional functioning	78.46 ± 13.8	75.00 (71.43-85.71)	0.073	77.98 ± 13.1	82.14 (67.86-84.82)	0.367
		Social functioning	81.11 ± 12.4	78.57 (71.43-92.86)	**0.033**	81.70 ± 10.2	82.14 (72.32-91.96)	0.277
		Close social functioning	85.22 ± 13.2	83.33 (75.00-100.00)	**0.003**	86.11 ± 8.0	83.33 (83.33-91.67)	**0.002**
		Far social functioning	77.82 ± 14.1	75.00 (68.75-87.50)	**0.012**	78.39 ± 14.4	75.00 (68.75-92.19)	**0.021**
		Total functioning	81.19 ± 12.4	81.00 (72.00-89.00)	0.283	79.04 ± 10.8	77.00 (69.75-89.75)	0.410

S3. Longitudinal comparison for PedsQL and DUX-25 scales

Supplementary Table S3.1. Longitudinal comparison (paired) for PedsQL and DUX-25 scales for children with oesophageal atresia at 8 and 12 years. SD = standard deviation, IQR = interquartile range. [A] Wilcoxon test

			8 years Median (IQR)	12 years Median (IQR)	Effect size (*r*)	p-value [A]	8 years Median (IQR)	12 years Median (IQR)	Effect size (*r*)	p-value [A]
PedsQL	**Self-reports**		*Male (n=24)*				*Female (n=21)*			
		Physical functioning	78.13 (63.28-84.38)	84.38 (75.78-96.88)	0.47	**0.021**	75.00 (65.63-82.81)	84.38 (75.00-90.63)	0.58	**0.008**
		Emotional functioning	67.50 (50.00-80.00)	85.00 (75.00-90.00)	0.53	**0.010**	65.00 (55.00-85.00)	70.00 (60.00-90.00)	0.24	0.264
		Social functioning	90.00 (70.00-95.00)	90.00 (85.00-95.00)	0.36	0.077	80.00 (70.00-90.00)	85.00 (80.00-95.00)	0.54	**0.014**
		School functioning	75.00 (56.25-88.75)	80.00 (66.25-85.00)	0.22	0.273	75.00 (67.50-85.00)	75.00 (65.00-85.00)	0.03	0.887
		Psychosocial health	78.33 (63.75-84.58)	84.17 (75.42-90.00)	0.54	**0.009**	76.67 (65.00-83.33)	76.67 (73.33-88.33)	0.52	**0.018**
		Total score	78.26 (63.32-82.61)	82.61 (76.09-91.85)	0.54	**0.009**	77.17 (64.13-80.43)	80.43 (73.91-88.04)	0.63	**0.004**
	Proxy-reports		*Male (n=23)*				*Female (n=21)*			
		Physical functioning	96.88 (84.38-100.00)	93.75 (84.38-100.00)	0.31	0.135	87.50 (78.13-95.31)	96.88 (83.04-100.00)	0.23	0.285
		Emotional functioning	80.00 (60.00-95.00)	90.00 (70.00-100.00)	0.14	0.493	75.00 (70.00-88.75)	80.00 (62.50-100.00)	0.18	0.418
		Social functioning	95.00 (65.00-100.00)	100.00 (80.00-100.00)	0.24	0.257	90.00 (77.50-100.00)	99.80 (72.50-100.00)	-0.07	0.752
		School functioning	85.00 (69.80-100.00)	85.00 (75.00-100.00)	0.17	0.419	80.00 (70.00-90.00)	95.00 (72.50-100.00)	0.46	**0.035**
		Psychosocial health	85.00 (70.00-95.00)	91.67 (75.00-96.67)	0.22	0.296	83.33 (77.50-88.33)	88.33 (66.67-98.33)	0.21	0.341
		Total score	87.38 (71.74-95.31)	91.51 (78.26-96.73)	0.40	0.058	83.70 (80.22-89.36)	91.25 (75.54-97.58)	0.25	0.259
DUX-25	**Self-reports**		*Male (n=20)*				*Female (n=19)*			
		Physical functioning	91.76 (71.88-95.83)	83.33 (70.83-90.63)	-0.37	0.102	87.50 (75.00-91.67)	75.00 (54.17-79.17)	-0.66	**0.004**
		Home functioning	90.00 (75.00-95.00)	90.00 (80.00-95.00)	0.07	0.754	90.00 (80.00-100.00)	90.00 (75.00-95.00)	-0.20	0.367
		Emotional functioning	75.00 (64.29-83.93)	75.00 (61.61-84.82)	0.05	0.836	71.43 (60.71-82.14)	75.00 (64.29-89.29)	0.16	0.494
		Social functioning	82.14 (73.21-89.29)	78.57 (71.43-84.82)	-0.13	0.568	78.57 (71.43-82.14)	75.00 (67.86-85.71)	-0.14	0.537
		Close social functioning	83.33 (83.33-97.92)	68.75 (68.75-82.81)	-0.70	**0.002**	83.33 (75.00-100.00)	75.00 (68.75-91.67)	-0.43	0.064
		Far social functioning	75.00 (68.75-81.25)	91.67 (83.33-91.67)	0.51	**0.023**	75.00 (62.50-81.25)	75.00 (68.75-91.67)	0.21	0.360
		Total functioning	84.00 (72.00-88.75)	80.00 (73.25-86.25)	-0.21	0.354	80.00 (70.00-87.00)	74.00 (66.00-87.00)	-0.32	0.158
	Proxy-reports		*Male (n=18)*				*Female (n=15)*			
		Physical functioning	89.58 (70.83-95.83)	83.33 (71.88-100.00)	-0.21	0.378	79.17 (62.50-87.50)	75.00 (54.17-83.33)	-0.59	**0.022**
		Home functioning	95.00 (75.00-100.00)	95.00 (80.00-100.00)	0.01	0.964	85.00 (85.00-100.00)	90.00 (75.00-100.00)	-0.05	0.858
		Emotional functioning	78.57 (70.54-89.29)	80.36 (75.00-96.43)	0.21	0.382	82.14 (75.00-89.29)	82.14 (60.71-96.43)	0.02	0.925
		Social functioning	80.36 (75.00-83.93)	78.57 (75.00-94.64)	0.21	0.363	85.71 (78.57-96.43)	82.14 (75.00-92.86)	-0.14	0.590
		Close social functioning	83.33 (75.00-93.75)	91.67 (75.00-100.00)	0.35	0.137	91.67 (83.33-91.67)	83.33 (83.33-91.67)	-0.09	0.725
		Far social functioning	75.00 (68.75-81.25)	75.00 (68.75-90.63)	0.15	0.530	87.50 (75.00-100.00)	75.00 (68.75-93.75)	-0.36	0.168
		Total functioning	83.00 (73.00-92.00)	82.00 (76.00-95.00)	-0.03	0.913	82.00 (77.00-94.00)	78.00 (69.00-91.00)	-0.15	0.550

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CHAPTER 10

Patient-reported outcome measures and clinical outcomes in children with foregut anomalies

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ABSTRACT

Increasing numbers of children and adults with chronic disease status highlight the need for a value-based healthcare system. Patient-reported outcome measures (PROMs) are essential to value-based healthcare, yet it remains unclear how they relate to clinical outcomes such as health and daily functioning. We aimed to assess the added value of self-reported PROMs for health status (HS) and quality of life (QoL) in the long-term follow-up of children with foregut anomalies. We evaluated data of PROMs for HS and/or QoL among eight-year-olds born with congenital diaphragmatic hernia (CDH), esophageal atresia (EA), or congenital lung malformations (CLM), collected within the infrastructure of a multidisciplinary, longitudinal follow-up program. Clinical outcomes were categorized into different outcome domains, and their relationships with self-reported HS and QoL were assessed through multivariable linear regression analyses. A total of 220 children completed HS and/or QoL self-reports. In children with CDH and EA, lower cognition was significantly associated with lower self-reported HS. Due to the low number of cases, multivariable linear regression analysis was not possible in children with CLM. HS, QoL, and clinical outcomes represent different aspects of a child's wellbeing and should be measured simultaneously to facilitate a more holistic approach to clinical decision making.

INTRODUCTION

Survival among children with congenital anomalies has greatly improved in recent decades. As a result, the numbers of children and adults living with a chronic condition are steadily increasing, causing significant financial strain on healthcare systems.[1-3] To optimize economic sustainability while improving health, emphasis must be placed on value-based healthcare (VBHC).[4] This can be achieved by focusing on the impact of disease-related morbidities on a patient's self-perceived health status (HS) and quality of life (QoL).[4-6] Patient-reported outcome measures (PROMs) are widely used tools for assessing long-term outcomes and are essential to value-based decision making.[5, 7] However, it remains unclear how PROMs relate to clinical outcomes such as health, morbidity, and daily functioning in children with chronic conditions.[5]

Our tertiary level European center of expertise for children born with congenital anomalies offers all children born with congenital anatomical anomalies a standardized multidisciplinary long-term follow-up program.[8] This unique infrastructure allows us to measure children's perceptions of their HS and QoL in a standardized manner, and to simultaneously monitor clinical outcomes throughout childhood.[9, 10]

To date, associations between clinical outcomes and self-perceived HS and QoL have never been investigated in children born with anatomical anomalies. Recognition of any relationship could serve to aid clinical decision making with respect to a given child's own experiences. In time, this might help to optimize the value of healthcare from the perspectives of cost-effectiveness and patient compliance.

We hypothesized that impairments in clinical outcomes would be associated with lower self-reported HS, but not with lower QoL. In this study, we aimed to assess the added value of PROMs for HS and QoL in the long-term follow-up of eight-year-old children with congenital foregut anomalies. Therefore, we studied the relationship between clinical outcomes and self-reported HS and QoL by evaluating clinical data from our longitudinal follow-up program.

MATERIALS AND METHODS

Study population

We analyzed available data from children born with congenital diaphragmatic hernia (CDH), esophageal atresia (EA), or congenital lung malformations (CLM) between January 1999 and December 2012, who joined our standardized prospective longitudinal follow-up program for children with congenital anomalies[8] and who underwent surgery within the first 28 days of life. Children with CLM were only included if they had undergone surgical resection of

the lesion ≤28 days after birth. Data were collected until June 2020. The Medical Research Involving Human Subjects Act did not apply to this study, as stated by the Institutional Ethics Review Board (MEC-2020-0551).

Data collection

For this study, we selected children who completed a Pediatric Quality of Life Inventory (PedsQL, a PROM for HS) and/or Dutch-Child-AZL-TNO-Quality-of-Life (DUX-25, a PROM for QoL) questionnaire during follow-up assessments at eight years of age. Standardized assessments of health and daily functioning were performed as standard of care using previously described instruments and methods.[11-14] Although our follow-up protocol was subject to changes in regular care throughout the study period, evaluation methods were considered interchangeable and the same outcomes were measured over the years.

Clinical outcomes were categorized into 14 domains (cognition, behavior, daily executive functioning, motor function, maximum exercise capacity, lung function, presence of gastroesophageal reflux (GER), respiratory morbidity, daily use of medication for either a physical or psychological condition, presence of scoliosis, need for tube feeding, and need for home oxygen), modified from previous outcome classification tables.[10, 15] Apart from behavior and daily executive functioning, all domains were assessed directly by our multidisciplinary follow-up team. A detailed description of all clinical outcome domains, instruments, and cut-off values is provided in Supplementary Materials S1.

During neuropsychological assessments, children filled in PedsQL and DUX-25 self-reports. Cognition was measured through intelligence testing. Emotional and behavioral problems (summarized as 'behavior') and daily executive functioning were evaluated using parent-proxy reports. Motor function was measured with the movement assessment battery for children. Maximum exercise capacity was evaluated using a treadmill test according to the Bruce protocol.

Lung function was tested using spirometry. Presence of GER was routinely determined by 24 hours pH-impedance testing in children with CDH and EA. Respiratory morbidity was defined as ≥3 lower respiratory tract infections in the past year requiring antibiotic therapy and/or hospitalization, or presence of vocal cord dysfunction or subglottic stenosis requiring regular follow-up. Treatment modalities affecting daily life, such as daily use of medication for physical and/or psychological conditions and the need for tube feeding and/or home oxygen, were registered. Scoliosis was defined as present when requiring regular follow-up and/or surgical intervention.

The following background data were retrieved from electronic patient records: sex, gestational age, birth weight, type and specification of anomaly,[16, 17] presence of associated

problems, type of primary surgery, duration of anesthetic exposure within the first 24 months of life, educational level, and highest maternal educational level (MEL). Preterm birth was defined as gestational age <37 weeks. Small for gestational age was defined as birth weight <10th percentile.[18] Duration of anesthetic exposure was defined as the time between induction and departure from the operating theater. VACTERL (vertebral, anorectal, cardiac, tracheoesophageal, renal, and limb malformations) association in children with EA was defined according to Solomon.[19] Associated anomalies were considered major if surgical intervention or regular hospital visits were required. MEL was recorded as a proxy for socioeconomic status.

Data analysis

Data are presented as frequencies (%) or median (interquartile range). Background data were compared between participants and non-participants using Mann–Whitney-U tests or Fisher's exact tests. Clinical outcomes were categorized as normal, borderline, or impaired (cognition, behavior, daily executive functioning), as normal or impaired (motor function, maximum exercise capacity, lung function), or as yes or no (GER, respiratory morbidity, daily use of medication for physical/psychological condition, scoliosis, tube feeding, home oxygen). See Supplementary Material S1 for appropriate cut-off values.

To assess the relationship between the different domains and the PedsQL and DUX-25 scores, multivariable linear regression analyses were performed for each diagnostic group. All PedsQL and DUX-25 subscales were selected as dependent variables, whereas cognition, behavior, daily executive functioning, motor function, maximum exercise capacity, lung function, and GER were selected as candidate independent variables. Subsequently, multiple imputation using fully conditional specification was implemented for all candidate independent variables, using 30 iterations and 50 imputations. Results were pooled over imputed data sets according to Rubin's rules. Variables with >35% missing values were excluded from the regression analysis. As a result, the regression models included the following independent variables: cognition, behavior, motor function, maximum exercise capacity, and lung function. For the final analyses, sex and MEL were added to the independent variables in order to rule out potential bias. Results are summarized as regression coefficients (B), 95% confidence intervals (CI), and p-values. A p-value < 0.05 was considered to indicate significance. All data were analyzed using SPSS version 25.0 (IBM, Chicago, IL, USA).

RESULTS

Patient characteristics

We identified 563 children with CDH, EA, or CLM born between 1999 and 2012, of whom 83% had survived. Of the survivors, 15 children (3%) had syndromes with severe intellectual

disability, 101 children with CLM had no surgery within 28 days after birth, and 1 child with CDH had an incidental finding of CLM, and was only analyzed in the CDH group, leaving 350 eligible children. A total of 220 children (63% of eligible number) completed the PedsQL and/or DUX-25 at 8 years of age (CDH n=114, EA n=93, CLM n=13) (see Figure 1). Participants had a median age of 8.2 years (range 7-9), and clinical characteristics did not differ significantly between participants and non-participants across all three diagnostic groups (see Table 1). In line with official data from the Statistics Netherlands' database,[20] 209 children (95%) attended regular education.

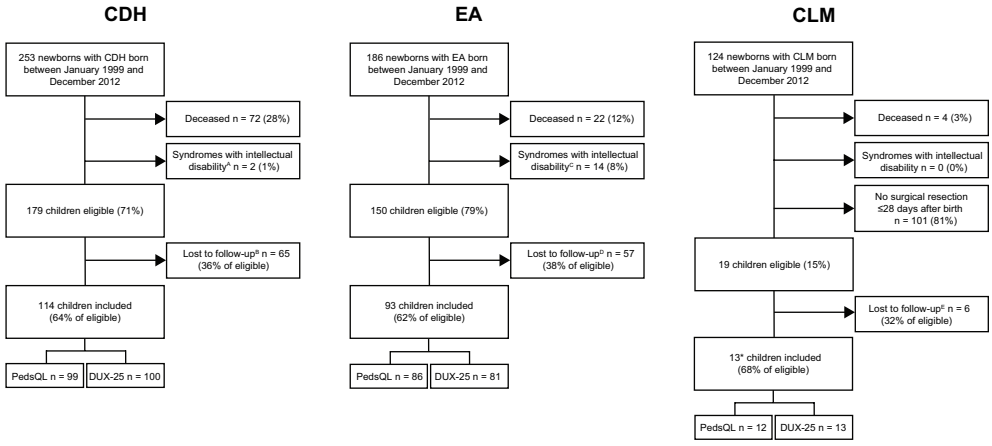

Figure 1. Flowcharts of included patients. CDH = congenital diaphragmatic hernia, EA = esophageal atresia, CLM = congenital lung malformation. [A] Wolf-Hirschhorn syndrome n=1, Simpson-Golabi-Behmel syndrome n=1. [B] Emigration n=5, organizational reasons n=27, no neuropsychological assessment n=7, no follow-up scheduled at 8 years n=2, no self-reports due to lack of time n=2, age ≥9 years due to postponement of follow-up visit n=1, refusal n=18, follow-up elsewhere n=1, untraceable n=2. [C] Down syndrome n=5, Opitz syndrome n=1, Goldenbar syndrome n=1, Wolf–Hirschhorn syndrome n=1, Mandibulofacial dysostosis Guion Almeida type n=1, 22q11 duplication syndrome n=1, other n=4. [D] Emigration n=6, organizational reasons n=4, no neuropsychological assessment n=6, no follow-up scheduled at 8 years n=8, no self-reports due to lack of time n=8, age ≥9 years due to postponement of follow-up visit n=4, refusal n=12, follow-up elsewhere n=5, untraceable n=4. [E] Organizational reasons n=2, no self-reports due to lack of time n=1, refusal n=2, untraceable n=1. * One child with CDH had an incidental finding of CLM and was not included in the CLM group.

CDH

The majority of the 114 children with CDH scored normal on cognition, behavior, daily executive functioning, motor function, and lung function. Out of these 114 children, 98 (86%) scored below normal scores for at least 1 of the 14 clinical outcome domains. Normal maximum exercise capacity was observed in 42% of children. GER was absent in the majority of children who underwent routine 24 hours pH-impedance testing. See Table 2 for more details on clinical outcomes.

Table 1A. Demographic variables of participating and non-participating children with congenital diaphragmatic hernia (CDH), esophageal atresia (EA), and congenital lung malformation CLM). Data are presented as n (%) or median (IQR). One patient had both CDH and an incidental finding of CLM, and was included in the CDH group. CPAM = congenital pulmonary airway malformation, BPS = bronchopulmonary sequestration, CLE = congenital lobar emphysema, BC = bronchogenic cyst, VACTERL = vertebral, anorectal, cardiac, tracheoesophageal, renal, and limb malformations, ISCED = International Classification of Education [21]. Asterisk indicates significance ($p < 0.05$). [A] Birth weight <10th percentile.[18] [B] According to Gross classification.[16] [C] Combination of CPAM and BPS. [D] According to Solomon criteria.[19] [E] Surgery abroad.

	CDH			**EA**		
	Participants (n=114)*	*Non-participants (n=65)*	*p-value*	*Participants (n=93)*	*Non-participants (n=58)*	*p-value*
Baseline characteristics						
Male	70 (61.4)	36 (55.4)	0.53	57 (61.3)	37 (63.8)	0.45
Gestational age in weeks	38.7 (37.7-39.6)	38.6 (38.0-39.9)	0.87	37.9 (36.2-40.0)	38.4 (36.1-40.0)	0.81
Birth weight in grams	3000 (2800-3445)	3000 (2503-3480)	0.71	2850 (2160-3180)	2735 (2108-3128)	0.38
Preterm birth	14 (12.3)	12 (18.5)	0.18	32 (34.4)	20 (34.5)	0.50
Small for gestational age [A]	28 (24.6)	10 (15.4)	0.55	36 (38.7)	21 (36.2)	0.57
Side of hernia						
Left	98 (86.0)	55 (84.6)	0.83			
Right	16 (14.0)	10 (15.4)	0.83			
Type repair						
Primary repair	40 (35.1)	24 (36.9)	0.70			
Patch	73 (64.0)	41 (63.1)	0.70			
Unknown	1 (0.9)	0	-			
Type of EA [B]						
Type A				7 (7.5)	6 (10.3)	0.56
Type B				0	2 (3.4)	-
Type C				82 (88.2)	43 (74.1)	0.06
Type D				0	1 (1.7)	-
Type E				3 (3.2)	4 (6.9)	0.43
Unknown				1 (1.1)	2 (3.4)	-
Staged repair				10 (10.8)	12 (20.7)	0.10
Associated problems						
VACTERL [D]	-	-	-	11 (11.8)	12 (20.7)	0.17
Major anomalies	26 (22.8)	13 (20·0)	0·71	35 (37.6)	29 (50.0)	0.18
Minor anomalies	5 (4.4)	6 (9·2)	0·21	28 (30.1)	23 (39.7)	0.29

Type of primary surgery						
Thoracotomy	1 (0.9)	0	-	63 (67.7)	43 (74.1)	0.36
Thoracoscopy	33 (28.9)	22 (33.8)	0.40	28 (30.1)	14 (24.1)	0.58
Laparotomy	67 (58.8)	30 (46.2)	0.21	0	0	-
Laparoscopy	1 (0.9)	3 (4.6)	0.13	0	0	-
Converted	10 (8.8)	6 (9.2)	1.00	2 (2.2)	0	-
Unknown [E]	1 (0.9)	4 (6.2)	-	0	1 (1.7)	-
Duration of anaesthetic exposure in the first 24 months of life in minutes	270 (184-422)	265 (170-505)	0.90	393 (261-786)	442 (299-798)	0.25
Characteristics at time of FU						
Age at FU	8.2 (8.1-8.3)			8.2 (8.1-8.3)		
Educational level						
Regular	97 (85.1)	26 (40.0)	0.05	71 (76.3)	30 (51.7)	0.53
Regular with help	11 (9.6)	4 (6.2)	0.75	17 (18.3)	3 (5.2)	0.12
Special education	6 (5.3)	7 (10.8)	0.19	5 (5.4)	7 (12.1)	0.05
Other	0	0	-	0	2 (3.4)	
Unknown	0	28 (43.1)	-	0	16 (27.6)	
Maternal educational level						
Low (ISCED 0-2)	8 (7.0)	2 (31)	1.00	15 (16.1)	8 (13.8)	1.00
Middle (ISCED 3-4)	43 (37.7)	11 (16.9)	0.53	37 (39.8)	20 (34.5)	0.86
High (ISCED 5-8)	47 (41.2)	17 (26.2)	0.53	39 (41.9)	19 (32.8)	0.86
Unknown	16 (14.0)	35 (53.8)	-	2 (2.2)	11 (19.0)	

Table 1B. Demographic variables of participating and non-participating children with congenital diaphragmatic hernia (CDH), esophageal atresia (EA), and congenital lung malformation CLM). Data are presented as n (%) or median (IQR). One patient had both CDH and an incidental finding of CLM, and was included in the CDH group. CPAM = congenital pulmonary airway malformation, BPS = bronchopulmonary sequestration, CLE = congenital lobar emphysema, BC = bronchogenic cyst, VACTERL = vertebral, anorectal, cardiac, tracheoesophageal, renal, and limb malformations, ISCED = International Classification of Education.[21] Asterisk indicates significance (p<0.05). [A] Birth weight <10th percentile.[18] [B] According to Gross classification.[16] [C] Combination of CPAM and BPS. [D] According to Solomon criteria.[19] [E] Surgery abroad.

	CLM		
	Participants (n=13)	*Non-participants (n=6)*	*p-value*
Baseline characteristics			
Male	10 (76.9)	3 (50.0)	0.32
Gestational age in weeks	38.7 (36.8-40.4)	38.4 (37.5-39.9)	0.58
Birth weight in grams	3265 (3070-3690)	3088 (2846-3888)	0.42
Preterm birth	3 (23.1)	1 (16.7)	1.00
Small for gestational age [A]	0	1 (16.7)	-
Type of CLM			
CPAM	7 (53.8)	5 (83.3)	0.04
BPS	2 (15.4)	1 (16.7)	1.00
CLE	3 (23.1)	0	-
BC	0	0	-
Hybrid [C]	1 (7.7)	0	-
Associated problems			
VACTERL [D]	-	-	-
Major anomalies	1 (7.7)	0	-
Minor anomalies	0	1 (16.7)	-
Type of primary surgery			
Thoracotomy	10 (76.9)	3 (50.0)	1.00
Thoracoscopy	2 (15.4)	1 (16.7)	1.00
Unknown [E]	1 (7.7)	2 (33.3)	-
Duration of anaesthetic exposure in the first 24 months of life in minutes	203 (184-229)	210 (58-264)	0.80
Characteristics at time of FU			
Age at FU	8.2 (8.1-8.3)		
Educational level			
Regular	11 (84.6)	1 (16.7)	0.37
Regular with help	2 (15.4)	1 (16.7)	0.37
Special education	0	0	-
Other	0	0	-
Unknown	0	4 (66.7)	-
Maternal educational level			
Low (ISCED 0-2)	2 (15.4)	0	-
Middle (ISCED 3-4)	2 (15.4)	1 (16.7)	1.00
High (ISCED 5-8)	7 (53.8)	3 (50.0)	1.00
Unknown	2 (15.4)	2 (33.3)	-

Table 2. Clinical outcomes of 8-year-old children with congenital diaphragmatic hernia (CDH, n=114), oesophageal atresia (EA, n=93) and congenital lung malformation (CLM, n=13), summarized per clinical domain. Data are presented as n (% of total cohort). Cognition, proxy-reported behaviour, and proxy-reported daily executive functioning were categorized as normal, borderline, or impaired. All other clinical domains were categorized as either normal or impaired or as yes or no. [A] Defined as ≥3 lower respiratory tract infections in the past year requiring antibiotic therapy and/or hospitalization, or presence of vocal cord dysfunction or subglottic stenosis requiring regular follow-up. See the Methods section and the Supplemental Material for a detailed description of the administered instruments.

		CDH				**EA**				**CLM**			
		Normal	*Borderline*	*Impaired*	*Missing*	*Normal*	*Borderline*	*Impaired*	*Missing*	*Normal*	*Borderline*	*Impaired*	*Missing*
Health and daily functioning	Cognition	87 (76.3)	21 (18.4)	4 (3.5)	2 (1.8)	82 (88.2)	9 (9.7)	1 (1.1)	1 (1.1)	13 (100.0)	0	0	0
	Proxy-reported behaviour	68 (59.6)	11 (9.6)	15 (13.2)	20 (17.5)	51 (54.8)	15 (16.1)	5 (5.4)	22 (23.7)	8 (61.5)	0	0	5 (38.5)
	Proxy-reported daily executive functioning	64 (56.1)	5 (4.4)	2 (1.8)	43 (37.7)	45 (48.4)	2 (2.2)	1 (1.1)	45 (48.4)	7 (53.8)	0	0	6 (46.2)
	Motor function	75 (65.8)		37 (32.5)	2 (1.8)	65 (69.9)		25 (26.9)	3 (3.2)	11 (84.6)		2 (15.4)	0
	Maximum exercise capacity	48 (42.1)		52 (45.6)	14 (12.3)	43 (46.2)		43 (46.2)	7 (7.5)	8 (61.5)		4 (30.8)	1 (7.7)
	Lung function	69 (60.5)		42 (36.8)	3 (2.6)	55 (59.1)		37 (39.8)	1 (1.1)	7 (53.8)		6 (46.2)	0
		No		*Yes*	*Missing*	*No*		*Yes*	*Missing*	*No*		*Yes*	*Missing*
	Gastroesophageal reflux	42 (36.8)		4 (3.5)	68 (59.6)	40 (43.0)		7 (7.5)	46 (49.5)	0		0	13 (100.0)
	Respiratory morbidity [A]	105 (92.1)		9 (7.9)	0	60 (64.5)		33 (33.5)	0	12 (92.3)		1 (7.7)	0
	Daily medication for physical condition	87 (76.3)		27 (23.7)	0	70 (75.3)		23 (24.7)	0	11 (84.6)		2 (15.4)	0
	Daily medication for psychological condition	111 (97.4)		3 (2.6)	0	92 (98.9)		1 (1.1)	0	12 (92.3)		1 (7.7)	0
	Scoliosis	108 (94.5)		6 (5.3)	0	88 (94.6)		5 (5.4)	0	13 (100.0)		0	0
	Tube feeding	112 (98.2)		2 (1.8)	0	89 (95.7)		4 (4.3)	0	13 (100.0)		0	0
	Home oxygen	114 (100.0)		0	0	92 (98.9)		1 (1.1)	0	13 (100.0)		0	0

Lower cognition was significantly associated with lower self-reported HS (see Table 3). Figure 2 presents the raw total PedsQL and DUX-25 scores for normal, borderline, and impaired cognition. Lower scores on behavior, motor function, maximum exercise capacity, and lung function were not associated with lower self-reported HS. We found no significant associations between clinical outcomes and QoL. These findings persisted after correction for sex and MEL (see Supplementary Material S2).

EA

The majority of the 93 children with EA scored normal on cognition, behavior, motor function, and lung function. Out of these 93 children, 83 (89%) had below normal scores for at least 1 of the 14 clinical outcome domains. Daily executive functioning was normal in 48% of children, and 46% had a normal exercise capacity. GER was absent in the majority of children who underwent routine 24 h pH-impedance testing. See Table 2 for more details on clinical outcomes.

Lower cognition and behavior were both significantly associated with lower self-reported HS (see Table 3). We found no significant associations between any clinical outcome and QoL. After correction for sex and MEL, the significant and self-reported HS disappeared

CLM

Out of the 13 children with CLM, 8 (62%) had below normal scores for at least one of the 14 clinical outcome domains. Due to the low number of cases, multivariable linear regression was not possible; therefore, the association between the health domains and self-perceived HS and QoL could not be evaluated in these children.

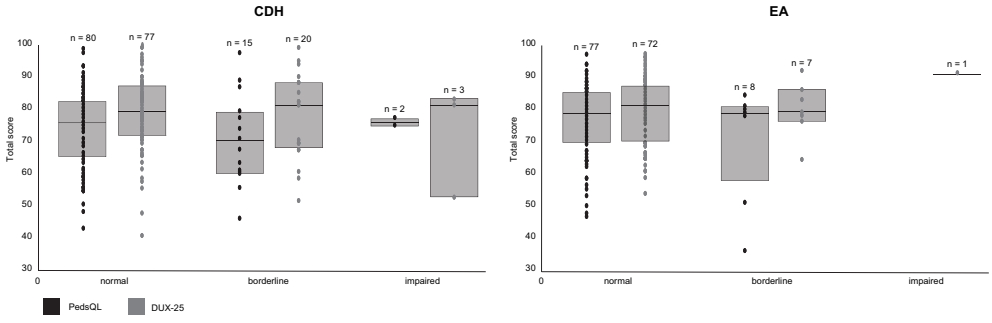

Figure 2. PedsQL and DUX-25 scores for normal, borderline, and impaired cognition. CDH = congenital diaphragmatic hernia, EA = esophageal atresia. Boxes indicate the interquartile range with median.

Table 3. Results of multiple linear regression analyses for PedsQL and DUX-25 scores and subscales of children with congenital diaphragmatic hernia (CDH, n=114) and oesophageal atresia (EA, n=93). Asterisk (*) and bold font indicate significance (p<0·05). B = unstandardized coefficient.

		Independent variables													
		Cognition				**Behaviour**				**Motor function**		**Maximum exercise capacity**		**Lung function**	
		Borderline (n=21)		*Impaired (n=4)*		*Borderline (n=11)*		*Impaired (n=15)*		*Impaired (n=37)*		*Impaired (n=52)*		*Impaired (n=42)*	
Dependent variables		*B*	*p-value*	*B*	*p-value*	*B*	*p-value*	*B*	*p-value*	*B*	*p-value*	*B*	*p-value*	*B*	*p-value*
CDH	**PedsQL**														
	Physical functioning	-16.57	**0.042 ***	-24.15	0.14	-7.02	0.51	-5.03	0.59	0.35	0.96	-10.15	0.15	-4.65	0.46
	Emotional functioning	-17.36	**0.031 ***	-23.17	0.15	-7.20	0.49	-5.79	0.53	2.11	0.76	-1.22	0.86	-5.09	0.41
	Social functioning	-19.91	**0.017 ***	-31.21	0.059	-6.89	0.51	-8.89	0.35	-2.53	0.72	-5.97	0.41	-5.75	0.38
	School functioning	-14.63	0.054	-31.79	**0.041 ***	-13.71	0.18	-8.39	0.33	-2.33	0.72	-0.14	0.98	-4.16	0.48
	Psychosocial health	-17.38	**0.021 ***	-28.60	0.056	-8.17	0.40	-7.84	0.36	-0.41	0.95	-3.72	0.57	-4.82	0.41
	Total score	-17.44	**0.022 ***	-26.76	0.079	-8.87	0.36	-6.63	0.44	-0.26	0.97	-5.06	0.43	-4.86	0.41
	DUX-25														
	Physical functioning	3.20	0.71	-13.24	0.45	9.56	0.36	-1.81	0.86	10.81	0.13	-9.83	0.18	-1.05	0.87
	Home functioning	-1.30	0.88	-20.49	0.28	17.58	0.096	1.14	0.91	12.41	0.086	-9.87	0.18	0.89	0.90
	Emotional functioning	-0.20	0.98	-20.42	0.19	7.39	0.44	-4.19	0.64	13.60	**0.037 ***	-7.04	0.29	1.24	0.84
	Social functioning	-6.50	0.44	-21.41	0.20	12.84	0.21	-0.49	0.96	11.51	0.097	-8.23	0.24	-0.08	0.99
	Close social functioning	-5.86	0.51	-32.69	0.065	14.10	0.19	-2.04	0.84	13.30	0.067	-11.42	0.12	1.11	0.87
	Far social functioning	-6.47	0.44	-14.27	0.41	12.54	0.21	2.68	0.78	10.27	0.14	-5.20	0.46	-0.63	0.92
	Total score	-0.82	0.92	-18.72	0.26	10.58	0.30	-2.01	0.83	12.00	0.071	-8.95	0.19	0.41	0.95

		Borderline (n=9)		*Impaired (n=1)*		*Borderline (n=15)*		*Impaired (n=5)*		*Impaired (n=25)*		*Impaired (n=43)*		*Impaired (n=37)*	
		B	*p-value*	*B*	*p-value*	*B*	*p-value*	*B*	*p-value*	*B*	*p-value*	*B*	*p-value*	*B*	*p-value*
EA	**PedsQL**														
	Physical functioning	-2.76	0.78	-62.34	**0.032 ***	-3.56	0.64	-23.65	0.092	-10.24	0.095	-3.10	0.63	-7.60	0.19
	Emotional functioning	-3.62	0.72	-56.38	0.089	4.67	0.54	-20.03	0.13	-1.43	0.82	4.83	0.45	-7.66	0.18
	Social functioning	1.82	0.87	-63.43	**0.043 ***	-5.67	0.49	-27.30	0.090	-6.46	0.34	-0.60	0.93	-5.02	0.42
	School functioning	-4.18	0.68	-52.31	0.055	-4.43	0.54	-30.54	**0.025 ***	-7.03	0.22	1.07	0.87	-7.62	0.16
	Psychosocial health	-1.44	0.89	-56.46	0.063	-0.94	0.90	-24.97	0.058	-4.29	0.47	1.14	0.85	-7.05	0.20
	Total score	-2.82	0.77	-58.90	**0·044 ***	-1.50	0.83	-29.16	**0.029 ***	-6.32	0.28	1.20	0.84	-6.50	0.23
	DUX-25														
	Physical functioning	-11.45	0.41	24.15	0.45	-4.88	0.67	-13.59	0.48	9.10	0.28	2.68	0.73	4.78	0.54
	Home functioning	-16.20	0.23	14.38	0.67	-7.52	0.52	-2.60	0.89	12.84	0.13	3.42	0.66	6.68	0.39
	Emotional functioning	-6.34	0.62	19.94	0.49	-7.69	0.43	-0.89	0.96	12.93	0.090	2.01	0.78	5.81	0.40
	Social functioning	-7.07	0.58	8.42	0.79	-4.57	0.67	-6.74	0.69	12.60	0.11	4.97	0.50	5.87	0.42
	Close social functioning	-6.19	0.66	7.97	0.80	-9.15	0.41	-5.19	0.79	12.85	0.12	3.40	0.60	10.03	0.19
	Far social functioning	-5.60	0.67	6.86	0.83	-1.77	0.87	-15.27	0.39	11.74	0.13	7.17	0.33	4.10	0.56
	Total score	-10.15	0.42	17.59	0.57	-6·44	0.55	-3.91	0.81	11.69	0.13	2.92	0.69	5.92	0.41

DISCUSSION

In this cohort of eight-year-old children born with foregut anomalies, we evaluated the relationship between generic PROMs and clinical outcomes. The standardized assessments of health and daily functioning indicated favorable clinical outcomes overall. In total, 189 (86%) out of 220 children with CDH, EA, or CLM had below normal scores for at least 1 of the 14 clinical outcome domains. In children with CDH, only lower cognition was associated with lower patient-reported HS. In children with EA, lower cognition and behavior were both associated with lower HS; although, after correction for sex and MEL, only the association with behavior remained. We found no associations between clinical outcomes and patient-reported QoL. Our results suggest that disease-related morbidity is not associated with self-reported HS, nor with self-reported QoL in school-aged children.

Determining what matters most to patients is essential to VBHC, the focus of which is improving quality of care while promoting cost-effectiveness.[4] When evaluating disease burden, both the physical and psychosocial impact of disease should be taken into account. Although PROMs play a central role in capturing patient experience, studies on the relationship between clinical outcomes and patient-reported HS and QoL remain scarce, and have mostly focused on clinical characteristics and symptoms rather than long-term outcomes.[22, 23]

Our findings are similar to those previously reported by our research group for other critical conditions. A study in five-year-old neonatal extracorporeal membrane oxygenation (ECMO) survivors also found a relationship between cognition and HS, although proxy-reports were used instead of self-reports.[10] Another study in eight-year-old neonatal ECMO survivors found no correlation between self-reported HS and actual motor performance, and self-perceived motor performance was found to be better than actual motor performance.[9] By contrast, a study in children with a history of laryngotracheal stenosis found that exercise capacity and lung function were positively associated with HS. However, this relationship was determined using parent-reported questionnaires, the results of which were found to differ significantly from self-reports.[24]

In the present study, we found an association between clinical outcome and patient-reported HS, but only for children with CDH and borderline cognition. The lack of relationships between other clinical outcomes and PROMs might be explained by several factors. First, it has been suggested that objective outcome measurements often focus on one physiological aspect, whereas PROMs are multifactorial.[25] Second, school-aged children with congenital anomalies tend to overestimate their performance, a phenomenon known as superiority bias.[26] However, despite the disparity between actual competence and self-reported competence, eight-year-olds have been found to be able to provide valid and reliable self-reports.[27]

The general disagreement between clinical outcomes and a child's own experiences found in our study indicates that identification of long-term morbidities cannot be based solely on PROMs for HS and QoL. Given that clinical outcomes tend to deteriorate with age in children born with foregut anomalies,[14, 28, 29] the importance of routinely measuring clinical outcomes becomes more apparent, as it allows for early identification of children at risk of declining performance. This should be communicated clearly to both children and their caregivers in order to support a discussion about the added value of complying to a timely intervention when problems arise. As such, PROMs may be used to support clinical decision making by prioritizing comorbidities in relation to what matters most to a given child. Clinicians with sufficient knowledge of long-term morbidities and their potential consequences later in life should counsel the child and their family about associated morbidities that do not directly affect self-perceived HS or QoL at school age. Moreover, since the wellbeing of children with lower IQ is particularly influenced by their cognitive disadvantage, clinicians must ensure that a child's level of academic performance matches their developmental level.

Here, we discuss the association between generic PROMs and clinical outcomes. Since condition-specific PROMs have been shown to provide more sensitive information than generic instruments,[30] studies using disease-specific PROMs might provide new insights into this relationship and help define a disease-specific minimal set of outcome measures.

Strengths of our study include the use of a relatively large and representative cohort of children with a rare congenital anomaly, whose data were collected within the infrastructure of a standardized longitudinal follow-up program. Several limitations need to be addressed. First, due to the small number of children with CLM who underwent surgery in the neonatal period, it was not feasible to assess the relationship between PROMs and clinical outcomes for these children. Nevertheless, we decided to show their data in order to cover the outcome results of a homogeneous group of children born with anatomical foregut anomalies. Second, a relatively large amount of data was missing for several clinical outcomes, notably behavior, daily executive functioning, and GER. These outcomes were added to our follow-up program at a later stage and might have caused confounding bias. Finally, our results may be subject to superiority bias. This tendency potentially disappears as children grow older. Longitudinal studies evaluating PROMs at 8 and 12 years of age are currently being performed by our department.

CONCLUSION

In conclusion, apart from an association between lower cognition or behavioral problems and lower patient-reported HS, we found limited relationships between clinical outcomes and PROMs for HS and QoL in school-aged children with foregut anomalies. Based on these

findings, HS, QoL and clinical outcomes should be regarded as different concepts that should be routinely measured in order to facilitate a more holistic approach to clinical decision making. PROMs may be useful to prioritize assessment and treatment of comorbidities in relation to a child's experiences and, as such, can promote VBHC in children with chronic conditions.

Our study provides a framework to assess the added value of PROMs in clinical decision making. Future studies should aim to broaden this perspective by including other chronic conditions and disease-specific PROMs. A clearer understanding of these relationships will help optimize healthcare systems while improving clinical outcomes, HS, and QoL in children with chronic disease status.

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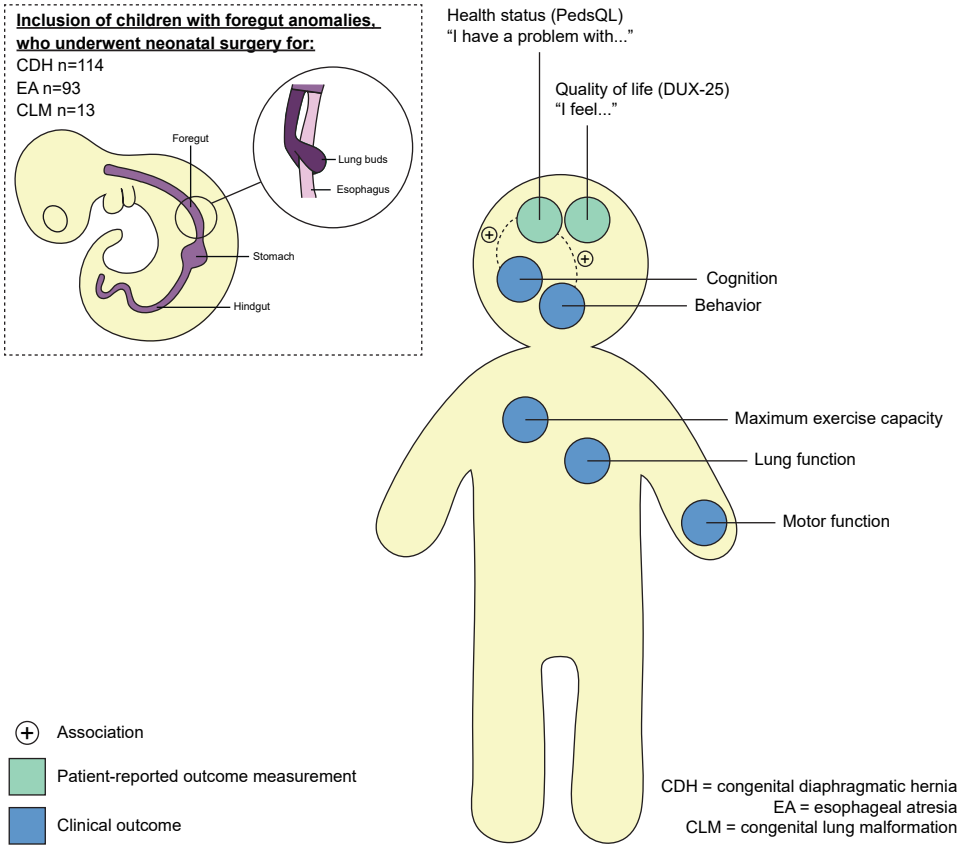
GRAPHICAL ABSTRACT



S1. Detailed description of instruments used to measure clinical outcomes

Health status

Pediatric Quality of Life Inventory (PedsQL)[1]

The PedsQL questionnaire is an instrument for assessing health status in children, adolescents, and adults, of which different age-appropriate versions are available. It consists of 23 items, divided into four subscales: physical functioning (eight items), emotional functioning (five items), social functioning (five items), and school functioning (five items). A fifth subscale, psychosocial health, is the sum of the emotional, social, and school functioning scales. The total score is calculated from all 23 items together. Answers range from 0 (never a problem) to 4 (almost always a problem) and are converted to a score between 0 and 100, with higher scores representing better health status.

In 2009, the PedsQL was validated in Dutch children aged 5-18 years,[2] and sex-specific norm values have recently been updated.[3] See Supplementary Table S1.1.

Quality of life

Dutch-Child-AZL-TNO-Quality-of-Life (DUX-25)[4]

The DUX-25 questionnaire is an instrument used to measure quality of life in children aged five years and older. It is comprised of 25 items within four domains: physical functioning (six items), emotional functioning (seven items), social functioning (seven items), and home functioning (five items). Social functioning can be divided into close and far social functioning. Close social functioning involves interaction with peers (e.g., friends or classmates). Far social functioning involves interaction with adults or teachers and the child's feelings towards other children. The total score is calculated from all 25 items together. Items are answered using a visual 5-point Likert scale, which are then converted to a score between 0-100. Higher scores indicate a better quality of life.

The DUX-25 has been modified from the original TNA-AZL Children's quality of life questionnaire (TACQOL), which contained 54 items for the self-report and 63 items for the parent-report.[5] Recently, the DUX-25 has been validated in Dutch children aged 8-17 years, with updated sex-specific norm values.[3] See Supplementary Table S1.2.

Cognition

Revised Amsterdam Intelligence Test (RAKIT)[6]

The RAKIT is an instrument for measuring intelligence (IQ) in children aged 4-12 years. It consists of 12 subtests, which provide – besides a total IQ score – scores for perceptual reasoning, verbal learning, spatial orientation, and verbal fluency.

The RAKIT was developed in the Dutch language and has been validated in Dutch children. In our hospital, the RAKIT was used until the end of 2009, after which it was replaced by the WISC. For this study, only total IQ scores were used. IQ >85 was considered normal, IQ 70-84 was considered borderline, and IQ <70 was considered impaired.

Wechsler Intelligence Scale for Children (WISC)[7]
The WISC is an instrument for measuring IQ in children aged 6-17 years. The Dutch WISC-III-NL consists of 13 subtests, which measure verbal, performance, and total IQ. The Dutch WISC-V-NL consists of 14 subtests, including eight tests from the 3rd version.

Both editions have been validated in Dutch children, with a mean (SD) of 100 (15).[7, 8] In our hospital, the WISC-III-NL was used until the end of 2019, after which it was replaced by the WISC-V-NL. For this study, only total IQ scores were used. IQ >85 was considered normal, IQ 70-84 was considered borderline, and IQ <70 was considered impaired.

Behavior
Strengths and Difficulties Questionnaire (SDQ)[9]
The SDQ is a brief emotional and behavioral screening tool for children aged between two and 17 years. A proxy-report for parents and teachers is available for children aged two years and older. The SDQ consists of 25 items divided between five domains: emotional symptoms (five items), conduct problems (five items), hyperactivity and inattention (five items), peer relationships (five items), and prosocial behavior (five items). A total score is calculated from all 25 items together.

For this study, we used the Dutch version of the parental proxy-reports.[10] The SDQ has been part of our follow-up program since 2011. We determined our cut-off values based on sex-dependent mean (SD) total scores. Up to +1SD was deemed normal, +1SD to +2SD was deemed borderline, and a score of >+2SD was deemed impaired.

Child Behavior Checklist (CBCL)[11, 12]
The CBCL is an instrument to rate behavioral and emotional problems in children, and is completed by parents or caretakers. Two versions are available: one for children aged 2-3 years and one for children aged 4-16 years. It consists of 20 items regarding activities, social interaction, and school functioning, 118 items regarding emotions and behavior, and two open questions on other behavioral problems. Answers are scored on a three-point scale and include not at all, sometimes, and often. The complete checklist results in a syntax-calculated total score.

For this study, we used parental proxy-reports for children aged 4-16 years. The CBCL was used in our follow-up program until the end of 2010. Based on Dutch norm values, behavior was scored as normal, borderline or impaired.[13]

Daily executive functioning

Behavior Rating Inventory of Executive Function (BRIEF)[14]

The BRIEF is a rating scale for assessing the everyday behavioral manifestations of the child's executive control functions. Three versions are available: a parent-reported version for children aged 5-17 years, a teacher-reported version for children aged 5-11 years, and a self-reported version for children aged 11-17 years. The proxy-reports consist of 75 items, whereas the self-report consists of 68 items. These items are divided into eight scales: inhibition, flexibility, regulation of emotions, initiative, working memory, planning, organization of materials, and monitoring. The scales are summarized into two indices (behavior and metacognition) and a total score. The BRIEF has been implemented in our follow-up program from 2015 onwards.

For this study, we evaluated the total scores of validated Dutch parental proxy-reports.[15] A total score of <60 is considered normal, a total score of 60-65 is considered borderline and a total score of >65 is considered impaired.

Motor function

Movement Assessment Battery for Children (MABC)[16]

The MABC is a motor function test and is used to evaluate a child's motor function. The MABC-I is used for children aged 4-12 years and the second version, the MABC-II, can be used for children aged 3-16 years. The checklist, that consists of 48 items for the MABC-I and 30 items for the MABC-II, covers activities in daily life and can be filled in by parents or teachers. The motor function test consists of eight items divided into three domains: three manual dexterity tasks, two ball-skill tasks, and three balance tasks. Each item is scored from 0 to 5 (good to very poor). This results in domain-specific scores and a total impairment score, which can be interpreted as a percentile using age-specific normative data tables.

In our follow-up program, the MABC-I was used until 2012, after which it was replaced by the MABC-II. Both editions contain similar content, are assumed to be comparable, and have been validated in Dutch children.[17, 18] We evaluated the percentiles of the total impairment scores. A percentile of ≥16 was considered normal and a percentile of ≤15 was considered impaired.

Maximum exercise capacity

Bruce protocol[19]

The Bruce protocol is a treadmill test for evaluating exercise capacity. During the test, the incline and speed are adjusted every three minutes according to the standardized Bruce

protocol and the child is encouraged to perform until exhaustion. Before and during the test, both heart rate and transcutaneous oxygen saturation are monitored. Maximal performance or exhaustion is defined as a heart rate of ≥185 beats per minute or loss of coordination.

In our study, the maximal endurance time in minutes was compared to recently defined age-specific reference values for Dutch children, leading to an SD score.[20] SD scores of ≥-1 was considered normal and <-1 was considered impaired.

Lung function

Lung function was tested according to criteria set by the European Respiratory Society.[21] Results were categorized as normal or impaired (standard deviation (SD) <-1.64 or >1.64) based on the forced expiratory volume (FEV1) before bronchodilation, in accordance with the Global Lung Initiative 2012.[22]

Maternal educational level (MEL)

MEL was used as a proxy for socioeconomic status and categorized according to the International Standard Classification of Education.[23]

Supplementary Table S1.1. Sex-specific Dutch norm values for PedsQL scales for 8-to-12-year-old healthy children. IQR = interquartile range.

		Median (IQR)	
		Male (n=93)	*Female (n=146)*
Self-reports	Physical functioning	93.75 (90.63-100.00)	90.63 (84.38-96.88)
	Emotional functioning	80.00-65.00-85.00)	75.00 (60.00-85.00)
	Social functioning	90.00 (75.00-100.00)	90.00 (80.00-100.00)
	School functioning	80.00 (75.00-90.00)	85.00 (75.00-95.00)
	Psychosocial health	81.67 (75.00-88.33)	83.33 (76.67-90.00)
	Total score	85.87 (80.44-91.30)	85.87 (79.35-91.30)
		Male (n=121)	*Female (n=179)*
Proxy-reports	Physical functioning	93.75 (84.38-100.00)	90.63 (84.38-96.88)
	Emotional functioning	75.00 (65.00-82.50)	75.00 (60.00-90.00)
	Social functioning	90.00 (75.00-100.00)	90.00 (75.00-100.00)
	School functioning	75.00 (65.00-90.00)	85.00 (75.00-95.00)
	Psychosocial health	78.33 (71.67-86.67)	81.67 (73.33-90.00)
	Total score	83.70 (77.17-90.22)	84.78 (77.17-91.30)

Supplementary Table S1.2. Sex-specific Dutch norm values for DUX-25 scales for 8-to-12-year-old healthy children. IQR = interquartile range.

		Median (IQR)	
Self-reports		*Male (n=92)*	*Female (n=145)*
	Physical functioning	89.58 (79.17-95.83)	87.50 (75.00-95.83)
	Home functioning	95.00 (90.00-100.00)	95.00 (90.00-100.00)
	Emotional functioning	78.57 (67.86-89.29)	82.14 (67.86-89.29)
	Social functioning	82.14 (71.43-89.29)	82.14 (75.00-89.29)
	Social close functioning	91.67 (83.33-100.00)	91.67 (83.33-100.00)
	Social far functioning	75.00 (62.50-87.50)	75.00 (68.75-87.50)
	Total functioning	85.50 (76.00-92.00)	85.00 (77.50-90.00)
Proxy-reports		*Male (n=121)*	*Female (n=179)*
	Physical functioning	91.67 (75.00-100.00)	91.67 (75.00-96.88)
	Home functioning	95.00 (80.00-100.00)	95.00 (80.00-100.00)
	Emotional functioning	82.14 (71.43-92.86)	85.71 (71.43-92.86)
	Social functioning	85.71 (71.43-92.86)	85.71 (75.00-96.43)
	Social close functioning	91.67 (75.00-100.00)	91.67 (75.00-100.00)
	Social far functioning	81.25 (62.50-93.75)	82.25 (68.75-93.75)
	Total functioning	86.00 (74.00-94.50)	87.00 (75.00-94.00)

S2. Results of multivariable linear regression analysis adjusted for sex and maternal educational level

Supplementary Table 3A. Results of multivariable regression analyses of PedsQL and DUX-25 scores and subscales of children with congenital diaphragmatic hernia (CDH, n=114) and esophageal atresia (EA, n=93). All models included sex, maternal education level, cognition, behavior, motor function, maximum exercise capacity and lung function as independent variables. *Asterisk indicates significance (p<0.05). B = unstandardized coefficient

		Sex		**Maternal educational level**				**Cognitive functioning**				**Behavior**			
		Male (n=70)		*Middle (n=43)*		*Low (n=8)*		*Borderline (n=21)*		*Impaired (n=4)*		*Borderline (n=11)*		*Impaired (n=15)*	
		B	*p-value*	*B*	*p-value*	*B*	*p-value*	*B*	*p-value*	*B*	*p-value*	*B*	*p-value*	*B*	*p-value*
CDH	**PedsQL**														
	Physical functioning	0.57	0.94	-2.62	0.74	-19.90	0.16	-16.14	0.056	-22.39	0.19	-4.11	0.70	-4.72	0.62
	Emotional functioning	-5.37	0.46	0.96	0.90	-3.13	0.83	-17.78	0.033 *	-26.13	0.12	-6.26	0.54	-6.25	0.53
	Social functioning	-3.08	0.68	-2.22	0.78	-22.03	0.11	-19.92	0.021 *	-29.10	0.091	-5.21	0.62	-10.65	0.28
	School functioning	-6.33	0.36	0.25	0.97	-17.69	0.20	-15.08	0.055	-32.54	0.049 *	-11.50	0.24	-9.63	0.29
	Psychosocial health	-5.19	0.46	-0.05	1.00	-14.06	0.34	-17.69	0.023 *	-30.56	0.061	-8.57	0.38	-8.48	0.35
	Total score	-3.01	0.66	-1.06	0.88	-15.57	0.23	-17.37	0.028 *	-25.91	0.19	-6.89	0.49	-7.62	0.40
	DUX-25														
	Physical functioning	11.28	0.14	8.23	0.32	1.28	0.93	0.03	1.00	-12.19	0.51	9.96	0.35	3.70	0.72
	Home functioning	9.01	0.25	8.19	0.32	-6.06	0.70	-3.58	0.70	-17.39	0.36	18.00	0.091	5.20	0.62
	Emotional functioning	4.99	0.48	8.15	0.28	-10.81	0.41	-2.81	0.73	-19.01	0.25	8.27	0.40	-1.39	0.89
	Social functioning	5.84	0.43	8.33	0.29	-9.18	0.51	-9.33	0.28	-19.86	0.27	13.56	0.18	1.82	0.86
	Close social functioning	5.29	0.50	4.65	0.54	-9.30	0.58	-7.83	0.39	-31.36	0.10	14.64	0.17	-1.26	0.91
	Far social functioning	6.95	0.35	12.49	0.11	-9.01	0.52	-10.67	0.22	-13.36	0.46	15.12	0.15	15.12	0.51
	Total score	7.86	0.28	9.70	0.20	-7.01	0.62	-4.83	1.00	-16.90	0.51	13.50	0.15	3.70	0.72

		Male (n=57)		*Middle (n=37)*		*Low (n=15)*		*Borderline (n=9)*		*Impaired (n=1)*		*Borderline (n=15)*		*Impaired (n=5)*	
		B	*p-value*	*B*	*p-value*	*B*	*p-value*	*B*	*p-value*	*p-value*	*B*	*B*	*p-value*	*B*	*p-value*
EA	**PedsQL**														
	Physical functioning	0.80	0.89	-7.32	0.24	-13.07	0.14	-1.57	0.88	-52.25	0.068	-1.28	0.87	-12.29	0.29
	Emotional functioning	-1.40	0.81	-9.46	0.12	-7.26	0.43	-3.78	0.74	-53.81	0.13	6.47	0.42	-15.19	0.18
	Social functioning	3.04	0.63	-12.11	0.069	-12.23	0.20	5.06	0.67	-62.10	0.053	-2.19	0.80	-16.62	0.15
	School functioning	-1.78	0.75	-8.46	0.15	-10.04	0.24	-0.97	0.93	-55.30	0.056	-2.02	0.78	-19.62	0.048 *
	Psychosocial health	0.05	0.99	-10.34	0.073	-10.42	0.22	0.89	0.94	-54.29	0.080	0.56	0.94	-16.02	0.16
	Total score	0.28	0.96	-9.21	0.12	-10.36	0.24	-0.52	0.96	-59.83	0.056	0.71	0.92	-15.15	0.19
	DUX-25														
	Physical functioning	1.00	0.90	3.48	0.68	-5.55	0.65	-10.56	0.48	27.00	0.43	-5.61	0.62	-15.70	0.37
	Home functioning	-4.52	0.58	0.64	0.94	-5.59	0.64	-16.01	0.30	17.51	0.61	-5.87	0.60	-3.15	0.88
	Emotional functioning	-1.12	0.88	3.72	0.64	-4.43	0.69	-6.66	0.65	25.60	0.40	-9.75	0.34	0.12	1.00
	Social functioning	-0.49	0.95	2.13	0.79	0.02	1.00	-2.76	0.86	8.17	0.79	-6.20	0.55	-23.50	0.18
	Close social functioning	-3.88	0.63	1.37	0.87	-3.46	0.77	-7.48	0.61	9.86	0.77	-9.72	0.42	-8.14	0.65
	Far social functioning	-0.16	0.98	0.23	0.98	2.85	0.80	-6.70	0.65	2.47	0.94	-3.07	0.77	-22.05	0.19
	Total score	-0.95	0.90	1.96	0.81	-4.75	0.67	-8.14	0.57	22.34	0.48	-6.35	0.54	-6.99	0.68

Supplementary Table 3B. Results of multivariable regression analyses of PedsQL and DUX-25 scores and subscales of children with congenital diaphragmatic hernia (CDH, n=114) and esophageal atresia (EA, n=93). All models included sex, maternal education level, cognition, behavior, motor function, maximum exercise capacity and lung function as independent variables. *Asterisk indicates significance (p<0.05). B = unstandardized coefficient

		Motor function		**Exercise capacity**		**Lung function**	
		Impaired (n=37)		*Impaired (n=52)*		*Impaired (n=42)*	
		B	*p-value*	*B*	*p-value*	*B*	*p-value*
CDH	**PedsQL**						
	Physical functioning	3.28	0.67	-9.83	0.11	-1.05	0.50
	Emotional functioning	2.43	0.75	-9.87	0.97	0.89	0.37
	Social functioning	1.07	0.89	-7.04	0.35	1.24	0.37
	School functioning	0.14	0.99	-8.23	0.87	-0.08	0.42
	Psychosocial health	1.03	0.89	-11.42	0.88	1.11	0.34
	Total score	2.08	0.77	-5.20	0.51	-0.63	0.39
	DUX-25						
	Physical functioning	7.76	0.31	-11.39	0.13	0.32	0.96
	Home functioning	10.46	0.18	-11.45	0.14	2.17	0.75
	Emotional functioning	12.49	0.08	-8.35	0.24	1.78	0.78
	Social functioning	10.25	0.18	-9.22	0.21	0.85	0.90
	Close social functioning	12.59	0.12	-12.72	0.10	1.69	0.81
	Far social functioning	7.60	0.32	-6.74	0.36	0.37	0.96
	Total score	9.77	0.18	-10.10	0.15	1.32	0.84
		Impaired (n=25)		*Impaired (n=43)*		*Impaired (n=37)*	
		B	*p-value*	*B*	*p-value*	*B*	*p-value*
EA	**PedsQL**						
	Physical functioning	-7.30	0.25	-4.16	0.50	-9.19	0.11
	Emotional functioning	0.07	0.99	4.82	0.44	-7.72	0.18
	Social functioning	-3.17	0.64	-1.84	0.79	-5.78	0.35
	School functioning	-5.02	0.39	-0.22	0.97	-8.96	0.092
	Psychosocial health	-2.79	0.64	1.07	0.86	-7.41	0.17
	Total score	-4.34	0.46	-0.51	0.93	-7.84	0.15
	DUX-25						
	Physical functioning	10.00	0.24	3.74	0.65	3.10	0.70
	Home functioning	13.46	0.12	4.50	0.59	5.85	0.47
	Emotional functioning	13.39	0.085	2.58	0.73	4.29	0.55
	Social functioning	11.97	0.13	8.08	0.31	6.67	0.37
	Close social functioning	13.34	0.12	5.05	0.53	9.38	0.24
	Far social functioning	11.17	0.16	9.81	0.22	4.18	0.56
	Total score	12.20	0.13	4.04	0.59	4.83	0.52

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CHAPTER 11

Psychometric performance of a condition-specific quality of life instrument for Dutch children born with esophageal atresia

Submitted

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ABSTRACT

Background

A condition-specific instrument (EA-QOL© questionnaires) to assess quality of life of children born with esophageal atresia (EA) has been developed in Sweden and Germany. Before implementing this instrument in clinical practice in the Netherlands, we evaluated its psychometric performance in Dutch children.

Methods

After Swedish-Dutch translation, cognitive debriefing interviews were conducted with a subset of children with EA and their parents. Next, feasibility, reliability (internal, comparison and retest) and validity (construct and convergent with the PedsQL questionnaire) were evaluated in a nationwide field test.

Results

Cognitive debriefing confirmed the predefined concepts, although some questions were deemed not generally applicable. Feasibility was poor to moderate. In 2-to-7-year-old children, 8/17 items had >5% missing values. In 8-to-17-year-old children, this concerned 3/24 items of the proxy-report and 5/14 items of the self-report. Internal reliability was good (Cronbach's alpha >0.7 for all total scores). Retest reliability showed good correlation (ICC respectively 0.86, 0.84, 0.72). Comparison reliability between self-reports and proxy-reports was strong (ICC 0.81). Construct validity was discriminative. Convergent validity was strong for the 2-to-7-year-old proxy-report (r_s=0.64, p<0.001), and weak to moderate for the 8-to-17-year-old proxy-report (r_s=0.39, p<0.001) and self-report (r_s=0.54, p<0.001).

Conclusions

Overall, the Dutch-translated EA-QOL questionnaires showed good reliability and validity. Given the poor to moderate feasibility, the questionnaires could be made more suitable for clinical practice in the Netherlands by using computer adaptive testing and thereby customizing the questionnaire to the individual patient. Furthermore, cross-cultural validation studies and evaluation of its implementation in clinical practice in different countries are needed.

INTRODUCTION

As more newborns with esophageal atresia (EA) survive, long-term morbidities and quality of life become more relevant. Reported morbidities include gastrointestinal and pulmonary problems (e.g. dysphagia, gastroesophageal reflux and/or recurrent airway infections),[1, 2] growth retardation,[3] reduced exercise capacity[4] and impaired motor function.[5] A significant long-term aspect is the burden of disease, reflected in patient-reported outcome measures (PROMs) on health status (HS) and quality of life (QoL). HS describes a patient's well-being in terms of functioning,[6] while QoL focusses on perception about one's functioning.[7] In literature, these two concepts are often combined to health-related QoL (HRQoL).[8]

Despite inconsistent findings, studies indicated that children with EA report lower generic HRQoL than do healthy children.[9-12] Condition-specific instruments tend to be more sensitive to detect and discriminate clinical morbidities, and more suitable to assess disease burden.[13] For that reason, Dellenmark-Blom and coworkers have developed a condition-specific instrument to measure HRQoL in children with EA: the EA-QOL© questionnaires.[14-16] This set of age-specific questionnaires covers multiple domains (see Supplementary Material S1) identified through focus group interviews with children and/or their parents.[16] Results of the protocolized validation process have been published for Swedish, German and Turkish children.[14, 17]

Assessing HRQoL benefits health care for chronic conditions and enhances communication between children and professionals.[18, 19] Therefore, generic questionnaires have been implemented in Dutch follow-up programs.[20] A condition-specific instrument could contribute to a better understanding of children's perception of their disease impact, enabling tailor-made intervention strategies. However, given the heterogeneity of EA, its comorbidities and follow-up strategies worldwide, the context in which an instrument would be implemented differs between countries. We assumed that the translated questionnaires would not necessarily frictionless fit our population. Before implementing the EA-QOL© questionnaire in clinical practice in the Netherlands, we evaluated its psychometric performance in Dutch children.

METHODS

This is a cross-sectional study, consisting of three phases (translation, cognitive debriefing and field testing), similar to the other EA-QOL© validation studies and following international guidelines.[14, 17, 21] The study has been approved by the participating institutional review boards (IRB) (MEC-2019-0521, MEC-20-564/C, MEC-2020-6961 and MEC-2019-631). See Supplementary Material S2 for a detailed description of methods and IRB-related data.

Translation and cognitive debriefing

A Swedish-Dutch forward-backward translation was conducted,[22] and reviewed by the Swedish developer to ensure conceptual equivalence. To ensure that all items were understood as intended by the Dutch target population,[23] cognitive debriefing was conducted in three groups, stratified by severity of complaints (see Supplementary Table S3.1), as described previously[15]: A) parents of 2-to-7-year-old children (proxy-report); B1) parents of 8-to-17-year-old children (proxy-report); and B2) 8-to-17-year-old children (self-report). Participants were interviewed face-to-face during an annual meeting of the Dutch patient support group in September 2019. Participants of groups B1 and B2 were related, and interviewed separately and simultaneously. Participants filled out the questionnaire on paper while giving feedback on the clarity and adequacy of the items. Results were analyzed using content analysis. If necessary, we slightly rephrased instructions and items, after having consulted the Swedish developer. We obtained consensus on the final questionnaires for the field test.

Field testing

A nationwide field test was conducted between August 2020 and April 2021 in the Netherlands. Participants without known intellectual disability who had sufficient command of the Dutch language were identified from the databases of four university hospitals that cover care for approximately 80% of the Dutch EA population. They were invited through a personal letter, containing a personal access code to fill out the questionnaires online (LimeSurvey GmbH version 2.06lts, Hamburg, Germany). Parents – who were supposed to be the child's primary care taker – and/or children ≥8 years old filled out age-appropriate proxy-reports or self-reports of the EA-QOL© questionnaire[14] and the Pediatric Quality of Life Inventory™ 4.0 (PedsQL) questionnaire[24] (see Supplementary Material S1). A general questionnaire on sociodemographic information and on digestive symptoms, feeding difficulties and respiratory symptoms in the past four weeks was obtained as proxy-report in children <12 years old and as both self-report and proxy-report in children ≥12 years old. Parental educational level was classified according to the International Standard Classification of Education.[25]

To examine the test-retest reliability, participants were invited to fill out the EA-QOL© questionnaire a second time three weeks after the initial response. If needed, reminders were sent twice maximally. The final reminder also included the questionnaire on paper with a pre-stamped envelope for reply.

The following data were retrieved from the patient records: sex, gestational age, birth weight, type of EA,[26] presence of VACTERL (vertebral, anorectal, cardiac, tracheoesophageal, renal and limb anomalies) association,[27] type of primary surgery, postoperative complications (anastomotic leakage, pneumothorax, sepsis, wound infection or recurrent fistula), history of gastrostomy and history of esophageal dilatation. EA was considered long gap if staged repair had been performed. Small for gestational age was defined as birth weight <10th percentile.[28] Pneumothorax was

defined as the need for a chest tube, sepsis as a positive blood culture and wound infection according to the surgical site infection criteria of Centers for Disease Control and Prevention.[29]

Statistical analysis

Data are presented as number (%), median (interquartile range) or mean ± SD. Items were answered on a 5-point Likert scale, and reversed linearly transferred to a 0-100 scale, with 100 as best possible score. Subscales and total scores were computed by the mean, with a maximum of 30% missing items per subscale. Items were described as mean ± SD (range). Feasibility (percentage of items with >5% missing values[15]) and psychometric criteria (skewness and kurtosis <2.0) were evaluated.[30] Feasibility was considered poor (>30%), moderate (10-30%) or good (<10%). Subscales and total scores were described as median (IQR) with floor and ceiling effects (percentage of respondents reporting respectively the minimum and maximum possible score <15%).[31]

Internal reliability was considered good if Cronbach's alpha ≥0.7 for the scales.[31] External reliability – both proxy-self and test-retest comparison – was evaluated using intra-class coefficients (ICCs), using a two-way random model, single measures and absolute agreement. It was considered poor (<0.50), moderate (0.50-0.74), good (0.75-0.90), or excellent (>0.90).[32]

Construct validity was determined through known-groups validity: Mann-Whitney-U tests served to assess differences in total scores between clinical subgroups: patients with and without primary repair, a gastrostomy or ≥1 esophageal dilatation in history, and with and without digestive symptoms, feeding difficulties and respiratory symptoms in the past four weeks. We applied a Bonferroni correction to account for multiple comparison. As we assessed differences for 20 different variables, alpha was set at 0.05/20=0.0025. Effect sizes (ESs) were calculated by converting z-scores of the Mann-Whitney-U tests ($r=z/\sqrt{n}$),[33] and considered strengthening the validity if moderate (>0.30) or large (>0.50). Children in clinical subgroups were hypothesized to have lower total scores.

Convergent validity was examined by correlating the proxy-reported and self-reported total scores with the concomitant PedsQL scores[24, 34] using Spearman's rho (rs), and concluded as poor (<0.40), moderate (0.40-0.59), good (0.60-0.79), or excellent (>0.80). Statistical analyses were performed using SPSS V.24.0 (IBM, Chicago, Illinois, USA), with a significance level of $p<0.05$.

RESULTS

Cognitive debriefing

Review of the translations confirmed the intended conceptual content. Twenty-nine participants (19 parents and 10 children) were recruited for cognitive debriefing. Group A

consisted of nine parents (11% male, age range 32-44 years) of children with mild (n=2), moderate (n=5) or severe (n=2) complaints. Group B1 contained ten parents (30% male, age range 41-61 years), and group B2 ten children (40% male, age range 9-17 years) with mild (n=4), moderate (n=4) or severe (n=2) complaints.

Supplementary Table S3.2 summarizes the cognitive debriefing results. Overall, participants understood the items correctly according to the predefined concepts. Parents considered two items (*Can your child eat at the same pace as other children his/her age?*; *Does your child need to think of drinking a lot when he/she eats?*) multi-interpretable. We rephrased those items as suggested. Although participants considered some items burdensome, none were rejected. Some items, e.g. questions on oral feeding in case of full dependency of (par)enteral feeding or questions on small stature while having physical height within normal ranges, were repeatedly considered not applicable and unable to answer properly. To keep the translated version in line with the original, we did not adjust the response scale but modified the instructions. In the field test, participants were instructed to omit questions if not applicable. Some participants indicated that they had missed certain topics (see Supplementary Table S3.3). To preserve the original structure of the questionnaire, we continued to the field test with the questionnaire in its current form.

Field test

Study population

In total, 101 parents of 2-to-7-year-old children, 136 parents of 8-to-17-year-old children and 130 8-to-17-year-old children participated in the field test (response rate respectively 51%, 41% and 39%, recruited nationwide[35]). Respectively, 26%, 38% and 39% of them returned the questionnaire on paper. The proportion of parents with high educational level was larger than that in the general population (58% vs. 36%).[36] See Table 1 for demographic characteristics and Table 2 for clinical symptoms of the participants.

Item evaluation

Feasibility was poor to moderate (see Supplementary Material S4). Of the 2-to-7-year-old proxy-report, 8/17 items had >5% (6.9-32.7%) missing values, including all items of 'Social isolation and stress'. Of the 8-to-17-year-old proxy-report, 5/24 items had >5% (5.8-28.7%) missing values. Of the 8-to-17-year-old self-report, 3/24 items had >5% (11.5-21.5%) missing values. Subscale and total scores are presented in Table 3. We did not observe any floor effects. Ceiling effects of >15% were found for 'Social isolation and stress' for the 2-to-7- year-old proxy-report, and for 'Social relationships', 'Body perception' and 'Health and well-being' of both the 8-to-17-year-old self-reports and proxy-reports.

Table 1. Basic characteristics of the respondents, presented as n (%) or median (interquartile range). ISCED = International classification of education, EA = esophageal atresia, VACTERL = vertebral, anorectal, cardiac, tracheoesophageal, renal or limb anomalies, ISCED = International Classification of Education.[25] [A] City with >100,000 citizens. [B] At time of filling out questionnaire. [C] Birth weight <10th percentile.[28] [D] According to Gross classification.[26] [E] According to Solomon criteria.[27] [F] Gastric pull-up n=1. [F] Gastric pull-up n=1, jejunal interposition n=5

	EAQOL 2-7 years proxy-report (n=101)	**EAQOL 8-17 years proxy-report (n=136)**	**EAQOL 8-17 years self-report (n=130)**
Demographic characteristics			
Region			
North	8 (7.9)	2 (1.5)	2 (1.5)
South	12 (11.9)	18 (13.2)	18 (13.8)
West	59 (58.4)	81 (59.6)	79 (60.8)
East	21 (20.8)	35 (25.7)	31 (23.8)
Foreign country	1 (1.0)	-	-
Urban area [A]	25 (24.8)	35 (25.7)	33 (25.4)
Parental characteristics			
Age (years) [B]	38.5 (35.4-41.7)	45.7 (41.9-49.4)	
Male	21 (20.8)	31 (22.8)	
Single caregiver	6 (5.9)	10 (7.4)	9 (6.9)
Born in the Netherlands	93 (92.1)	120 (88.2)	114 (87.7)
Parental educational level			
Low (ISCED 0-2)	7 (6.9)	18 (13.2)	18 (13.8)
Middle (ISCED 3-4)	28 (27.7)	46 (33.8)	43 (33.1)
High (ISCED 5-8)	66 (65.3)	72 (52.9)	68 (52.3)
Parent with chronic condition	8 (7.9)	13 (9.6)	13 (10.0)
Child characteristics			
Age (years) [B]	5.0 (3.5-6.5)	13.6 (10.9-15.9)	13.8 (11.0-15.9)
Male	60 (59.4)	85 (62.5)	83 (63.8)
Gestational age (weeks)	37.7 (35.8-39.9)	38.0 (35.6-39.3)	38.0 (35.6-39.3)
Birth weight (grams)	2790 (1978-3300)	2740 (2200-3149)	2750 (2215-3200)
Preterm birth	36 (35.6)	47 (34.6)	46 (35.4)
Small for gestational age [C]	38 (37.6)	49 (36.0)	46 (35.4)
Type of EA [D]			
Type A	4 (4.0)	11 (8.1)	11 (8.5)
Type B	2 (2.0)	2 (1.5)	2 (1.5)
Type C	87 (86.1)	116 (85.3)	110 (84.6)
Type D	-	2 (1.5)	2 (1.5)
Type E	4 (4.0)	3 (2.2)	3 (2.3)
Unknown	4 (4.0)	2 (1.5)	2 (1.5)
Staged repair	8 (7.9)	14 (10.3)	14 (10.8)
VACTERL association [E]	16 (15.8)	16 (11.8)	15 (11.5)
Type of repair			
Primary anastomosis	85 (84.2)	120 (88.2)	115 (88.5)
Delayed primary anastomosis	10 (9.9)	10 (7.4)	9 (6.9)
Esophageal replacement [F]	1 (1.0) [E]	6 (4.4) [F]	6 (4.6) [F]
Unknown	5 (5.0)	-	-
Postoperative complications			
Anastomotic leakage	15 (14.9)	16 (11.8)	16 (12.3)
Pneumothorax	32 (31.7)	46 (33.8)	43 (33.1)
Sepsis	13 (12.9)	11 (8.1)	11 (8.5)
Wound infection	7 (6.9)	5 (3.7)	5 (3.8)
Recurrent fistula	1 (1.0)	5 (3.7)	6 (4.6)
History of gastrostomy	15 (14.9)	18 (13.2)	17 (13.1)
History of ≥1 dilatation	53 (52.5)	68 (50.0)	66 (50.8)
Siblings	77 (76.2)	110 (80.9)	106 (81.5)

Table 2. Digestive symptoms, respiratory symptoms and feeding difficulties in the four weeks prior to filling out the questionnaires, presented as n (%). [A] Only children ≥12 years old reported these items.

		EAQOL 2-7 years proxy-report (n=101)	EAQOL 8-17 years proxy-report (n=136)	EAQOL 8-17 years self-report (n=84) [A]
Symptoms	Heartburn	18 (17.8)	17 (12.5)	12 (14.3)
	Vomiting during or after meals	21 (20.8)	6 (4.4)	2 (2.4)
	Difficulty to swallow food	40 (39.6)	30 (22.1)	24 (28.6)
	Food getting stuck	45 (44.6)	39 (28.7)	30 (35.7)
	Complaints of pain while swallowing	16 (15.8)	9 (6.6)	8 (9.5)
	Coughing	64 (63.4)	63 (46.3)	42 (50.0)
	Wheezing	26 (25.7)	9 (6.6)	11 (13.1)
	Dyspnea at rest	10 (9.9)	6 (4.4)	7 (8.3)
	Dyspnea during physical activity	12 (11.9)	20 (14.7)	25 (29.8)
	Chest tightness	3 (3.0)	9 (6.6)	14 (16.7)
	Airway infections	14 (13.9)	12 (8.8)	3 (3.6)
	Recurrent pulmonary problems	34 (33.7)	32 (23.5)	14 (16.7)
Feeding difficulties	Avoiding food that is difficult to swallow	35 (34.7)	31 (22.8)	12 (14.3)
	Eating small portions	43 (42.6)	26 (19.1)	13 (15.5)
	Requiring energy-enriched food	21 (20.8)	8 (5.9)	5 (6.0)
	Requiring adjusted food consistency	25 (24.8)	1 (0.7)	-
	Needing >30 minutes to finish a meal	36 (35.6)	17 (12.5)	8 (9.5)
	Requiring increased fluid intake	45 (44.6)	43 (31.6)	31 (36.9)
	Nutrition through tube or gastrostomy	12 (11.9)	2 (1.5)	1 (1.2)
	Receiving nutrition through infusion pump	-	-	-
	Needing adult support while eating	28 (27.7)	4 (2.9)	14 (16.7)

Internal and external reliability

Internal reliability was good for the total scores, but the Cronbach's alpha for 'Health and well-being' was <0.7. For the proxy-self comparison, 128 child-parent couples were available, with good correlation for all subscales (see Supplementary Table S5.1) and the total score (ICC 0.81). In the retest, 70 parents (69% of the original sample) of 2-to-7-year-old children, 82 parents (60%) of 8-to-17-year-old children and 71 8-to-17-year-old children (55%) responded. Basic characteristics did not differ between respondents and non-respondents. Respectively 6%, 17% and 16% of the questionnaires were returned on paper. Clinical symptoms of none of the children differed from that in the initial test. Test-retest agreement was good for the total scores and most of the subscales (see Table 3). Agreement was moderate for 'Social isolation and stress' in the 2-to-7-year-old proxy-report, and 'Social relationships' and 'Body perception' in the 8-to-17-year-old self-report.

Table 3. Descriptive values of the EA-QOL© questionnaires. SD = standard deviation, IQR = interquartile range, ICC = intra-class correlation coefficient, CI = confidence interval. [A] Digestive symptoms, feeding difficulties and respiratory symptoms in the 4 weeks prior to the retest did not differ from those in the 4 weeks prior to the initial test. [B] Median interval of 39 days (range 20-91). [C] Median interval of 50 days (range 20-138). [D] Median interval of 51 days (range 20-138).

	Items (n)	Respondents (n)	Median (IQR)	Floor, n (%)	Ceiling, n (%)	Cronbach's alpha	Respondents (n) [A]	Level of agreement, ICC (95% CI)
Children 2-7 years old (proxy-report)								
Eating	7	101	82.14 (65.48-92.86)	-	6 (5.9)	0.85	68	0.77 (0.65-0.85
Physical health and treatment	6	92	75.00 (65.50-90.00)	-	9 (8.9)	0.79	62	0.84 (0.75-0.90)
Social isolation and stress	4	71	93.75 (68.75-100.00)	-	30 (29.7)	0.77	42	0.66 (0.44-0.0.80)
Total score	17	101	79.69 (66.99-91.07)	-	5 (5.0)	0.90	70 [B]	0.86 (0.78-0.91)
Children 8-17 years old (proxy-report)								
Eating	8	128	81.70 (71.88-90.63)	-	18 (14.1)	0.74	76	0.74 (0.62-0.83)
Social relationships	7	134	89.29 (81.25-100.00)	-	44 (32.8)	0.75	80	0.81 (0.72-0.87)
Body perception	5	133	100.00 (85.00-100.00)	-	68 (51.1)	0.79	79	0.71 (0.56-0.81)
Health and well-being	4	134	91.67 (79.69-100.00)	-	37 (27.6)	0.59	80	0.80 (0.70-0.87)
Total score	24	136	87.50 (79.21-93.28)	-	6 (4.4)	0.86	82 [C]	0.84 (0.77-0.90)
Children 8-17 years old (self-report)								
Eating	8	129	81.25 (68.75-92.19	-	17 (13.2)	0.69	69	0.78 (0.66-0.86)
Social relationships	7	129	89.29 (76.79-100.00)	-	37 (28.7)	0.73	70	0.68 (0.53-0.79)
Body perception	5	129	100.00 (90.00-100.00)	-	77 (59.7)	0.76	69	0.54 (0.35-0.69)
Health and well-being	4	128	89.58 (81.25-100.00)	-	38 (29.7)	0.39	68	0.70 (0.56-0.81)
Total score	24	130	86.71 (78.72-94.75)	-	8 (6.2)	0.85	71 [D]	0.72 (0.85-0.81)

Construct validity

Total scores of the 2-to-7-year-old proxy-report were lower for symptomatic children, with moderate to large ESs – except for children with heartburn, chest tightness and airway infections. Total scores of the 8-to-17-year-old proxy-report were lower for children avoiding certain food, adjusting their portions or increasing their fluid intake during meals, with moderate ESs. Total scores of the 8-to-17-year-old self-report were lower for children with dysphagia or dyspnea during physical activity, and for children adjusting their portions or increasing their fluid intake during meals, with moderate ESs. See Table 4.

Convergent validity

Total PedsQL scores showed a strong correlation with total EA-QOL© score of the 2-to-7-year-old proxy-report (n=100, rs=0.64, p<0.001), a weak correlation with total score of the 8-to-17-year-old proxy-report (n=135, rs=0.39, p<0.001), and a moderate correlation with the total score of the 8-to-17-year-old self-report (n=130, rs=0.54, p<0.001). See Table S6.1 for complete subscale and total PedsQL scores.

DISCUSSION

In this nationwide validation study of a condition-specific PROM for children with EA, we evaluated the psychometric performance of the Dutch-translated EA-QOL© questionnaires. Cognitive debriefing confirmed good understanding of the items according to the predefined concepts, but not all questions were deemed applicable for each child. Overall, the field test showed good internal and retest reliability for the total scores and most of the subscales. Construct validity was slightly discriminative. Convergent validity was variable, from weak to strong correlations.

In general, Dutch participants reported higher EA-QOL© scores than those in the Swedish-German validation study. From a clinical perspective, this could be explained by differing perceptions of symptoms – or perhaps fewer comorbidities. In our population, 2-to-7-year-olds tended to have fewer airway infections, and 8-to-17-year-olds had fewer complaints of heartburn and vomiting than in the Swedish-German study population. None of the Dutch children required parenteral nutrition in the field test, in contrast to 4 out of 124 Swedish-German children.[14] Considering the psychological distress of parenteral feeding,[37] this difference could have contributed to the higher EA-QOL scores in the Dutch population.

Table 4a. Comparison between clinical subgroups of total scores of the EA-QOL© questionnaire for children aged 2-7 years old (proxy-reports). Only patients for whom both clinical data and total scores EA-QOL© were available were included in the analyses. Asterisks indicate Bonferroni-adjusted significance p<0.0025.

	2-7 years old (proxy-report)					
	Yes		**No**			
	n	*Median (IQR)*	*n*	*Median (IQR)*	*p-value*	*Effect size (r)*
Surgical characteristics						
Primary repair	85	81.25 (68.30-92.42)	11	66.18 (46.88-76.47)	0.007	0.27
History of gastrostomy	15	66.18 (50.00-82.14)	82	82.03 (69..03-92.65)	0.003	-0.30
History of ≥1 dilatation	53	80.00 (67.16-92.52)	47	79.41 (67.31-87.50)	0.785	-0.03
Digestive symptoms						
Heartburn	18	69.79 (60.00-81.99)	66	84.69 (72.52-93.08)	0.005	-0.31
Dysphagia [A]	60	72.39 (62.68-85.88)	41	88.24 (77.99-94.85)	<0.001 *	-0.42
Vomiting	21	69.12 (61.65-78.24)	80	83.32 (70.21-92.65)	0.002 *	-0.31
Respiratory symptoms						
Coughing	64	72.39 (62.68-85.88)	37	89.71 (78.31-95.71)	<0.001 *	-0.43
Wheezing	26	68.45 (58.46-80.35)	73	84.38 (71.45-93.02)	<0.001 *	-0.37
Dyspnea at rest	10	60.66 (44.28-72.93)	90	82.58 (69.03-92.30)	<0.001 *	-0.36
Dyspnea during physical activity	12	61.08 (46.09-68.53)	88	82.58 (70.55-92.06)	<0.001 *	-0.37
Chest tightness	3	45.31	84	82.81 (69.34-95.53)	0.041	-0.22
Airway infections	14	72.39 (57.58-82.26)	85	82.35 (68.30-92.65)	0.014	-0.25
Feeding difficulties						
Avoiding certain food	35	66.18 (53.85-76.92)	61	84.39 (74.26-93.93)	<0.001 *	-0.54
Eating small portions	43	70.00 (54.69-82.81)	55	85.94 (73.44-93.75)	<0.001 *	-0.45
Energy-enriched food	21	58.82 (51.47-76.63)	79	83.83 (70.45-92.65)	<0.001 *	-0.42
Adjusted food consistency	25	67.65 (53.68-76.63)	73	85.29 (72.57-93.02)	<0.001 *	-0.44
Needing >30 minutes to finish a meal	36	67.75 (55.35-79.89)	61	85.94 (75.74-93.93)	<0.001 *	-0.50
Increased fluid intake during meals	45	75.00 (83.28-86.76)	51	85.00 (70.45-94.12)	0.015 *	-0.25
Nutrition through tube or gastrostomy	12	53.39 (45.70-61.10)	87	82.81 (70.45-92.65)	<0.001 *	-0.50
Adult support while eating	28	65.00 (50.74-78.96)	71	85.00 (72.06-92.65)	<0.001 *	-0.41

Table 4b. Comparison between clinical subgroups of total scores of the EA-QOL© questionnaire for children aged 8-17 years old, proxy-reports (left) and self-reports (right) between clinical subgroups. Only patients for whom both clinical data and total scores EA-QOL© were available were included in the analyses. Asterisks indicate Bonferroni-adjusted significance p<0.0025.

	8-17 years old (proxy-report)						**8-17 years old (self-report)**					
	Yes		**No**				**Yes**		**No**			
	n	*Median (IQR)*	*n*	*Median (IQR)*	*p-value*	*Effect size (r)*	*n*	*Median (IQR)*	*n*	*Median (IQR)*	*p-value*	*Effect size (r)*
Surgical characteristics												
Primary repair	120	87.50 (80.43-93.46)	16	84.71 (65.70-89.84)	0.058	0.16	115	87.50 (79.17-95.46)	15	81.52 (68.48-90.63)	0.118	0.14
History of gastrostomy	18	84.71 (65.84-89.06)	118	87.50 (80.43-93.55)	0.042 *	-0.17	17	82.29 (70.70-90.63)	113	87.50 (79.17-95.55)	0.114	-0.14
History of ≥1 dilatation	68	88.35 (74.22-93.21)	68	87.20 (80.43-93.28)	0.689	-0.03	66	84.38 (73.95-95.01)	64	87.50 (81.32-94.23)	0.318	-0.09
Digestive symptoms												
Heartburn	17	83.70 (76.04-87.13)	114	87.83 (79.99-93.48)	0.080	-0.15	12	82.43 (80.73-88.72)	66	90.63 (79.30-96.63)	0.028	-0.25
Dysphagia [A]	51	83.33 (72.92-90.63)	85	88.54 (83.33-95.61)	0.001 *	-0.29	39	81.25 (72.73-93.48)	44	91.49 (84.48-97.92)	0.001 *	-0.36
Vomiting	6	73.08 (53.13-84.62)	130	87.50 (80.16-93.00)	0.167	-0.12	2	86.03	81	89.13 (78.27-95.83)	0.801	-0.03
Respiratory symptoms												
Coughing	63	85.42 (73.96-91.30)	72	88.88 (82.95-95.13)	0.009	-0.22	42	85.10 (76.56-95.50)	41	90.63 (81.52-97.25)	0.078	-0.15
Wheezing	9	85.42 (74.40-91.06)	127	87.50 (80.21-93.42)	0.451	-0.06	11	81.25 (77.08-93.18)	72	89.68 (79.21-96.28)	0.181	-0.19
Dyspnea at rest	6	79.94 (72.66-88.94)	129	87.50 (79.78-93.48)	0.202	-0.11	7	79.17 (72.73-86.46)	75	89.58 (79.35-96.43)	0.106	-0.18
Dyspnea during physical activity	20	82.95 (72.06-87.50)	112	88.54 (80.26-93.75)	0.023	-0.20	25	80.68 (72.23-90.49)	57	91.30 (84.04-96.59)	0.002 *	-0.35
Chest tightness	9	87.50 (78.46-91.74)	125	87.50 (79.78-93.48)	0.776	-0.02	14	81.97 (72.48-93.75)	68	89.58 (79.68-96.28)	0.089	-0.19
Airway infections	12	72.40 (68.95-85.64)	123	88.16 (80.43-93.75)	0.002 *	-0.27	3	80.68	79	89.58 (79.67-95.83)	0.095	-0.18
Feeding difficulties												
Avoiding certain food	31	80.68 (72.62-86.46)	104	88.54 (83.42-94.79)	<0.001 *	-0.33	12	79.17 (71.17-92.71)	69	89.58 (80.01-96.21)	0.064	-0.21
Eating small portions	26	78.65 (68.23-87.09)	108	88.88 (81.32-94.79)	<0.001 *	-0.37	13	73.96 (69.70-87.73)	69	90.63 (81.91-96.51)	0.001 *	-0.35
Energy-enriched food	8	70.55 (65.89-81.30)	128	87.50 (80.43-93.46)	0.005	-0.24	5	82.29 (71.33-90.63)	77	89.58 (79.17-95.83)	0.241	-0.13
Adjusted food consistency	1	80.43	134	87.50 (79.30-93.44)	0.426	-0.07	0		83	89.13 (79.17-95.83)	-	-
Needing >30 minutes to finish a meal	17	78.13 (69.27-84.69)	119	88.54 (80.68-93.75)	<0.001 *	-0.19	8	79.69 (73.44-85.50)	71	89.77 (79.35-96.59)	0.025	-0.25
Increased fluid intake during meals	43	81.25 (71.88-86.46)	90	90.63 (83.24-95.50)	<0.001 *	-0.42	31	79.35 (71.74-90.63)	50	92.19 (84.21-97.92)	<0.001 *	-0.44
Nutrition through tube or gastrostomy	2	53.68	134	87.50 (80.00-83.44)	0.027	-0.19	1	68.75	82	89.36 (79.17-95.83)	0.138	-0.16
Adult support while eating	4	66.15 (54.69-76.26)	132	87.50 (80.43-93.46)	0.006	-0.24	0		83	89.13 (79.17-95.83)	-	-

Another clinical explanation is the potential influence of the COVID-19 pandemic. The second lockdown in the Netherlands overlapped with the field test period. Closure of primary and secondary schools for three and six months, respectively,[38] significantly impacted children's social life. Reduced social activities may have resulted in less negative confrontation with impairments of their chronic condition and leading to items being less applicable, while healthy children's QoL was negatively affected by COVID-19.[39]

One could argue that the higher Dutch scores are related to test characteristics. Ceiling effects were present in both study populations, but floor effects (all <15%) were observed only in the Swedish-German population. However, validation of the well-established DISABKIDS, CHQ-CF87 and PedsQL instruments showed similar results, with rare floor effects but ceiling effects up to 86%.[24, 30, 40] Validation of the Dutch version of the CHQ-CF87, cross-culturally adapted from the United States, even showed no floor effects at all,[40] like in our study. Moreover, the high-level child-parent agreements favor a clinical rather than a technical explanation for the differences between the Dutch and Swedish-German population.

Next, the proportion of items with missing values in our study (up to 32.7%) was larger than that in previous studies.[14, 17] Considering the cognitive debriefing results in our study (see Supplementary Table S3.2), this was anticipated. We instructed participants in the field test to omit the questions they considered not applicable, and noted that the omitted questions corresponded with those commented on during cognitive debriefing. Soyer and coworkers – who performed the Turkish field test of the EA-QOL© questionnaires in the outpatient clinic – did not share data on cognitive debriefing.[17] Differences in study design could explain the above-mentioned differences.

The wide – slightly skewed – age range within the groups could explain this poor feasibility. Toddlers' perception of potential problems in daily functioning differs from that of school-aged children, and toddlers may be less capable to express their burden verbally. Moreover, not every toddler attends daycare, which may differ amongst countries. In the Netherlands, daycare attendance was even less during the COVID-19 pandemic. In a longitudinal cohort study we showed that growth is slightly below the norm in younger children with EA, but normalizes at 12 years.[3] This may explain the frequently omitted question on perception of having a short stature and emphasizes the need for cross-cultural adaptation of the questionnaire.

Differences in clinical presentation and follow-up care in different centers, could impact the rating of a child's QoL. Furthermore, one's health perception might be subject to cultural differences between countries.[41] Culture is multi-aspect concept which requires further exploration in this context. For example, adequate coping skills may lead to positive illusionary bias[42] and hence to considering chronic healthcare problems and concomitant lifestyle

factors such as dietary restrictions normal. This phenomenon as well as differential item functioning[43] – measuring different aspects in subgroups of participants due to perceptional differences – should be taken into account when implementing the EA-QOL© questionnaires in clinical practice, and during cross-cultural evaluation.

Moreover, small sample sizes and heterogeneity (to which cross-cultural differences contribute) are known challenges for the soundness of PROMs in rare diseases. A possible solution might be computer adaptive testing (CAT), enabling customization of a questionnaire to an individual's situation by using skip patterns that based on the individual's prior responses administer items from an item bank.[44] For generic PROMs, the Patient-Reported Outcome Measure Information System contains item banks for physical, mental and social health in adult and pediatric populations, selected from literature and tested through various extensive item-response theory (IRT) models.[45] CATs have been developed to measure HRQoL in children with chronic conditions,[46] but not in rare diseases such as EA. Generating an item bank for condition-specific items, requires large sample sizes recruited from multiple countries.[21] Given the strong correlation of condition-specific scores with generic PedsQL scores, the added value of implementing the EA-QOL© questionnaires in clinical practice should first be established. A possible approach is to correlate scores to clinical outcomes, like has been done for the PedsQL and DUX-25.[47] A next step could be to combine the internationally obtained validation results into an IRT model, using the original EA-QOL© items available before item reduction[16] with the addition of topics brought up during cognitive debriefing in multiple countries. However, further research in additional countries is needed to evaluate the potential of CAT for the EA-QOL© questionnaires in daily practice.

One of the strengths of this study is the relatively large sample size considering that EA is a rare condition. Furthermore, response rates were high and participants were recruited nationwide. Some limitations should be addressed. We recruited participants from hospital databases and not only those who participated in follow-up programs. We did not collect data from non-participants, thereby selection bias cannot be ruled out. Furthermore, the parental educational level was higher than in the general population. Although this is a common finding in the EA population[12, 47] and in psychometric evaluation in general,[48] it should be taken into account when interpreting the results. Moreover, the online study set-up and some statistical assumptions differed from earlier EA-QOL© validation studies. Next, investigating sex-specific EA-QOL© scores was beyond the scope of this study. Still, it is recognized that females report lower QoL than do males.[34, 49] In future cross-cultural evaluation, sex should therefore be considered as a potential confounder.[50] Lastly, the COVID-19 pandemic could have influenced the field test results.

CONCLUSION

The Dutch-translated EA-QOL© questionnaires showed good reliability and validity. Feasibility was most likely affected by items not deemed applicable to an individual child's situation, as the cognitive debriefing made clear. Leading from this, CAT could be a potential solution to make the questionnaires more suitable for clinical practice in the Netherlands. Cross-cultural evaluation of the validation results obtained in multiple countries should further explore this.

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S1. Description of measurement instruments

The EA-QOL© questionnaires[1]

The EA-QOL© questionnaire for children aged 2-7 years old (proxy-report) consists of 17 items in three domains: eating (7 items), physical health and treatment (6 items), and social isolation and stress (4 items). The EA-QOL© questionnaire for children aged 8-17 years old (proxy-report and self-report) consists of 24 items in four domains: eating (8 items), social relationships (7 items), body perception (5 items) health and well-being (4 items). The total score is calculated from all items (respectively 17 or 24) together. Subscales and total scores can only be calculated if ≤30% of the items is missing. All items are asking about problems in the past 4 weeks, and are answered on a 5-point Likert scale. Total and subscale scores are rescaled to a score between 0 (worst) and 100 (best).

The Paediatric Quality of Life Inventory™ 4.0 (PedsQL) questionnaire[2]

The PedsQL questionnaire is available in different age-appropriate versions. For children aged 2-4 years old, the parent-proxy-report version consists of 21 questions within four domains: physical functioning (8 items), emotional functioning (5 items), social functioning (5 items), and school functioning (3 items). A fifth domain, psychosocial health, is calculated as the sum of the emotional, social, and school functioning subscales.

For children aged 5-7 years old, a parent-proxy-report and self-report version is available. In this study, we only used the parent-proxy-report, which consists of 23 questions within four domains: physical functioning (8 items), emotional functioning (5 items), social functioning (5 items), and school functioning (5 items). A fifth domain, psychosocial health, is calculated as the sum of the emotional, social, and school functioning subscales.

For children aged 8-12 years old and 13-17 years old, age-specific proxy-reports and self-reports are available. We used both the proxy-report and the self-report for these age groups in this study. Both versions consist of 23 questions within four domains: physical functioning (8 items), emotional functioning (5 items), social functioning (5 items), and school functioning (5 items). A fifth domain, psychosocial health, is calculated as the sum of the emotional, social, and school functioning subscales.

The total score is calculated from all items together. Subscales and total scores can only be calculated if ≤50% of the items is missing. All items are asking about problems in the past 4 weeks, and are answered on a 5-point Likert scale. Total and subscale scores are rescaled to a score between 0 (worst) and 100 (best).

S2. Detailed methodological description of the translation, cognitive debriefing and field testing phases

Ethical approval

The study has been approved by the participating institutional review boards of the Erasmus Medical Center (MEC-2019-0521), the University Medical Center Utrecht (MEC-20-564/C), the Radboud University Medical Center (MEC-2020-6961) and the University of Amsterdam (MEC-2019-631).

Translation

A Swedish-Dutch forward-backward translation has been conducted according to the 'Translation and Cultural Adaptation of Patient Reported Outcomes Measures - Principles of Good Practice'.[3] Two forward translations have been developed by two certified native Dutch-speaking translators, independently, who were recruited from a professional agency. To avoid any ambiguities, they were provided with a document containing the explanations and concepts of the questions. After review of the translations by two members of the research team (CtK and MvW), a reconciliation meeting was planned together with one of the translators wherein discrepancies between the two forward translations were solved. All reconciliation decisions were documented.

Next, one backward translation was performed by a third certified translator recruited from the professional agency, who was a native Swedish speaker and fluent in the Dutch language and who had not seen the original questions. To ensure the conceptual equivalence, this backward translation was reviewed by the original, Swedish developer (MDB) of the EA-QOL© questionnaire. During a virtual cross-cultural meeting between CtK, MvW and MDB, all translations were systematically discussed and the last discrepancies were solved.

Cognitive debriefing

To ensure that the instructions, items and response scale were understood by the respondents as intended, cognitive debriefing was applied through face-to-face interviews with children with EA and one of their parents.[4] To avoid exchange of information about the questionnaire, interviews with 8-to-17-year old children were performed with the child separated from the parent, simultaneously at the same time in different rooms.

Three groups were selected: group A) parents of children with EA between the age of 2 and 7 years old (proxy-report), group B1) parents of children with EA between the age of 8 and 17 years old (proxy-report), and group B2) children with EA between the age of 8 and 17 years old (self-report).

To assure the representation of the EA population, each group consisted of a predefined stratified sample of children who were categorized into patients with mild, moderate or severe complaints (Supplementary Table 1) modified from the severity criteria used in the pilot testing in Sweden.[5] Children in the category 'mild' did not suffer from additional associated anomalies and had no complaints at all, or only one of the following symptoms: dysphagia, gastroesophageal reflux disease (GERD), or a chronic pulmonary condition for which medication is not required (for example upper respiratory tract infections that do not require antibiotic treatment). Children with associated anomalies, automatically fell into the category 'moderate' or 'severe'. Furthermore, children in the category 'moderate' had one or more of the following symptoms: actual dysphagia, GERD, a history of esophageal dilatation, or a chronic pulmonary condition for which daily medication was required. Children in the category 'severe' had all of the above medical symptoms: actual dysphagia, GERD, a history of esophageal dilatation and clinically significant chronic pulmonary condition.

The interviews were held during an annual meeting of the Dutch patient support group VOKS (Vereniging voor Ouderen en Kinderen met een Slokdarmatresie). Participants were initially approached by a member of the board of the patient support group by telephone in the weeks prior to the meeting. If participants were interested, a member of the research team called one of the parents to explain the aim of the study. After verbal informed consent, the researcher recorded the presence of the above-mentioned medical conditions. Children were categorized into 'mild', 'moderate' or 'severe' based on the parental proxy-report obtained during this initial telephone call.

Participants filled out the questionnaire on paper at the same time as they gave verbal feedback on the clarity and adequacy of the items. They indicated if an item was easy to understand, and if an item was sensitive for answer, for example because it raised certain emotions like sadness or fear. If an item could not be answered because it was not a applicable to a child's situation, too difficult, or too sensitive, the item was left empty and registered as 'missing'. Finally, they were asked if they had missed any items in this questionnaire. Field notes were made by the interviewer, including observations of non-verbal language. In 8-to-17-year-olds, the interviews were performed individually, separately and simultaneously with the child and the parent. The child and the parent were not able to exchange information about the questionnaire. If two parents were present, only one parent participated in the cognitive debriefing. Parents were free to decide themselves which parent would participate. The interviewer was either a member of the research team (CtK or MvW) or another researcher in the field of EA. None of the interviewers was involved as care provider of the participants.

The results from the cognitive debriefing were analyzed using manifest content analysis. The participants' understanding of the items was compared with the predefined explanations and concepts of the items. This content analysis was performed by two members of the research

team (CtK and MvW) and discussed during a meeting with the original developer (MDB). Instructions and items were adjusted if necessary until consensus was reached.

Field testing

Finally, the feasibility, validity and reliability of the translated questionnaires was statistically evaluated. Participants were recruited via the routine care of four university hospitals in The Netherlands. Participants were eligible if they had sufficient command of the Dutch language, and the child was aged 2-17 years old and born with EA. Children with known intellectual disability were excluded. They either did not get an invitation, or were excluded afterwards if parents informed us after filling out the questionnaire.

All participants were invited to participate through a personal letter. Children ≥12 years old received their own personal letter, since from this age forward children have to consent to participate in research themselves as well. Only one parents filled out the questionnaire; parents were free to decide who of them would participate.

The letter contained a personal code, which gave access to the online questionnaires (LimeSurvey GmbH version 2.06lts, Hamburg, Germany). By filling out the questionnaires, participants automatically gave consent for the study. This was also explained in the invitation letter. All parents and children aged ≥8 years old filled out the age-appropriate version of the Dutch-translated EA-QOL© questionnaire and the PedsQL questionnaire.[2] Additionally, all parents and children aged ≥12 years old filled out a short questionnaire on sociodemographic items and presence of digestive symptoms, feeding difficulties and respiratory symptoms in the past 4 weeks. To maximize the response rate, parents and/or patients received maximally two reminders. With the last reminder, they also received the questionnaires on paper with a pre-stamped envelope for reply.

To examine the reliability of the EA-QOL© questionnaires over time, all parents and/or patients who participated in the initial test received a second invitation letter three weeks after the initial response. This letter contained a new personal code, which was used to fill out the EA-QOL© questionnaires a second time. Participants were also asked about potential differences in the presence of digestive symptoms, feeding difficulties and respiratory symptoms during the recall period. Participants again received a maximum of two reminders.

S3. Cognitive debriefing

Supplementary Table S3.1. Stratified sample size for the cognitive debriefing. Children were categorized into 'mild', 'moderate' or 'severe' based on parental proxy-report obtained during a telephone call with the researcher prior to the cognitive debriefing interviews. Predefined = ideal number of participating patients based on the study protocol and normal distribution in the esophageal atresia population, included = actual number of participating patients. Group A = children with esophageal atresia aged 2-7 years old, group B = children with esophageal atresia aged 8-17 years old. GERD = gastroesophageal reflux disease

	Clinical characteristics of the patient	**Group A (2-7 years)**		**Group B (8-17 years)**	
		Predefined	*Included (n=9)*	*Predefined*	*Included (n=10)*
Mild	No associated anomalies and no complaints at all *Or* No associated anomalies and one of the following symptoms: · Dysphagia · GERD · Chronic pulmonary condition for which medication is not required	2 patients	2 patients	2 patients	4 patients
Moderate	Associated anomalies *or* One or more of the following symptoms: · Dysphagia · GERD · History of esophageal dilatation · Chronic pulmonary condition for which daily medication is required	4-5 patients	5 patients	4-5 patients	4 patients
Severe	Associated anomalies *and* All of the following symptoms: · Dysphagia · GERD · History of esophageal dilatation · Chronic pulmonary condition for which daily medication is required	2-3 patients	2 patients	2-3 patients	2 patients

Supplementary Table S3.2. Summary of the results of the cognitive debriefing. The bold item numbers correspond with the items of the Dutch EA-QOL questionnaire. The item numbers in brackets after the aim of the item correspond with the items of the original Swedish-German pilot questionnaire before item reduction. The complete English questions can be found in the supplementary material of the original article.[5] The last column shows a selection of the most important comments on the items, which have led to a change. Q20 is missing from the self-reports for 8-17 years old, due to administrative problems. [A] Answered with yes or no. [B] For example because it raised certain emotions like sadness or fear, answered with yes or no. [C] If an item could not be answered because it was not a applicable to a child's situation, too difficult, or too sensitive, the item was left empty and registered as 'missing'.

			Response (n)								
		Aim of the item, to measure perceived impact on… (reference)	*Never*	*Seldom*	*Sometimes*	*Often*	*Always*	*Missing* [C]	**Easy to understand (n)** [A]	**Sensitive to answer (n)** [B]	**Important comments**
2-7 years old (parent-report, n=9)	**Q1**	Eating problems, due to food sticking in the throat (Q1)	1	2	4	1	0	1	7 (78%)	2 (22%)	My child is not fed orally, only tube feeding.
	Q2	The child's ability to eat a large portion or full meal (Q2)	0	1	4	2	1	1	7 (78%)	1 (11%)	
	Q3	Eating-related stress on the child (Q3)	4	1	3	1	0	0	9 (100%)	0 (0%)	
	Q4	The child's satisfaction with their eating pace (Q4)	2	2	4	0	1	0	7 (78%)	0 (0%)	Differs between home and school. Time pressure experienced by the parents or the child itself?
	Q5	Emotional impact, in terms of worry, of the risk of choking on food (Q5)	6	1	1	0	1	0	8 (89%)	0 (0%)	
	Q6	Degree of the child's problem with vomiting (Q7)	5	1	1	0	2	0	8 (89%)	0 (0%)	My child never vomits.
	Q7	Limitations on social activities and events that include eating with peers (Q8)	2	1	1	4	0	1	7 (78%)	1 (11%)	
	Q8	Physical limitation on playing games or sports (Q9)	3	1	3	2	0	0	9 (100%)	1 (11%)	
	Q9	Physical ability of the child to perform physically demanding activities in daily life compared to healthy peers (Q10)	3	0	3	2	1	0	9 (100%)	1 (11%)	
	Q10	Respiratory symptoms on the child's daily life (Q11)	1	2	5	1	0	0	9 (100%)	1 (11%)	
	Q11	Problematic respiratory infections on the child's daily life (Q12)	2	3	3	1	0	0	6 (67%)	1 (11%)	
	Q12	Severity of the emotional impact of the need for medical treatment (Q13)	6	1	1	0	1	0	9 (100%)	0 (0%)	All medication is administered by gastrostomy tube.
	Q13	EA related morbidity on the child's sleep (Q14)	3	2	3	1	0	0	8 (89%)	2 (22%)	
	Q14	School absence and social isolation with those of peers (Q15)	3	2	4	0	0	0	7 (78%)	1 (11%)	
	Q15	Social stress of explaining one's condition to other people at very young age (Q16)	6	0	1	0	1	1	8 (89%)	0 (0%)	My child is too young to explain this.
	Q16	Social stigma and stress in the child (Q17)	3	1	4	0	0	1	8 (89%)	1 (11%)	
	Q17	Social exclusion and stress in the child's life (Q18)	6	0	1	0	0	2	6 (67%)	0 (0%)	My child is too young to understand this.

8-17 years old (parent-report, n=10)	**Q1**	Food getting stuck in the throat, from the child's perspective (Q1)	3	4	1	0	0	2	10 (100%)	2 (20%)	My child is not fed orally, only parenterally.
	Q2	Restriction on food intake on the child (Q2)	2	3	1	0	2	2	7 (70%)	3 (30%)	
	Q3	Pain during food and fluid intake due to the child's condition (Q3)	5	0	1	1	0	3	9 (90%)	1 (10%)	
	Q4	The need to drink a lot when eating (Q4)	3	0	2	0	2	3	5 (50%)	3 (30%)	Does my child need to drink to be able to eat or do I need to remind my child?
	Q5	Emotional impact of fear of choking (Q5)	5	3	0	0	0	2	9 (90%)	1 (10%)	My child never chokes.
	Q6	Impact of choking on the child's eating situation (Q6)	3	3	0	0	0	4	8 (80%)	2 (20%)	
	Q7	Child's ability to eat at the same pace as children their own age, from their perspective (Q7)	0	1	0	0	6	3	7 (70%)	1 (10%)	
	Q8	The degree of the child's problem with vomiting (Q8)	1	1	0	0	2	6	6 (60%)	2 (20%)	My child is unable to vomit due to fundoplication surgery.
	Q9	Experience of emotional isolation (Q9)	3	3	1	1	0	2	8 (80%)	0 (0%)	
	Q10	Social stress related to severity of explaining EA to others (Q10)	2	2	4	0	0	2	9 (90%)	1 (10%)	
	Q11	Social exclusion in terms of being called names by others (Q11)	4	3	1	0	0	2	10 (100%)	1 (10%)	
	Q12	Social exclusion in terms of perceiving that others stare at you (Q12)	3	2	2	1	0	2	10 (100%)	1 (10%)	
	Q13	Social stress related to the need to explain their scar(s) to other people (Q13)	4	3	1	0	0	2	10 (100%)	0 (0%)	My child never gets any questions.
	Q14	Children's perception of others saying mean things about them from the perspective of social exclusion (Q14)	5	2	1	0	0	2	9 (90%)	3 (30%)	
	Q15	Strain of reacting to other people's questions (Q16)	4	3	1	0	0	2	10 (100%)	0 (0%)	
	Q16	Experience of feeling different due to surgical scar(s) (Q17)	3	2	2	1	0	2	10 (100%)	0 (0%)	
	Q17	Concern regarding what to wear because of the surgical scar(s) (Q18)	4	0	3	1	0	2	10 (100%)	0 (0%)	
	Q18	Unease related to the scar(s) being visible to others (Q19)	4	0	2	2	0	2	10 (100%)	2 (20%)	
	Q19	Impact of scar(s) on children's perception of their looks (Q20)	3	3	1	1	0	2	9 (90%)	2 (20%)	
	Q20	Impact of being small for age (Q21)	1	1	2	0	0	6	9 (90%)	3 (30%)	My child is not smaller than other children of his/her age.
	Q21	Impact of breathing difficulties on exercise and play, from the perspective of physical performance (Q23)	3	0	2	3	0	2	10 (100%)	1 (10%)	
	Q22	Impact of EA related morbidity on sleep (Q24)	3	3	2	0	0	2	10 (100%)	0 (0%)	
	Q23	Emotional impact with regard to worries about the future due to EA (Q25)	5	2	1	0	0	2	10 (100%)	1 (10%)	
	Q24	Emotional impact of EA in terms of feelings of sadness (Q26)	5	2	0	0	1	2	9 (90%)	1 (10%)	

8-17 years old (self-report, n=10)	**Q1**	Food getting stuck in the throat, from the child's perspective (Q1)	2	3	3	0	0	2	6 (60%)	1 (10%)	Food never gets stuck.
	Q2	Restriction on food intake on the child (Q2)	5	1	1	1	1	1	10 (100%)	1 (10%)	
	Q3	Pain during food and fluid intake due to the child's condition (Q3)	6	1	2	0	0	1	10 (100%)	2 (20%)	
	Q4	The need to drink a lot when eating (Q4)	4	1	1	1	1	1	8 (80%)	2 (20%)	
	Q5	Emotional impact of fear of choking (Q5)	4	3	3	0	0	0	8 (80%)	0 (0%)	
	Q6	Impact of choking on the child's eating situation (Q6)	8	1	1	0	0	1	8 (80%)	0 (0%)	
	Q7	Child's ability to eat at the same pace as children their own age, from their perspective (Q7)	2	1	2	1	3	1	10 (100%)	1 (10%)	
	Q8	The degree of the child's problem with vomiting (Q8)	4	2	1	1	1	1	5 (50%)	2 (20%)	I never have to vomit.
	Q9	Experience of emotional isolation (Q9)	6	3	1	0	0	0	10 (100%)	0 (0%)	
	Q10	Social stress related to severity of explaining EA to others (Q10)	1	3	4	1	1	0	9 (90%)	1 (10%)	
	Q11	Social exclusion in terms of being called names by others (Q11)	8	2	0	0	0	0	10 (100%)	2 (20%)	
	Q12	Social exclusion in terms of perceiving that others stare at you (Q12)	5	2	2	1	0	0	10 (100%)	0 (0%)	
	Q13	Social stress related to the need to explain their scar(s) to other people (Q13)	8	1	1	0	0	0	9 (90%)	1 (10%)	Nobody can see my scars, they are covered by my swimming suit.
	Q14	Children's perception of others saying mean things about them from the perspective of social exclusion (Q14)	8	2	0	0	0	0	9 (90%)	1 (10%)	
	Q15	Strain of reacting to other people's questions (Q16)	4	1	4	0	0	1	9 (90%)	1 (10%)	
	Q16	Experience of feeling different due to surgical scar(s) (Q17)	9	0	1	0	0	0	10 (100%)	0 (0%)	
	Q17	Concern regarding what to wear because of the surgical scar(s) (Q18)	7	1	0	1	1	0	8 (80%)	1 (10%)	
	Q18	Unease related to the scar(s) being visible to others (Q19)	7	0	3	0	0	0	9 (90%)	0 (0%)	
	Q19	Impact of scar(s) on children's perception of their looks (Q20)	9	1	0	0	0	0	9 (90%)	1 (10%)	
	Q21	Impact of breathing difficulties on exercise and play, from the perspective of physical performance (Q23)	2	2	0	4	1	1	9 (90%)	0 (0%)	
	Q22	Impact of EA related morbidity on sleep (Q24)	7	1	1	0	1	0	8 (80%)	1 (10%)	
	Q23	Emotional impact with regard to worries about the future due to EA (Q25)	8	1	0	0	0	1	9 (90%)	0 (0%)	I never think about the future.
	Q24	Emotional impact of EA in terms of feelings of sadness (Q26)	7	2	1	0	0	0	9 (90%)	1 (10%)	

Supplementary Table S3.3. Overview of topics parents and children missed in the EA-QOL© questionnaire. Group A = parents of children with EA aged 2-7 years old (proxy-report) Group B1 = parents of children with EA aged 8-17 years old (proxy-report), group B2 = children with EA aged 8-17 years old (self-report). EMDR = eye movement desensitization and reprocessing

Group A	Group B1	Group B2
Physical height Hospital visits, hospitalizations Trauma and need for EMDR therapy (Self-imposed) dietary restrictions	Physical condition, exercise endurance, lung capacity Comorbidities, associated anomalies Medical equipment (gastrostomy, aerosol therapy, central venous line) Hospital visits, hospitalizations Concerns about a child's psychosocial wellbeing (trauma) Social contacts Cultural differences, challenges for immigrants Transition to adulthood	Visible deformities Comorbidities, associated anomalies Coughing in public, using medication in public

S4. Item evaluation of the field test

Supplementary Table S4.3. Feasibility of the EA-QOL© questionnaire for 2-7 year old children (proxy-report, n=101). The bold item numbers correspond with the items of the Dutch EA-QOL questionnaire. The item numbers number in brackets after the topics correspond with the items of the original Swedish-German pilot questionnaire before item reduction. The complete English questions can be found in the supplementary material of the original article.[5] Items were answered on a 5-point Likert scale, ranging from never (1) to always (5). Raw, untransformed scores are presented in this table. Feasibility (percentage of items with >5% missing values[5]) was considered poor (>30%), moderate (10-30%) or good (<10%).

		Topic (reference)	Missing values, n (%)	Mean ± SD	Range	Skewness	Kurtosis
2-7 years old proxy-report (n=101)	**Q1**	Food getting stuck (Q1)	-	2.2 ± 0.9	1-5	0.6	0.2
	Q2	Eating full meals (Q2)	1 (1.0)	2.3 ± 1.3	1-5	0.7	-0.6
	Q3	Eating is stressful (Q3)	-	1.5 ± 0.9	1-5	2.0	4.3
	Q4	Pace of eating (Q4)	5 (5.0)	4.0 ± 1.1	1-5	-1.0	0.5
	Q5	Choking (Q5)	7 (6.9)	1.6 ± 0.9	1-5	1.7	2.6
	Q6	Vomiting (Q7)	9 (8.9)	1.9 ± 1.0	1-5	1.0	0.6
	Q7	Eating with friends (Q8)	5 (5.0)	2.0 ± 1.2	1-5	1.1	0.2
	Q8	Tired (Q9)	1 (1.0)	2.2 ± 1.2	1-5	0.6	-0.7
	Q9	Strength (Q10)	5 (5.0)	2.2 ± 1.2	1-5	0.8	-0.5
	Q10	Respiratory problems (Q11)	-	2.2 ± 1.1	1-5	0.4	-1.0
	Q11	Respiratory infections (Q12)	22 (21.8)	2.0 ± 1.1	1-5	0.7	-0.7
	Q12	Medicine (Q13)	16 (15.8)	1.9 ± 1.0	1-5	1.0	0.8
	Q13	Sleeping (Q14)	1 (1.0)	1.6 ± 0.9	1-5	1.7	2.5
	Q14	Absence from school (Q15)	23 (22.8)	1.7 ± 1.0	1-5	1.4	1.5
	Q15	Explaining to others (Q16)	33 (32.7)	1.7 ± 1.0	1-5	1.4	1.4
	Q16	Comments (Q17)	26 (25.7)	1.6 ± 1.0	1-4	1.2	-0.0
	Q17	Noises (Q18)	25 (24.8)	1.6 ± 0.9	1-4	1.2	0.4

Supplementary Table S4.4. Feasibility of the EA-QOL© questionnaire for 8-17 year old children (proxy-report, n=136 and self-report, n=130). The bold item numbers correspond with the items of the Dutch EA-QOL questionnaire. The item numbers number in brackets after the topics correspond with the items of the original Swedish-German pilot questionnaire before item reduction. The complete English questions can be found in the supplementary material of the original article.[5] Items were answered on a 5-point Likert scale, ranging from never (1) to always (5). Raw, untransformed scores are presented in this table. Feasibility (percentage of items with >5% missing values[5]) was considered poor (>30%), moderate (10-30%) or good (<10%).

		Topic (reference)	Missing values, n (%)	Mean ± SD	Range	Skewness	Kurtosis
8-17 years old proxy-report (n=139)	**Q1**	Food getting stuck (Q1)	27 (19.9)	2.8 ± 1.4	1-5	0.4	-1.0
	Q2	Restricting from food (Q2)	3 (2.2)	1.6 ± 0.9	1-5	1.6	2.6
	Q3	Pain (Q3)	6 (4.4)	1.6 ± 0.8	1-5	1.4	1.8
	Q4	Drinking (Q4)	2 (1.5)	2.4 ± 1.4	1-5	0.6	-1.0
	Q5	Afraid of choking (Q5)	3 (2.2)	1.3 ± 0.7	1-5	2.7	8.6
	Q6	Hard to eat due to choking (Q6)	5 (3.7)	1.2 ± 0.5	1-3	2.3	4.7
	Q7	Pace of eating (Q7)	1 (0.7)	3.6 ± 1.4	1-5	-0.6	1.0
	Q8	Vomiting (Q8)	39 (28.7)	1.4 ± 1.0	1-5	2.5	6.0
	Q9	Loneliness (Q9)	8 (5.9)	1.8 ± 1.2	1-5	1.2	0.3
	Q10	Explaining to others (Q10)	3 (2.2)	1.8 ± 1.1	1-5	1.2	0.6
	Q11	Name-calling (Q11)	1 (0.7)	1.2 ± 5.2	1-4	3.3	12.5
	Q12	Staring (Q12)	3 (2.2)	1.7 ± 1.0	1-5	1.1	0.0
	Q13	Scars (Q13)	8 (5.8)	1.4 ± 0.8	1-4	1.9	2.7
	Q14	Saying mean things (Q14)	1 (0.7)	1.2 ± 0.5	1-3	2.2	3.7
	Q15	Feeling awkward (Q16)	3 (2.2)	1.4 ± 0.8	1-5	2.0	3.6
	Q16	Feeling different (Q17)	1 (0.7	1.5 ± 0.9	1-5	1.8	2.1
	Q17	Adjusting cloths (Q18)	2 (1.5)	1.4 ± 0.9	1-5	1.8	3.8
	Q18	Visible scars (Q19)	3 (2.2)	1.4 ± 0.8	1-5	1.9	2.7
	Q19	Feeling imperfect (Q20)	3 (2.2)	1.4 ± 0.8	1-5	2.5	6.4
	Q20	Smaller than peers (Q21)	28 (20.6)	1.6 ± 1.0	1-5	1.5	1.2
	Q21	Breathing difficulties (Q23)	1 (0.7)	2.0 ± 1.1	1-5	0.9	-0.1
	Q22	Sleeping (Q24)	4 (2.9)	1.7 ± 1.0	1-5	1.2	0.8
	Q23	Worried about future (Q25)	4 (2.9)	1.2 ± 0.6	1-5	3.4	13.8
	Q24	Sad (Q26	2 (1.5)	1.3 ± 0.6	1-3	2.1	3.1

8-17 years old self-report (n=131)	**Q1**	Food getting stuck (Q1)	15 (11.5)	3.0 ± 1.5	1-5	0.1	-1.3
	Q2	Restricting from food (Q2)	-	1.6 ± 0.9	1-5	1.6	1.7
	Q3	Pain (Q3)	2 (1.5)	1.7 ± 0.9	1-5	1.2	0.8
	Q4	Drinking (Q4)	1 (0.8)	2.4 ± 1.4	1-5	0.6	-1.0
	Q5	Afraid of choking (Q5)	1 (0.8)	1.5 ± 0.9	1-5	1.8	2.4
	Q6	Hard to eat due to choking (Q6)	4 (3.1)	1.2 ± 0.5	1-4	3.2	10.6
	Q7	Pace of eating (Q7)	-	3.8 ± 1.5	1-5	-0.8	-0.8
	Q8	Vomiting (Q8)	28 (21.5)	1.4 ± 1.0	1-5	2.8	6.7
	Q9	Loneliness (Q9)	1 (0.8)	2.0 ± 1.3	1-5	1.0	-0.4
	Q10	Explaining to others (Q10)	1 (0.8	1.7 ± 1.1	1-5	1.2	0.2
	Q11	Name-calling (Q11)	2 (1.5)	1.2 ± 0.6	1-4	3.3	10.9
	Q12	Staring (Q12)	1 (0.8)	1.6 ± 1.0	1-5	1.7	2.6
	Q13	Scars (Q13)	4 (3.1)	1.5 ± 1.0	1-5	1.9	3.1
	Q14	Saying mean things (Q14)	-	1.2 ± 0.5	1-4	3.0	10.1
	Q15	Feeling awkward (Q16)	1 (0.8)	1.6 ± 0.9	1-5	1.3	0.7
	Q16	Feeling different (Q17)	2 (1.5)	1.4 ± 0.8	1-5	2.5	6.8
	Q17	Adjusting cloths (Q18)	1 (0.8)	1.3 ± 0.8	1-5	2.9	7.7
	Q18	Visible scars (Q19)	3 (2.3)	1.4 ± 0.9	1-5	2.5	5.6
	Q19	Feeling imperfect (Q20)	1 (0.8)	1.2 ± 0.6	1-5	3.8	17.4
	Q20	Smaller than peers (Q21)	28 (21.5)	1.4 ± 1.0	1-5	2.2	4.0
	Q21	Breathing difficulties (Q23)	3 (2.3)	2.0 ± 1.2	1-5	0.8	-0.3
	Q22	Sleeping (Q24)	2 (1.5)	1.5 ± 0.8	1-4	1.2	0.0
	Q23	Worried about future (Q25)	4 (3.1)	1.2 ± 0.5	1-4	3.1	11.5
	Q24	Sad (Q26	3 (2.3)	1.2 ± 0.5	1-3	2.5	5.7

S5. Reliability of the field test

Supplementary Table S5.1. Comparison reliability (child-parent agreements) between proxy-reports and self-reports of the EA-QOL© questionnaire for 8-17 years old. The level of agreement can be considered poor (<0.50), moderate (0.50-0.74), good (0.75-0.90), or excellent (>0.90).[6] ICC = intra-class correlation coefficient, CI = confidence interval.

	Child-parent pairs (n)	**Level of agreement, ICC (95% CI)**
Eating	122	0.78 (0.71-0.84)
Social relationships	126	0.69 (0.59-0.77)
Body perception	125	0.76 (0.67-0.83)
Health and well-being	124	0.67 (0.57-0.75)
Total score	128	0.81 (0.74-0.86)

S6. Results of the PedsQL questionnaire

Supplementary Table S6.1. Subscale and total scores of the previously validated PedsQL questionnaire.

	2-7 year proxy-reports (n=100)	**8-17 year olds proxy-reports (n=135)**	**8-17 year olds self-reports (n=130)**
	Median (IQR)	*Median (IQR)*	*Median (IQR)*
Physical functioning	93.75 (81.25-100.00)	93.75 (84.38-100.00)	93.75 (87.50-100.00)
Emotional functioning	75.00 (65.00-90.00)	90.00 (75.00-100.00)	90.00 (80.00-100.00)
Social functioning	95.00 (75.00-100.00)	100.00 (85.00-100.00)	100.00 (90.00-100.00)
School functioning	90.00 (71.25-100.00)	80.00 (60.00-100.00)	80.00 (70.00-95.00)
Psychosocial Health	84.17 (71.67-95.00)	85.00 (76.67-96.67)	88.33 (78.33-96.67)
Total score	85.87 (76.11-94.14)	88.04 (80.43-96.74)	89.13 (82.61-96.74)

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APPENDIX

DCEA Study Group – pediatric surgeons and pediatric gastroenterologists of the participating centers:

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- S. (Sjoerd) de Beer
- R. (Ramon) Gorter
- M.P. (Michiel) van Wijk

S1. Pilot questionnaire

1. Do you enjoy eating?
2. How often does food get stuck in the esophagus?
3. Do you need to drink water with the meal to help the food go down?
4. How long does it take you to finish a meal?
5. How do you divide the meals over the day?
6. Are you able to eat everything you would like to eat? Specifically in regard to the food texture?
7. To what extent do you take into account your choice of certain foods in everyday life? Specifically in regard to the food texture?
8. Does your esophageal atresia limit you to go to a restaurant?
9. Do you have trouble to make a food choice in a restaurant?
10. Does your esophageal atresia limit your choice for a quick bite on the street? For example, a snack or a sandwich.
11. Do you ever experience problems with eating at a party or another occasion where people eat standing up?
12. Are you avoiding parties or occasions where you can't eat sitting down?
13. How much does it bother you when food gets stuck in your esophagus?
14. Do you feel fearful when food gets stuck in your esophagus?
15. Do you feel like you're being watched when you eat?
16. Do you feel uncomfortable while eating?
17. Do you ever get comments about your eating behavior?
18. Do you experience pain while eating?
19. Do you experience difficulties with swallowing your food?
20. Do you experience acid reflux (heartburn)?
21. Do you experience a burning sensation on the chest?
22. Do you experience regurgitation (food or fluid rising back up through the esophagus into the mouth)?
23. Do you experience burping after eating?
24. Do you experience feeling bloated after eating?
25. Do you experience nausea throughout the day?
26. Do you experience stomach ache and intestinal cramps after eating?
27. Do you experience palpitations or dizziness after meals?
28. How much do your esophageal complaints bother you?
29. To what extent do esophageal complaints affect your daily life?
30. Do your esophageal complaints limit you in your daily life?
31. How much do your stomach and/or intestinal complaints bother you?
32. To what extent do the complaints of the stomach and intestines affect your daily life?

33. Do your stomach and/or intestinal complaints limit you in your daily life?
34. How many days of the week do you experience respiratory complaints?
35. How often do you cough?
36. How often do you cough up phlegm (sputum)?
37. How often do you experience shortness of breath at rest?
38. How often do you experience shortness of breath at exertion?
39. How often do you experience wheezing?
40. How often do you suffer from airway infections?
41. Do you ever get comments about the fact that you are coughing?
42. Do you feel like you're being watched when you cough?
43. Do you feel uncomfortable while coughing?
44. How much do your respiratory complaints bother you?
45. To what extent do the respiratory complaints affect you in your daily life?
46. Do your respiratory complaints limit you in your daily life?
47. Do physical respiratory complaints disrupt your sleep?
48. How is your stamina?
49. How much does it bother you that you have reduced stamina?
50. To what extent does your reduced stamina affect you in your daily life?
51. Does your reduced stamina limit you in your daily life?
52. Do you feel disabled by your stamina?
53. How often can you perform the normal daily activities? For example going to school, work or doing household chores.
54. How often can you practice your hobbies and/or sports?
55. How is your muscle strength?
56. Does your muscle strength limit you in your daily life?
57. Do you feel tired or fatigued?
58. How are your gross motor skills (big movements such as walking, running and balance)?
59. How are your fine motor skills (small movements such as writing, drawing or pouring coffee)?
60. Do your motor skills (gross and fine) limit you in your daily life?
61. Do you feel disabled by your muscle strength or motor skills?
62. Esophageal atresia may be associated with visible birth defects. To what extent do these defects affect you in your daily life?
63. How much do these visible birth defects bother you?
64. How uncomfortable do you feel because of these visible birth defects?
65. Do you ever get comments about these visible birth defects?
66. To what extent do these visible birth defects affect you in your daily life?
67. Do these visible birth defects limit you in your daily life?
68. How much do your scars bother you?

69. Do your scars make you feel uncomfortable?
70. Do you ever get comments about your scars?
71. To what extent do your scars affect you in your daily life?
72. Do your scars limit you in your daily life?
73. Do you ever feel like you are being looked at because of your scars or other visible birth defects?
74. Do you take medication for symptoms related to your esophageal atresia?
75. Do you take medication for respiratory complaints (e.g. inhalers))?
76. How much does it bother you to take medication for our esophageal atresia or for respiratory complaints?
77. To what extent does the medication you take affect you in your daily life?
78. Does your medication limit you in your daily life?
79. Do you ever have bad recollections of past events?
80. How much do these recollections bother you?
81. To what extent do these recollections affect you in your daily life?
82. Do these recollections limit you in your daily life?
83. Is there anyone you can talk to about past events?
84. Do your parents ever have bad recollections of past events?
85. To what extent do your parents' bad recollection of past events affect you in your daily life?
86. Are your parents' recollections of past events limiting you in your daily life?
87. Do you worry about complaints of your esophagus?
88. Do you worry about complaints of your stomach and intestines?
89. Do you worry about complaints of your lungs or airways?
90. Do you worry about your stamina?
91. Do you worry about your movement skills (gross and fine motor skills)?
92. Do you worry about whether esophageal atresia is hereditary?
93. Do you worry about the impact of your esophageal atresia on taking out a mortgage or insurance?
94. Do you sleep less well because you are worried about your esophageal atresia or one of the things that may have to do with it?
95. To what extent do these worries affect you in your daily life?
96. Do you worry when you need to have a follow-up visit with the gastroenterologist?
97. Do you worry when you have to undergo an endoscopy of the esophagus and stomach?
98. Do you worry about the development of damage to the lining of the esophagus or the development of esophageal cancer?
99. Do you feel anxious or tense because of your esophageal atresia in general?
100. Do you feel anxious or tense when you go to the GP?
101. How anxious or tense do you feel when you visit your GP?

102. Do you feel anxious or tense when you go to the hospital for an examination or treatment?
103. How anxious or tense do you feel when you go to the hospital for an examination or treatment?
104. Do you feel anxious or tense when you are in the hospital as a visitor?
105. How anxious or tense do you feel when you are in the hospital as a visitor?
106. Do you feel anxious or tense with minor medical procedures, such as having blood drawn?
107. How anxious or tense do you feel with minor medical procedures, such as having blood drawn?
108. Are you afraid of getting sick?
109. How much do these anxieties bother you?
110. To what extent do these anxieties affect you in your daily life?
111. Do these anxieties limit you in your daily life?
112. Do you feel happy (with regard to life in general)?
113. Are you sad about your esophageal atresia?
114. Does your esophageal atresia ever cause frustration?
115. Do comments about your esophageal atresia make you sad?
116. To what extent have you been given an explanation about what esophageal atresia is and what complaints may be associated with it?
117. To what extent did you understand this explanation?
118. Do you feel guilty towards your parents for being born with an esophageal atresia?
119. Do you feel guilty towards your siblings for being born with an esophageal atresia?
120. To what extent do your parents still influence your daily decisions?
121. What was it like for you to inform your partner about your esophageal atresia?
122. To what extent does your esophageal atresia affect the relationship with your partner?
123. To what extent does your esophageal atresia interfere with your desire to have children (or has it done so in the past)?
124. What was it like for you to inform your children about your esophageal atresia?
125. To what extent does your esophageal atresia affect your relationship with your children?
126. What was it like for you to inform other family members about your esophageal atresia?
127. To what extent does your esophageal atresia affect your relationship with your other family members?
128. Do you have enough time to finish your meal during your daytime activities?
129. Do you come home after your daytime activities without having eaten, because there was no time for that?
130. Do you ever have to miss a day because of your esophageal atresia?

131. Do you feel that your colleagues can empathize with your esophageal atresia during your daytime activities (e.g. when you need more time to eat or have to go to the hospital)?
132. Has your esophageal atresia ever hindered your career?
133. What was it like for you to inform your friends about your esophageal atresia?
134. To what extent does your esophageal atresia affect your relationship with your friends?
135. To what extent do culture, or origin, play a role in the choice of whether you tell someone about your esophageal atresia?
136. Are you quickly at ease with people who are new to you?
137. Does your esophageal atresia prevent you from showing your emotions?
138. Do you feel that you are more combative in life than someone else?
139. Do you feel like you get emotional sooner than someone else?
140. How much does it bother you when people ask questions about your esophagus atresia?
141. How much does it bother you to explain what esophageal atresia is?
142. Do you feel like a 'patient'?
143. How much does it bother you when people talk about you as patient?
144. Do you ever get negative comments about your esophageal atresia in general?
145. Are you in contact with other patients with an esophageal atresia?
146. Are you in need for contact with other patients?
147. Is there a single physician in charge of the monitoring of your esophageal atresia and any additional problems?

S2. Description of measurement instruments

Gastrointestinal Quality of Life Index (GIQLI)[1]

Description: The GIQLI is a health-related quality of life questionnaire containing 36 questions, each with five response categories graded from 0 to 4. Responses to all items sum up to a total score ranging from 0 to theoretical maximum score of 144, with a higher score indicating a higher QoL. It was originally developed for assessing quality of life in adults with a broad spectrum of benign and malign gastrointestinal disorders.
Validated: This questionnaire has been validated in the Dutch language.[2]

St. George Respiratory Questionnaire (SGRQ)[3]

Description: The SGRQ is a health-related quality of life questionnaire containing 50 questions regarding three domains: symptoms (assessing the frequency and severity of respiratory symptoms), activity (assessing the effects of breathlessness on mobility and physical activity), and impact (assessing the psychosocial impact of disease). The number of response categories varies per question. Responses are weighted and summarized using an Excel-based scoring calculator, resulting in domain scores and a total score ranging from 0 to a theoretical maximum of 100, with a higher score indicating a poorer QoL. It was originally developed for use in patients with chronic obstructive pulmonary disease (COPD) and asthma, but was since used in a broad range of respiratory conditions.
Validated: This questionnaire has been validated in the Dutch language.[4]

RAND-36[5]

Description: The RAND-36 is a generic, health-related quality of life questionnaire containing 36 questions in eight domains: physical functioning, physical role functioning, emotional role functioning, bodily pain, general health, vitality, social functioning and mental health. All responses are weighted and summarized, resulting in domain scores ranging from 0 to a theoretical maximum of 100, with a higher score indicating a higher QoL. All domain scores are standardized, aggregrated and transformed to calculate the Physical Component Score (PCS) and the Mental Component Score (MCS), each weighted domain score contributing differently to each component score. These component scores have a mean of 50 and a standard deviation of 10, with a higher score again indicating a higher quality of life. It was originally developed as an instrument for measuring health perception in the general population.[6]
Validated: This questionnaire has been validated in the Dutch language.[7]

S3. IRT models

Methods – two IRT models

We considered different item-response theory (IRT) models, namely a partial credit model (PCM) and a generalized partial credit model (GPCM). These models contain difficulty (or threshold) parameters, which describe the HRQoL level needed to obtain a certain response, and discrimination parameters, which indicate how well a statement differentiates between subjects with high and low HRQoL. In the PCM, items vary in their difficulty using a difficulty parameter for each category of each item, but all items share the same discrimination parameter. The GPCM provides additional flexibility compared to the PCM, by allowing items to differ in the discrimination, using item-specific discrimination parameters.

Both IRT models were assessed using individual fit statistics (infit or the inlier-sensitive fit, and outfit or the outlier-sensitive fit) and overall model fit statistics. Infit and outfit statistics indicate how accurately the data for each item fit the model, with a value of 1 indicating a perfect fit, and values between 0.5 to 1.5 considered productive for measurement. A likelihood ratio test statistic was calculated to compare the fit of the PCM and GPCM, but this result was used for indicative purposes only. Both the PCM and GPCM can be used to calculate patient-specific HRQoL estimates, but only in the PCM is this estimate a function of the sum score of all items. To assess the consequences of differential discrimination of items for the HRQoL estimates, the similarity of the patient-specific HRQoL estimates between the PCM and GPCM was calculated using Spearman's rho.

The item-specific discrimination parameters in the GPCM were assessed. Category probability curves were examined for each item. These should demonstrate that for patients with the lowest HRQoL, the lowest answer is the most probable, and that with improving HRQoL, each category of each item sequentially becomes the most probable answer. When this does not occur, an item demonstrates disordered thresholds. Differential item functioning (DIF) occurs when two or more groups of respondents of the same ability level respond differently to an item based on a factor other than HRQoL. DIF was examined on the basis of age, sex, and educational level using the lordif package in R. To identify items with DIF, a cut-off of 0.02 was used for the difference in the McFadden R^2 between groups.

Results - initial IRT results and item selection

All psychometric models were initially estimated on a data set of 447 subjects with 36 questions. In the exploratory bifactor analysis, items Q30 ('*Do you feel that having esophageal atresia has made you stronger as a person?*') and Q35 ('*Do you feel that your colleagues can empathize with your esophageal atresia during your daytime activities (e.g. when you need more time to eat or have to go to the hospital)?*') had standardized loadings on the common factor below 0.20, and the other items had loadings of 0.30 or greater. The results of infit

and outfit indices in the PCM showed three items with scores greater than 1.5 (Q30, Q33 '*Does your esophageal atresia interfere with your desire to have children (or has it done so in the past)?*', and Q35). For these items, variability between observed responses and those predicted by the model was larger than expected , which made these items less useful for the measurement of HRQoL. The poor fit of these three items was confirmed by Mokken scale analysis, which showed that these three items, as well as item Q21 ('*Do your scars make you feel uncomfortable?*', had item scalability coefficients (H values) lower than 0.2, suggesting relatively low correlations with the total SQEA score.

The model assumption of the PCM that all items have equal discrimination was assessed by comparing the PCM with a GPCM, which showed a better fit for the GPCM $\chi^2_{df=35}$=820.48 ($p<0.001$). To further assess the discrimination of the items in-depth, we evaluated the discrimination parameters in the GPCM. The discrimination parameters of the items Q30, Q33 and Q35 were 0.09, 0.34 and 0.109, respectively, whereas the discrimination parameters of the other items ranged from 0.44 to 2.45. Figure 3 presents the category probability curves of the PCM for items Q30, Q33 and Q35. Considering all of these findings, we decided to remove items Q30, Q33 and Q35 from the final SQEA scale.

S4. Pilot testing

Supplementary Table S4.1. Presentation of missing scores and distribution of the participants (n=42) over the response categories of the SQEA pilot questionnaire. Questions with an asterix have been preserved in the first phase of item reduction due to their clinical relevance. Question numbers correspond with the pilot questionnaire in Supplementary Material S1.

	Missing scores (%)	**Distribution of participants (n=42) over the response options**						
		5 (%)	*4 (%)*	*3 (%)*	*2 (%)*	*1 (%)*	*Skewness*	*Kurtosis*
Q1	-	45.2	45.2	4.8	2.4	2.4	1.797	4.608
Q2	-	21.4	23.8	4.8	21.4	28.6	-0.089	-1.636
Q3	-	21.4	19.0	7.1	19.0	33.3	-0.223	-1.606
Q4	-	7.1	4.8	38.1	35.7	14.3	-0.617	0.471
Q5	-	88.1	9.5	2.4	-	-	3.105	9.857
Q6	-	59.5	31.0	7.1	2.4	-	1.429	1.878
Q7	-	50.0	19.0	21.4	9.5	-	0.722	-0.858
Q8	-	92.9	7.1	-	-	-	3.453	10.416
Q9	-	66.7	19.0	11.9	-	2.4	1.995	4.521
Q10	-	64.3	9.5	19.0	7.1	-	1.101	-0.276
Q11	-	64.3	19.0	4.8	11.9	-	1.485	0.901
Q12	-	92.9	4.8	-	2.4	-	5.051	27.501
Q13	-	23.8	26.2	19.0	19.0	11.9	0.289	-1.122
Q14	-	45.2	26.2	23.8	4.8	-	0.614	-0.817
Q15	-	61.9	11.9	21.4	2.4	2.4	1.292	0.954
Q16	-	64.3	23.8	4.8	7.1	-	1.708	2.203
Q17	2.4	61.9	21.4	9.5	2.4	2.4	1.870	3.568
Q18	-	52.4	26.2	19.0	2.4	-	0.843	-0.442
Q19	2.4	28.6	38.1	21.4	9.5	-	0.473	-0.637
Q20	-	31.0	28.6	23.8	7.1	9.5	0.717	-0.336
Q21	-	54.8	16.7	19.0	9.5	-	0.869	-0.655
Q22	-	35.7	26.2	28.6	7.1	2.4	0.575	-0.362
Q23	-	35.7	26.2	26.2	11.9	-	0.366	-1.113
Q24	-	26.2	35.7	26.2	9.5	2.4	0.541	-0.210
Q25	-	66.7	14.3	16.7	2.4	-	1.289	-0.366
Q26	-	42.9	28.6	16.7	9.5	2.4	0.913	-0.005
Q27	-	81.0	11.9	7.1	-	-	2.174	3.678
Q28	2.4	38.1	26.2	23.8	4.8	4.8	0.876	0.216
Q29	-	61.9	23.8	11.9	2.4	-	1.324	0.932
Q30	-	66.7	11.9	21.4	-	-	1.041	-0.716
Q31	-	50.0	23.8	2.4	9.5	14.3	1.042	-0.468
Q32	-	61.9	16.7	14.3	2.4	4.8	1.614	2.031
Q33	-	57.1	14.3	21.4	4.8	2.4	1.115	0.364
Q34	-	73.8	7.1	-	9.5	9.5	1.619	0.958
Q35	4.8	19.0	23.8	19.0	23.8	9.5	0.099	-1.156
Q36	2.4	40.5	26.2	2.4	11.9	16.7	0.756	-1.037
Q37	-	59.5	23.8	7.1	2.4	7.1	1.789	2.508
Q38	-	42.9	7.1	7.1	23.8	19.0	0.186	-1.728
Q39	2.4	66.7	19.0	4.8	2.4	4.8	2.215	4.572
Q40	2.4	50.0	40.5	4.8	2.4	-	1.249	2.012
Q41	-	38.1	35.7	11.9	2.4	11.9	1.196	0.491
Q42	-	50.0	14.3	16.7	14.3	4.8	0.788	-0.723
Q43	-	66.7	9.5	14.3	4.8	4.8	1.540	1.386

Q44	-	45.2	33.3	4.8	2.4	14.3	1.309	0.451
Q45	-	66.7	19.0	2.4	-	11.9	1.922	2.417
Q46	-	61.9	21.4	4.8	2.4	9.5	1.764	2.040
Q47	-	64.3	11.9	19.0	2.4	2.4	1.435	1.413
Q48	-	4.8	19.0	38.1	19.0	19.0	0.035	-0.696
Q49	-	59.5	14.3	2.4	11.9	11.9	1.120	-0.383
Q50	-	66.7	16.7	4.8	-	11.9	1.827	2.055
Q51	-	66.7	14.3	7.1	4.8	7.1	1.720	1.856
Q52	2.4	78.6	7.1	4.8	7.1	-	2.244	3.848
Q53	-	83.3	7.1	4.8	4.8	-	2.616	6.060
Q54	-	61.9	23.8	2.4	11.9	-	1.539	1.211
Q55	-	4.8	14.3	50.0	23.8	7.1	-0.103	-0.361
Q56	-	69.0	11.9	9.5	7.1	2.4	1.684	1.855
Q57	-	42.9	42.9	4.8	4.8	4.8	1.629	2.600
Q58	2.4	2.4	9.5	64.3	14.3	7.1	0.361	1.677
Q59	-	7.1	2.4	81.0	7.1	2.4	-0.844	4.627
Q60	-	66.7	11.9	14.3	7.1	-	1.332	0.417
Q61	-	78.6	9.5	7.1	4.8	-	2.180	3.838
Q62	-	66.7	21.4	7.1	4.8	-	1.723	2.355
Q63	-	64.3	26.2	4.8	4.8	-	1.774	2.893
Q64	-	64.3	23.8	11.9	-	-	1.176	0.062
Q65	-	54.8	38.1	4.8	2.4	-	1.355	2.218
Q66	-	78.6	16.7	-	4.8	-	2.819	8.277
Q67	-	73.8	16.7	7.1	2.4	-	2.010	3.627
Q68	-	42.9	38.1	9.5	7.1	2.4	1.271	1.314
Q69	-	50.0	33.3	11.9	4.8	-	1.081	0.516
Q70	-	19.0	59.5	16.7	4.8	-	0.624	0.736
Q71	-	76.2	16.7	4.8	2.4	-	2.302	5.374
Q72	-	85.7	4.8	7.1	2.4	-	2.715	6.658
Q73	-	45.2	40.5	9.5	2.4	2.4	1.538	3.104
Q74	-	71.4	4.8	2.4	16.7	4.8	1.430	0.097
Q75	2.4	78.6	9.5	-	2.4	7.1	2.581	5.506
Q76	-	81.0	7.1	4.8	-	7.1	2.606	5.891
Q77	-	90.5	2.4	4.8	-	2.4	3.989	16.937
Q78	-	92.9	2.4	2.4	-	2.4	4.787	24.247
Q79 *	-	61.9	23.8	14.3	-	-	1.050	-0.323
Q80	-	66.7	16.7	7.1	4.8	4.8	1.852	2.656
Q81	-	85.7	9.5	2.4	2.4	-	3.321	11.810
Q82	-	88.1	4.8	7.1	-	-	2.835	6.852
Q83	-	85.7	7.1	4.8	-	2.4	3.593	14.275
Q84	-	31.0	47.6	11.9	7.1	2.4	1.111	1.236
Q85	-	71.4	21.4	4.8	2.4	-	2.039	4.285
Q86	-	78.6	11.9	4.8	4.8	-	2.362	4.963
Q87	-	47.6	31.0	14.3	2.4	4.8	1.419	1.834
Q88	-	54.8	23.8	11.9	2.4	7.1	1.530	1.650
Q89	-	52.4	28.6	11.9	2.4	4.8	1.575	2.269
Q90	-	61.9	19.0	11.9	7.1	-	1.317	0.591
Q91	-	76.2	11.9	11.9	-	-	1.702	1.417
Q92	2.4	47.6	33.3	9.5	4.8	2.4	1.449	2.019
Q93	-	95.2	2.4	2.4	-	-	5.111	26.980
Q94	-	78.6	16.7	4.8	-	-	2.028	3.388
Q95	-	76.2	16.7	4.8	2.4	-	2.302	5.374
Q96	-	38.1	28.6	14.3	9.5	9.5	0.868	-0.355

Q97	-	31.0	23.8	14.3	21.4	9.5	0.361	-1.222
Q98	-	33.3	47.6	14.3	2.4	2.4	1.219	2.319
Q99 *	-	69.0	21.4	9.5	-	-	1.413	0.788
Q100	-	50.0	28.6	19.0	-	2.4	1.265	1.850
Q101	-	50.0	35.7	11.9	2.4	-	0.996	0.449
Q102	-	23.8	23.8	28.6	11.9	11.9	0.365	-0.825
Q103	-	19.0	28.6	33.3	14.3	4.8	0.263	-0.509
Q104	-	66.7	16.7	11.9	2.4	2.4	1.831	3.147
Q105	2.4	69.0	16.7	9.5	-	2.4	1.731	2.247
Q106	-	42.9	23.8	14.3	7.1	11.9	0.916	-0.401
Q107	-	45.2	21.4	19.0	4.8	9.5	0.999	-0.023
Q108	-	50.0	42.9	4.8	2.4	-	1.208	1.968
Q109	-	61.9	16.7	4.8	7.1	9.5	1.448	0.726
Q110	-	85.7	11.9	2.4	-	-	2.726	7.393
Q111	-	88.1	7.1	4.8	-	-	3.028	8.583
Q112	-	42.9	52.4	2.4	-	2.4	2.081	8.140
Q113	-	76.2	21.4	2.4	-	-	1.733	2.306
Q114	-	57.1	28.6	11.9	2.4	-	1.184	0.681
Q115	-	76.2	19.0	-	2.4	2.4	3.121	10.854
Q116	2.4	33.3	33.3	14.3	14.3	2.4	0.718	-0.461
Q117	-	59.5	31.0	4.8	4.8	-	1.621	2.488
Q118	-	83.3	4.8	7.1	2.4	2.4	2.723	7.153
Q119	-	85.7	7.1	2.4	-	4.8	3.422	11.518
Q120	-	69.0	19.0	7.1	4.8	-	1.804	2.586
Q121	-	90.5	9.5	-	-	-	2.861	6.492
Q122	-	92.9	7.1	-	-	-	3.453	10.416
Q123	2.4	73.8	11.9	4.8	2.4	4.8	2.406	5.231
Q124	-	95.2	2.4	2.4	-	-	5.111	26.980
Q125	-	92.9	-	4.8	2.4	-	3.723	13.253
Q126	-	90.5	7.1	2.4	-	-	3.584	13.351
Q127	-	95.2	4.8	-	-	-	4.408	19.296
Q128 *	-	57.1	38.1	4.8	-	-	0.828	-0.238
Q129	-	81.0	16.7	2.4	-	-	3.171	12.290
Q130	-	90.5	7.1	-	2.4	-	4.537	22.867
Q131	4.8	69.0	4.8	19.0	2.4	-	1.340	0.212
Q132	-	90.5	2.4	2.4	2.4	2.4	3.692	13.478
Q133	-	83.3	7.1	7.1	2.4	-	2.550	5.875
Q134	-	97.6	2.4	-	-	-	6.481	42.000
Q135	-	97.6	2.4	-	-	-	6.481	42.000
Q136	-	42.9	35.7	14.3	4.8	2.4	1.191	1.280
Q137	-	38.1	23.8	23.8	11.9	2.4	0.577	-0.687
Q138	-	14.3	7.1	26.2	31.0	21.4	-0.555	-0.632
Q139	-	23.8	16.7	28.6	23.8	7.1	-0.004	-1.091
Q140	-	83.3	16.7	-	-	-	1.856	1.514
Q141	-	95.2	4.8	-	-	-	4.408	18.296
Q142	-	57.1	28.6	7.1	4.8	2.4	1.714	2.815
Q143	-	69.0	7.1	9.5	11.9	2.4	1.398	0.524
Q144	-	97.6	2.4	-	-	-	6.481	42.000
Q145	-	2.4	2.4	11.9	-	83.3	-3.805	16.756
Q146	2.4	47.6	16.7	23.8	4.8	4.8	0.952	0.081
Q147	2.4	33.3	4.8	21.4	7.1	33.3	-0.039	-1.660

Supplementary Table S4.2. Overview of deleted questions during the second phase of item reduction. PCA = principal component analysis, N/A = not applicable. [A] PCA not possible, Kaiser-Meyer-Olkin test <0.5. Question numbers correspond with the pilot questionnaire in Supplementary Material S1.

Phase	Domain	N	Deleted questions
1. Missing scores		None.	
2. Distribution		27	Q5, Q8, Q12, Q27, Q30, Q64, Q66, Q82, Q91, Q93, Q94, Q110, Q111, Q113, Q121, Q122, Q124, Q125, Q126, Q127, Q129, Q130, Q134, Q135, Q140, Q141, Q144
3. PCA per domain	Eating and drinking	4	Q3, Q7, Q10, Q11
	Complaints of the esophagus	2	Q19, Q21
	Complaints of the stomach and bowel	2	Q31, Q32
	Complaints of the lungs	5	Q34, Q35, Q36, Q37, Q45
	General physical functioning	5	Q49, Q50, Q52, Q54, Q61
	Appearance	3	Q62, Q64, Q66
	Medicine	3	Q74, Q77, Q78
	Memories	3	Q80, Q83, Q86
	Fears and worries	6	Q100, Q101, Q103, Q104, Q106, Q107
	Relationships	N/A [A]	
	Daily activities	0	
	Distinction form others	N/A [A]	
4. Not part of any domain		5	Q116, Q117, Q145, Q146, Q147
5. Reliability analysis per domain	Eating and drinking	4	Q2, Q14, Q15, Q17
	Complaints of the esophagus	4	Q28, Q20, Q22, Q23
	Complaints of the stomach and bowel	3	Q24, Q25, Q26
	Complaints of the lungs	5	Q38, Q39, Q40, Q41, Q43
	General physical functioning	5	Q48, Q55, Q57, Q58, Q59
	Appearance	4	Q65, Q70, Q71, Q73
	Medicine	1	Q75
	Memories	2	Q79, Q84
	Fears and worries	10	Q90, Q92, Q96, Q97, Q98, Q102, Q105, Q108, Q109, Q115
	Relationships	2	Q120, Q133
	Daily activities	0	
	Distinction form others	4	Q138, Q139, Q142, Q143
Total		109	
Remaining questions		38	

Supplementary Table S4.3. Summary of total item reduction. [A] In these clinical domains, items were merged or wording was adapted for uniformization or clarification after review by the expert team. The Cronbach's alpa presented here, is before final item merging.

Domain	Number of items at start	Number of items after reduction	Reliability (Cronbach's alpha)
Eating and drinking	17	6	0.873[A]
Esophageal complaints	9	2	0.881[A]
Complaints of the stomach and/or intestines	7	4- 1	0.830[A]
Respiratory complaints	14	9- 3	0.786
Physical performance in general	14	4	0.889
Appearance	12	5	0.721
Medication	5	1	0.880[A]
Recollections of past events	8	2	0.868
Fears and worries	29	5	0.865
Relationships	13	3	0.709
Daytime activities	5	3	0.701
Distinction from others (not born with EA)	9	2	0.821

S5. Concept questionnaire

1. Do you enjoy eating?
2. How long does it take you to eat a meal?
3. Are you able to eat everything you would like to eat? Specifically in regard to the food texture?
4. Is your choice of food during social events limited by your esophageal atresia?
5. How much does it bother you when food gets stuck in your esophagus?
6. Do you feel fearful when food gets stuck in your esophagus?
7. Do you feel uncomfortable while eating?
8. How much do your esophageal complaints bother you?
9. Do your esophageal complaints limit you in your daily life?
10. How much do your stomach and/or intestinal complaints bother you?
11. Do your stomach and/or intestinal complaints limit you in your daily life?
12. Do you feel uncomfortable while coughing?
13. How much do your respiratory complaints bother you?
14. Do your respiratory complaints limit you in your daily life?
15. Do these complaints, related to your esophageal atresia, disrupt your sleep?
16. How is your stamina compared to others of your age?
17. How is your strength compared to others of your age?
18. Does your esophageal atresia limit you in your daily activities? For example going to school, work or doing household chores?
19. Does your esophageal atresia limit you while doing hobbies or sports?
20. Do you have visible birth defects and/or scars that limit you in your daily life?
21. Do your scars make you feel uncomfortable?
22. How much does it bother you to take medication for your esophageal atresia or related respiratory complaints?
23. To what extent do bad recollections of past events affect you in your daily life?
24. To what extent do your parents or caretakers' bad recollections of past events affect you in your daily life?
25. Do you worry about your esophagus, stomach and/or intestines?
26. Do you worry about your lungs and/or airway?
27. Do you feel anxious or tense because of your esophageal atresia in general?
28. Does your esophageal atresia ever cause frustration?
29. Does having an esophageal atresia prevent you from showing your emotions?
30. Do you feel that having esophageal atresia has made you stronger as a person?
31. Do you feel guilty towards your parents for being born with an esophageal atresia?
32. Do you feel guilty towards your siblings for being born with an esophageal atresia?
33. Does your esophageal atresia interfere with your desire to have children (or has it done so in the past)?

34. Do you have enough time to finish your meal during your daytime activities?
35. Do you feel that your colleagues can empathize with your esophageal atresia during your daytime activities (e.g. when you need more time to eat or have to go to the hospital)?
36. Has your esophageal atresia ever hindered your career?

S6. Self-reported reasons for regular physician visits

Number of patients reporting regular physician visits: 79 (17.7% of total number of participants); one patient can have multiple reasons for regular physician visits.

Related to esophageal atresia:

- Acid reflux (n=2)
- Acid reflux, obesity
- Acid reflux, pulmonary complaints
- Chronic obstructive pulmonary disease, esophageal carcinoma
- Gastrointestinal problems
- Impaired lung function, Barrett's esophagus
- Pulmonary complaints, Barrett's esophagus
- Pulmonary complaints, gastrointestinal complaints
- Recurrent esophageal dilatations

Related to associated morbidities of esophageal atresia:

- Pulmonary complaints (n=5)
- Asthma (n=3)
- Scoliosis (n=3)
- Cardiac problems (n=2)
- Impaired lung function (n=2)
- Kidney failure (n=2)
- Check-ups after cardiac surgery
- Cystic kidney disease
- Kidney transplantation
- Kidney transplantation, colostoma
- Scoliosis, asthma, rheumatic disorder
- Spina bifida
- Tetralogy of Fallot
- Valvular heart disease
- VSD, astma

Other problems:

- Vitamin B12 deficiency (n=5)
- Thyroid disease (n=3)
- Breast cancer (n=2)
- Joint problems (n=2)
- Visual impairment (n=2)
- Abdominal hernia

- Aneurysm
- Allergies
- Bronchopulmonary dysplasia
- Ulcerative colitis (n=2)
- Cerebral infarction
- Cholesteatoma
- Chronic fatigue syndrome, diabetes mellitus, asthma
- Conversion disorder
- Dermatitis
- Fibromyalgia, irritable bowel syndrome
- Hearing problems
- Hypermobile Ehlers-Danlos syndrome
- Hypertension
- Hypertension, asthma
- Irritable bowel syndrome
- Irritable bowel syndrome, pulmonary complaints
- Myocardial infarction
- Obesity
- Pituitary macroprolactinoma
- Psoriasis
- Retinopathy, sclerosis hepatoportale
- Rheumatic disorder
- Rheumatic disorder, fibromyalgia, arthrosis, lipedema, asthma
- Sarcoidosis
- Subfertility
- Tracheal stenosis, irritable bowel syndrome

S7. Self-reported mental health

Number of patients reporting mental problems: 85 (19% of total number of participants); one patient can have multiple self-reported mental problems

Mental problems in history (n=35):

- Depression (n=16)
- Anxiety disorder (n=10)
- Occupational burnout (n=4)
- Attention deficit hyperactivity disorder (n=2)
- Obsessive-compulsive disorder (OCD, n=2)
- Post-traumatic stress disorder (n=2)
- Eating disorder
- Social anxiety disorder
- Substance use disorder
- Tics

Mental problems currently (n=50):

- Pervasive developmental disorder not otherwise specified (n=21)
- Attention deficit hyperactivity disorder (n=13)
- Depression (n=10)
- Anxiety disorder (n=7)
- Autism Spectrum Disorder (ASD) (n=3)
- Obsessive-compulsive disorder (OCD, n=2)
- Social anxiety disorder
- Personality disorder
- Personality disorder
- Worrying excessively

S8. Item evaluation of the field test

These are the results of the field test of the final SQEA questionnaire of 33 items, after reduction of three items based on the item-response theory results.

Supplementary Table S8.1. Item evaluation of the SQEA questionnaire (n=447). Raw, untransformed scores are presented in this table. Items were answered on a 5-point Likert scale, ranging from never (1) to always (5). Zero (0) represents the option 'not applicable'.

	Mean ± SD	Range	Skewness	Kurtosis
Q1	4.5 ± .07	1-5	-1.4	2.7
Q2	2.7 ± 0.9	1-5	0.4	0.3
Q3	4.5 ± 0.8	1-5	-2.1	5.5
Q4	4.5 ± 0.8	1-5	-2.1	4.5
Q5	2.2 ± 1.5	0-5	-0.1	-1.2
Q6	3.2 ± 1.8	0-5	-0.8	-0.7
Q7	4.5 ± 0.8	1-5	-1.8	2.6
Q8	2.9 ± 1.8	0-5	-0.6	-1.0
Q9	3.6 ± 1.9	0-5	-1.2	-0.2
Q10	2.1 ± 2.0	0-5	0.1	-1.7
Q11	2.6 ± 2.2	0-5	-0.2	-1.8
Q12	3.2 ± 1.8	0-5	-0.7	-0.8
Q13	2.4 ± 1.9	0-5	-0.1	-1.5
Q14	2.9 ± 2.1	0-5	-0.5	-1.4
Q15	3.2 ± 2.0	0-5	-0.8	-1.1
Q16	2.8 ± 1.0	1-5	0.3	0.1
Q17	2.9 ± 0.9	1-5	0.1	0.9
Q18	4.7 ± 0.7	1-5	-3.2	11.2
Q19	4.6 ± 0.8	1-5	-2.4	6.1
Q20	4.5 ± 1.0	0-5	-2.9	9.0
Q21	4.4 ± 0.9	1-5	-1.6	2.3
Q22	1.2 ± 2.0	0-5	1.1	-0.6
Q23	3.0 ± 2.2	0-5	-0.5	-1.6
Q24	3.7 ± 1.9	0-5	-1.2	-0.3
Q25	3.6 ± 1.5	0-5	-1.5	1.2
Q26	2.4 ± 1.8	0-5	-1.0	-0.5
Q27	4.7 ± 0.6	2-5	-2.0	4.6
Q28	4.5 ± 0.7	1-5	-1.6	2.6
Q29	4.9 ± 0.5	2-5	-4.0	16.8
Q31	4.8 ± 0.6	1-5	-3.9	17.6
Q32	4.4 ± 1.5	0-5	-2.4	4.2
Q34	4.5 ± 0.8	1-5	-2.1	5.1
Q36	4.8 ± 0.6	1-5	-3.7	15.0

S9. Sex-specific validity results

Supplementary Table S9.1. Comparison between clinical subgroups of total SQEA scores Only patients for whom both clinical data and SQEA scores were available were included in the analyses. Asterisks indicates significance p<0.05.

		Yes		**No**			
		n	*Median (IQR)*	*n*	*Median (IQR)*	*p-value*	*Effect size (r)*
Male	Primary repair	188	87.88 (80.49-92.42)	27	87.12 (79.55-91.67)	0.607	0.04
	Esophageal replacement	11	82.58 (75.00-87.12)	204	88.64 (81.25-92.42)	**0.026 ***	0.15
	Major surgical revision	30	86.74 (79.36-90.53)	185	87.88 (81.44-92.42)	0.290	0.07
	History of severe tracheomalacia	17	84.09 (65.15-91.29)	198	88.64 (81.06-92.42)	0.100	0.11
	Other disabling congenital condition	14	78.79 (60.23-85.23)	201	88.64 (81.82-92.42)	**0.002 ***	0.21
	History of anti-reflux surgery	42	84.09 (77.84-91.86)	173	88.63 (81.06-92.80)	0.217	0.08
	History of ≥1 dilatation	113	86.36 (78.41-91.67)	102	89.39 (83.33-93.18)	**0.012 ***	0.17
	Presence of dysphagia	90	84.09 (75.00-89.39)	127	90.15 (84.85-93.93)	**<0.001 ***	0.41
	Impaired lung function	7	78.03 (73.48-83.33)	30	89.39 (83.90-92.61)	**0.004 ***	0.47
Female	Primary repair	178	82.58 (75.00-87.88)	29	78.79 (71.97-85.98)	0.081	0.12
	Esophageal replacement	16	74.62 (69.70-79.36)	191	82.58 (75.00-87.88)	**0.006 ***	0.19
	Major surgical revision	23	79.55 (73.48-88.64)	184	82.58 (75.00-87.88)	0.700	0.03
	History of severe tracheomalacia	10	76.14 (63.26-83.71)	197	82.58 (75.00-87.88)	0.064	0.13
	Other disabling congenital condition	5	84.09 (78.03-89.02)	202	81.82 (74.81-87.88)	0.496	0.05
	History of anti-reflux surgery	26	78.79 (64.96-85.04)	181	82.58 (75.00-87.88)	0.085	0.12
	History of ≥1 dilatation	93	82.58 (75.76-89.39)	102	89.39 (83.33-93.18)	0.281	0.07
	Presence of dysphagia	100	78.79 (70.64-83.33)	111	85.61 (78.03-90.91)	**<0.001 ***	0.39
	Impaired lung function	12	82.58 (78.03-85.23)	40	81.82 (71.97-89.20)	0.672	0.06

Supplementary Table S9.2. Correlation between dysphagia and an impaired lung function with the different questionnaires. SQEA = Specific Quality of life in Esophageal atresia Adults, GIQLI = Gastrointestinal Quality of Life Index, SGRQ = St. George Respiratory Questionnaire, PCS = Physical Component Scale, MCS = Mental Component Scale

		AUC (95% CI)	
		Dysphagia (n=90)	*Impaired lung function (n=7)*
Male	SQEA	0.738 (0.672-0.803	0.855 (0.734-0.976)
	GIQLI	0.654 (0.578-0.729)	0.670 (0.483-0.874)
	SGRQ	0.661 (0.585-0.736)	0.824 (0.684-0.964)
	PCS	0.562 (0.484-0.640)	0.738 (0.519-0.958)
	MCS	0.611 (0.535-0.688)	0.552 (0.330-0.775)
		Dysphagia (n=100)	*Impaired lung function (n=13)*
Female	SQEA	0.727 (0.660-0.795)	0.541 (0.376-0.706)
	GIQLI	0.654 (0.579-0.729)	0.585 (0.376-0.795)
	SGRQ	0.575 (0.496-0.653)	0.601 (0.435-0.766)
	PCS	0.533 (0.453-0.612)	0.400 (0.221-0.579)
	MCS	0.625 (0.548-0.702)	0.547 (0.367-0.727)

Supplementary Table S9.3. Descriptive values of the SQEA questionnaire, Gastrointestinal Quality of Life Index (GIQLI) questionnaire, the George Respiratory Questionnaire (SGRQ) and the RAND-36. Correlations of the GIQLI, SGRQ and RAND-36 scores with the SQEA scores. SD = standard deviation, IQR = interquartile range, r_s = Spearman's rho. Agreements between questionnaires were concluded as poor (<0.40), moderate (0.40-0.59), good (0.60-0.79), or excellent (>0.80).

		Respondents (n)	**Mean ± SD**	**Median (IQR)**	**Level of agreement**	
					r_s	*p-value*
Male	SQEA	227	84.90 ± 11.48	87.88 (81.06-92.42)		
	GIQLI					
	Symptoms	222	66.69 ± 8.98	69.00 (62.00-74.00)	0.672	p<0.001
	Emotional	224	17.50 ± 2.68	18.00 (17.00-19.00)	0.514	p<0.001
	Physical	224	24.45 ± 3.03	25.00 (23.00-17.00)	0.548	p<0.001
	Social	225	18.34 ± 2.69	20.00 (17.00-20.00)	0.518	p<0.001
	Total score	220	127.00 ± 14.70	131.00 (122.00-138.00)	0.685	p<0.001
	SGRQ					
	Symptoms	222	19.35 ± 19.43	14.08 (4.42-27.94)	-0.527	p<0.001
	Activity	226	8.64 ± 11.34	5.96 (0.00-12.17)	-0.403	p<0.001
	Impact	226	4.30 ± 7.62	0.00 (0.00-5.45)	-0.548	p<0.001
	Total score	222	8.18 ± 9.47	5.04 (1.97-10.77)	-0.595	p<0.001
	RAND-36					
	Physical functioning	222	94.14 ± 11.80	100.00 (95.00-100.00)	0.506	p<0.001
	Social functioning	222	90.77 ± 17.73	100.00 (87.50-100.00)	0.475	p<0.001
	Role limitations (physical)	222	92.60 ± 18.52	100.00 (100.00-100.00)	0.397	p<0.001
	Role limitations (emotional)	222	91.48 ± 19.73	100.00 (100.00-100.00)	0.294	p<0.001
	Mental health	221	79.52 ± 16.41	84.00 (75.00-90.00)	0.454	p<0.001
	Vitality	221	72.05 ± 18.46	75.00 (62.50-87.50)	0.437	p<0.001
	Bodily pain	220	69.24 ± 21.08	62.50 (50.00-87.50)	-0.144	p<0.001
	General health	220	66.68 ± 18.70	70.00 (55.00-80.00)	0.545	p<0.001
	Physical Component Scale	222	52.36 ± 5.28	52.13 (49.87-56.06)	0.210	p<0.001
	Mental Component Scale	222	52.39 ± 9.92	55.23 (49.53-58.79)	0.452	p<0.001

Female	SQEA	220	79.76 ± 11.65	82.58 (75.00-87.88)		
	GIQLI					
	Symptoms	215	64.32 ± 9.54	67.00 (60.00-71.00)	0.639	p<0.001
	Emotional	217	16.94 ± 2.87	18.00 (16.00-19.00)	0.492	p<0.001
	Physical	217	23.12 ± 3.78	24.00 (21.00-26.00)	0.549	p<0.001
	Social	214	17.79 ± 3.08	19.00 (17.00-20.00)	0.535	p<0.001
	Total score	213	122.11 ± 16.70	125.00 (114.00-134.00)	0.700	p<0.001
	SGRQ					
	Symptoms	212	24.19 ± 21.41	18.48 (6.60-38.06)	-0.529	p<0.001
	Activity	212	16.75 ± 16.81	12.17 (5.97-23.72)	-0.555	p<0.001
	Impact	212	8.33 ± 11.05	3.90 (0.00-12.63)	-0.603	p<0.001
	Total score	212	13.50 ± 12.79	9.47 (3.64-19.99)	-0.645	p<0.001
	RAND-36					
	Physical functioning	217	88.09 ± 15.30	95.00 (80.00-100.00)	0.612	p<0.001
	Social functioning	217	87.04 ± 19.80	100.00 (75.00-100.00)	0.462	p<0.001
	Role limitations (physical)	216	89.32 ± 23.35	100.00 (93.75-100.00)	0.440	p<0.001
	Role limitations (emotional)	216	87.77 ± 22.59	100.00 (83.00-100.00)	0.374	p<0.001
	Mental health	217	76.63 ± 16.73	80.00 (65.00-90.00)	0.471	p<0.001
	Vitality	217	64.09 ± 20.50	65.00 (50.00-80.00)	0.524	p<0.001
	Bodily pain	215	67.52 ± 21.97	62.50 (50.00-87.50)	-0.017	p<0.001
	General health	217	61.64 ± 19.08	65.00 (45.00-75.00)	-0.526	p<0.001
	Physical Component Scale	217	50.61 ± 6.54	51.28 (47.75-54.87)	0.357	p<0.001
	Mental Component Scale	217	50.48 ± 10.07	52.78 (46.28-57.36)	0.461	p<0.001

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APPENDIX

DCEA Study Group – pediatric surgeons and pediatric gastroenterologists of the participating centers:

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- S.H.A.J. (Stefaan) Tytgat
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- R.H.J. (Roderick) Houwen
- A. (Annemone) van den Berg

Radboud University Medical Center, Amalia Children's Hospital:

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- H. (Horst) Scharbatke
- M. (Maarten) Schurink
- G. (Gerard) Damen
- N. (Nicole) Gierenz

Amsterdam UMC – Emma Children's Hospital:

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- S. (Sander) Zwaveling
- S. (Sjoerd) de Beer
- R. (Ramon) Gorter
- M.P. (Michiel) van Wijk

DISCUSSION AND SUMMARY

CHAPTER 13

General Discussion

ABSTRACT

Every year, around 40 children in the Netherlands are born with esophageal atresia (EA).[1] Between 1950 and 1970, the survival rate spectacularly improved and has been relatively high for decades now.[2] The present-day survival rates are over 90%, and even approach 100% in EA without associated anomalies.[3] Consequently, more children born with EA than before are growing up. This, however, creates new challenges in the health care of these children. After surgical correction of the esophageal discontinuity, most children need long-term care for morbidities caused by the anomaly itself or for associated comorbidities, and – as such – EA can be considered an anatomical congenital anomaly that results in a chronic health condition. Compared with children with other embryonic foregut anomalies, the proportion of children born with EA patients currently reaching adulthood is high. Mortality rates of, for example diaphragmatic hernia (25-30%[4, 5]) are much higher. Therefore, the lessons learned from EA can serve as a blueprint for life-course medicine in other anatomical conditions.

Although multidisciplinary follow-up during childhood is well established during childhood – a longitudinal follow-up program was set-up in our center in 1999[6] – this multidisciplinary approach should also be implemented and optimized in the follow-up for adult patients. Since 2013, a standardized endoscopic surveillance program for the early detection of Barrett's esophagus (BE) and esophageal carcinoma is run in our institution for adults born with EA.[7] We have learned that adults born with EA, besides problems with the gastrointestinal tract, may have also other medical and psychosocial needs that require attention. In 2019, we have therefore initiated a standardized screening program for pulmonary abnormalities. Last but not least, the burden of a disease – reflected as health status (HS) and quality of life (QoL) – should always be taken into consideration in the management of a chronic condition as well. As illustrated in this thesis and to be discussed in this chapter, our EA research group has grown into a unique collaboration between the departments of Pediatric Surgery and Intensive Care, Clinical Genetics, Gastroenterology and Pulmonology.

When optimizing health care for patients with EA, multiple factors should be taken into account. As physicians, we intend to obtain the best possible outcome. Complications are treated, and interventions are offered whenever possible for comorbidities detected during follow-up. However, just as important as the clinical outcome is the patients' own perception – and that of the parents – of their health and daily functioning, both in childhood and adulthood. As we learned from Chapter 10, clinical outcomes do not always correlate well with a patient's perception of level of functioning. It is important to combine both aspects, taking into consideration what matters most to them. Health care possibilities should be facilitated in a way one feels comfortable with and that ties in with one's experiences and expectations in daily life. In this era of shared clinical decision-making, the perspective of patients on the organization of health care becomes more and more important. In early

childhood, however, only proxy-reported outcomes are available, what makes it challenging to interpret the perspective of pediatric patients. When a child gets older, self-reported and proxy-reported opinions will have to be combined.

The research presented in this thesis aimed to optimize the health care for patients born with EA of all ages, with the unique input of the perspectives of both patients and professionals (see Figure 1). This research goes beyond the previously discussed long-term gastrointestinal morbidity[8] by combining contributions of multiple disciplines (Pediatrics, Pediatric Surgery, Clinical Genetics, Gastroenterology and Pulmonology) throughout life. Besides, we introduce a prelude to first-level evidence with the set-up up of a randomized controlled trial for the treatment of refractory anastomotic stenosis (Chapter 6), and acknowledge the pulmonary problems which adults born with EA encounter (Appendix). The challenge lies in minimizing the burden of disease, thereby sustaining or improving quality of life. In this chapter, we discuss the findings reported in this thesis in a broader perspective, and give recommendations for daily practice and future research.

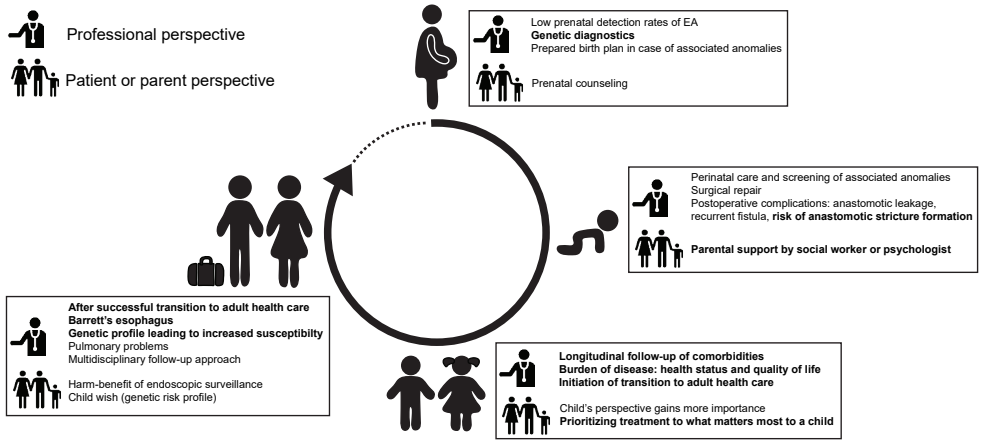

Figure 1. Health care needs throughout life, from a professional and a patient or parent perspective. The topics in bold are addressed in this thesis.

Prenatal counseling – care for the parents as well

Health care for EA actually already starts before birth. Prenatal detection of EA remains difficult. Ultrasound has an overall sensitivity of only 32% in detecting EA.[9] When a magnetic resonance imaging (MRI) is performed in fetuses with suspicious sonographic signs, the sensitivity increases to 95% with a specificity of 89%.[9] Neonates who prenatally have been diagnosed with EA by ultrasound have higher mortality rates than those diagnosed after birth. However, the former group has more severe additional anomalies.[10] Ideally, both isolated and

non-isolated EA are diagnosed prenatally, thereby offering the opportunity to counsel the parents and to optimize perinatal care.

Prenatal counseling is offered when EA is suspected by ultrasound (e.g., small or absent fetal stomach or polyhydramnios). The addition of MRI, which offers high sensitivity and specificity, might benefit the counselling process. At that moment, a pediatric surgeon will counsel the parents on the surgery and postoperative care by, while a pediatrician informs them on the life-long follow-up of their child, and a clinical geneticist offers genetic counselling. After birth, every child will be evaluated by a clinical geneticist. In some instances, a monogenetic disorder is suspected based on the child's physical appearance. If a monogenetic syndrome is not immediately suspected, a standard protocol is followed. The most prevalent disease genes are screened using an exome sequencing based panel of six genes (*CHD7*, *EFTUD2*, *GLI3*, *MID1*, *MYCN* and *SOX2*) and copy number profiles are evaluated to exclude large pathogenic changes. If pathogenic changes are not detected, the remainder of the coding region is evaluated. In the last two decades, multiple PhD theses of the Department of Pediatric Surgery of the Erasmus MC have been devoted to discovering genetic defects causing EA.[11-13] Overall, multiple contributing genomic variations – chromosomal anomalies, copy number variations, single nucleotide variants – as well as monogenetic syndromes have been detected. Moreover, EA can be part of many syndromes (see Table 2 of Chapter 2).

Unfortunately, the genetic diagnostic yield is low due to the uncertain heritability, large locus heterogeneity and a broad phenotypical spectrum of associated anomalies seen in patients with EA.[14] De *novo* changes are enriched in patients with EA,[15] affecting specific biological pathways.[16] Therefore, to discover genetic alteration, large sample sizes as well as detailed phenotyping are crucial.

We hypothesized that by combining two rare foregut-derived defects, genetic alterations in genes important for foregut morphogenesis would surface. The pyloric muscle of the stomach develops around the 6th week of gestation,[17] while the esophagus separates from the trachea between the 4th and 6th week of gestation as well.[18] Infantile hypertrophic pyloric stenosis has a 30-times higher prevalence (7.5%) in patients with EA than in the normal population (0.25%).[19] Moreover, both pyloric stenosis and EA can be present in phenotypically overlapping syndromes (see Table 2 of Chapter 2). However, we were not able to detect a central causative gen when investigating patients with this combination of defects (Chapter 2). Therefore, we proposed two multifactorial models (see Figure 3 of Chapter 2) in which both genetic, mechanical and environmental factors contribute. Altogether, given the tremendous amount of research over the years, it may be time to shift the focus of research from causative genetic defects towards these multifactorial models. Large cohorts are needed to explore these theories, which can only done in large multicenter cohorts. The

Congenital Esophageal and Airway Defect Research) CLEAR consortium is an example of such collaborative research programs.[20, 21]

After the corrective surgery a pediatrician of the long-term follow-up team provides – prior to discharge of the initial hospitalization – information about the longitudinal follow-up program with standardized visits[6] and tells the parents how their child can be expected to function in daily life. At this point, the endoscopic and pulmonary follow-up in adulthood is also briefly mentioned.

The vivid memories of parents of adult patients with EA (Chapter 7) illustrate that parental anxiety is an important topic to address. It is important to realize that these patients were born several decades ago, when health care was organized completely different without specific attention for the parents or for parent-child bonding. Despite the improvements over time – such as rooming-in around-the-clock and family-centered care – parental anxiety remains a significant problem. Parents of a child born with EA describe infancy as one of the most challenging periods in their life.[22] Although this applies to almost all new parents, parents of a child with a congenital malformation are confronted with more insecurities that require more coping mechanisms. It is known that parents of children with a chronic illness face several stressors, both in a practical (e.g. managing daily routines) and an emotional sense (e.g. worrying and falling prey to psychosocial problems such as anxiety and depression).[23] Compared with most other congenital malformations – such as congenital diaphragmatic hernia, anorectal malformation, Hirschsprung's disease, myelomeningocele, gastroschisis or omphalocele – parents of children with EA report the highest scores on psychological stress,[24] and even in a recent study, more than half of the parents of children with EA fulfilled the criteria of posttraumatic stress disorder.[25] Psychological problems of parents with children with chronic conditions are associated with less parental involvement and more hostility towards the child.[26, 27] This emphasizes the importance of optimizing health care not only for the infant born with EA, but for the parents as well. Intervention sessions that teach parents how to use adaptive disease-related coping skills have proven effective to ameliorate parental anxiety and depression.[23] However, this has not yet been investigated in the context of a child's admission to the intensive care unit, or in children with rare diseases. Besides the support of a social worker with practical issues such as their jobs or care for other children at home, it is important to offer parents guidance in trauma processing as well. Furthermore, the parent support association can support in coping behavior by stimulating exchange between parents of children with EA.

Recommendations:

- When EA is suspected by ultrasound, MRI should be offered during prenatal counselling to improve the prediction precision and thereby the counselling process.
- Research is required towards multifactorial models as a cause of EA. This requires large patient cohorts, recruited in international collaborations.
- Offer timely parental support. During initial hospitalization, support of a social worker should be offered, and contact with the parent support association should be encouraged.
- If parents have psychological problems, intervention sessions can be helpful in teaching parents coping skills. The additional effects of these interventions need to be studied in parents of children born with EA.

Management of postoperative complications

The stressful period of infancy can be a strain on the child's mental health. The challenges around corrective surgery, such as in long gap EA, premature birth or being born small for gestational age, are beyond the scope of this thesis. However, once an anastomosis has been established, postoperative complications remain lurking, such as anastomotic leakage, recurrent tracheoesophageal fistula and – most frequently – anastomotic stricture formation.[28] In Chapters 4, 5 and 6, we focused on the management of anastomotic strictures. We confirmed with an international survey results that consensus for stricture management is currently lacking (Chapter 4), while, especially in rare diseases, standardization of health care is important. We made several recommendations to optimize the treatment. First, we advise to only dilate a stricture in symptomatic patients, since this will reduce the number of dilatations and, consequently, the anesthetic exposure. Each dilatation requires hospitalization and therefore adds significantly to the burden of disease. The importance of minimizing the number of hospitalizations is supported by the fact that many patients at adult age still experience hospital anxiety (Chapter 7). Second, we advise to standardize the dilatation procedure within a hospital. At the moment, there is no hard evidence for preferring balloon dilatation or bougienage, although comparative studies are ongoing. Therefore, at this point we suggest at least adhering to a single technique. Standardization provides the opportunity to evaluate the efficacy of techniques over time. Last, we advocated for expertized health care. A center should perform minimally 10 dilatations in children with EA per year, and otherwise refer the patient.

Refractory strictures form a special challenge in anastomotic stricture management. Our research group started to investigate this problem by describing risk factors associated with the development of refractory strictures: EA type A, anastomotic leakage and the need for

a first dilatation ≤28 days after surgery.[29] The association between anastomotic leakage and stricture formation is thought to be caused by the enhancement of inflammation and therefore scarring of the anastomotic area.[30, 31] The same effect has been identified for GER.[32, 33] This made us presume that if we can prevent scar development, we could potentially prevent stricture formation. It has been hypothesized that steroids can prevent the regeneration of hypertrophic scar tissue by the inhibition of collagen formation, the enhancement of collagen breakdown and the decreased fibrotic healing,[34, 35]

After our successful initial experience with intralesional steroid injections prior to endoscopic dilatation to clear recurrent strictures (Chapter 5), our research group has taken the next step; i.e., determining the effectiveness and safety of this treatment in an international, multicenter, randomized controlled trial. The study (Chapter 6) is currently up and running within the European Reference Network on Inherited and Congenital Abnormalities (ERNICA). Considering the rarity of the disease, we expect to complete the inclusions and present the first results of this study in five years. If we can prove our hypothesis that intralesional steroid injections can prevent refractory strictures, it is most likely that this treatment will be implemented into the standard of care for patients with EA.

Recommendations:

- Standardization of the management of anastomotic strictures implies that a dilatation procure only is in performed in symptomatic patients, that this procedure is standardized within a hospital, and that only expert centers should perform the procedure. Standardization provides the opportunity to evaluate stricture management in future research.
- Intralesional steroid injections may be a potential adjuvant treatment prior to endoscopic dilatation in refractory strictures.

Management of comorbidities during childhood

As mentioned above, parents are informed about the longitudinal follow-up program of our hospital. All children born with congenital anomalies – including EA – are routinely enrolled in this program. The structured set-up with standardized assessments at predetermined time points provides insight in the morbidities of children with EA and even more important, the longitudinal course as children grow older. Previous studies from our research group have taught us about esophageal dysmotility, GER, impaired motor function, reduced exercise capacity and airflow obstruction.[36-40] In Chapter 10, we evaluated several of those comorbidities – assessed routinely within the standardized infrastructure of the long-term follow-up program and outlined as clinical outcomes – in a cohort of 8-year-old children born with anatomical foregut anomalies including EA. Overall, 86% of the included children scored below normal for at least one clinical outcome. Looking specifically at children with EA in this

chapter, 27% of the children scored at least one standard deviation below normal for motor function, 46% for maximum exercise capacity, and 40% for lung function. Twenty-five percent of the children with EA used daily medication.

One can imagine that such comorbidities contribute to the burden of disease for these children. Patient-reported outcome measures (PROMs) can be used to evaluate if HS and/or QoL is indeed affected. Interestingly, in Chapter 10, only behavioral problems reported by parents were associated with lower self-reported HS, and no associations were found with self-reported QoL. When we compare the HS and QoL of children with EA with those of healthy children, as illustrated in Chapter 9, self-reported HS was below normal for boys and girls at 8 years old but normalized at 12 years. Self-reported QoL was below normal for girls at 8 and 12 years old but normal for boys at both ages. HS improved significantly between 8 and 12 years – possibly explained by an improvement of their clinical condition over time – while QoL declined. Since QoL was only below normal for girls and a declining QoL over time is a phenomenon also common in healthy teenagers as they get older,[41, 42] one could wonder whether children with EA are actually burdened by their disease.

Besides, as emphasized in this thesis, it is important to consider HS and QoL as two separate concepts. Where HS describes a person's functioning in daily life, QoL describes how they perceive their functioning and if they are bothered by potential limitations.[43, 44] Having comorbidities does not necessarily affect one's self-perceived QoL. It is possible that a child has certain comorbidities – for example reduced exercise capacity – but is not bothered by this. The question which additional burden EA brings to the daily life of these children is strengthened by the relatively high QoL scores presented in Chapter 9. On the other hand, the PROMs currently used in the longitudinal follow-up program in our hospital – and presented in this chapter – are generic questionnaires. Condition-specific instruments have been shown to be more sensitive in detecting clinical morbidities[45] and might provide new insights.

This piqued our interest in the EA-QOL© questionnaire: a Swedish-German questionnaire to measure health-related QoL in children with EA.[46] The results of translation and validation of this questionnaire in the Dutch language are presented in Chapter 11. Interestingly, Dutch children and their parents reported higher EA-QOL© scores than did children and their parents in Sweden, Germany and Turkey.[47, 48] Moreover, we found a strong correlation between EA-QOL© scores and simultaneously obtained PedsQL scores – as was found in previous validation studies in Sweden, Germany and Turkey. One could argue that this finding questions the added value of a condition-specific instrument, which we will discuss later on.

Although the validation process took place during the COVID-19 pandemic – which potentially has influenced the results – the higher EA-QOL© scores in our population could be due to cultural differences between countries, which has been acknowledged to affect one's health

perception.[49] Furthermore, differences in clinical presentation and follow-up care in different centers can affect a child's coping skills, which may result in differential item functioning (DIF). DIF means that items are perceived differently in subgroups of participants (i.e. living in different countries), which implies that an item could measure different aspects.[50] The results of the cognitive debriefing underline that questions can be interpreted differently between countries, which may require adaption of the EA-QOL© instrument. By comparing validation studies performed in different countries, items may be identified that are perceived differently across countries or cultures and thus are answered differently. Although such cross-cultural evaluation is essential, one may consider implementing the EA-QOL© questionnaire in its current form in the Dutch follow-up programs to evaluate its added value in clinical practice.

So, what does all of this imply for the follow-up of children with EA? How should we translate the findings in this thesis into the daily management of these children's comorbidities? First of all, we advocate standardized longitudinal follow-up programs. It provides unique insights in the morbidities and development of each individual patient that may require early tailor-made interventions. However, this brings us directly to another point of discussion: when is intervention needed? As debated in Chapter 10, the increasing number of patients living with a chronic condition and the resulting financial strain on the health care system, urges us to consider value-based health care.[51-53] The limited relationships between clinical outcomes and PROMs for HS and QoL make it important to routinely measure all three concepts simultaneously during follow-up. By means of this holistic approach, PROMs may be useful to prioritize treatment of comorbidities in relation to what matters most to a child. However, we should bear in mind that children might not always oversee the consequences of certain comorbidities. For example, reduced exercise capacity might not bother a child but can lead to physical inactivity and secondary morbidities such as obesity, hypertensions and diabetes at adult age. Therefore, PROMs may be useful to prioritize treatment but should never be leading. Even more, on the long road this same approach could help which assessments provide meaningful input in this process of clinical decision-making.

Recommendations:

- Standardized, longitudinal follow-up programs provide unique insights in the morbidities and development of each individual patient that may require early tailor-made interventions.
- Health status and quality of life, assessed through PROMs, are two different concepts that together reflect the burden of disease. This does not always correlate well with the clinical outcome. Therefore, all three should be measured simultaneously during follow-up.
- Considering value-based health care, treatment of comorbidities should be prioritized in relation to what matters most to the child.
- Cross-cultural evaluation – by comparing validation studies of a PROM performed in different countries – may identify items that are perceived differently across countries or cultures.

Management of health care in adulthood

First, let us discuss the endoscopic surveillance program that was initiated in 2013 in addition to the multidisciplinary follow-up program during childhood, to early detect BE and esophageal carcinoma. In Chapter 8, we evaluated the yield of surveillance during the first seven years of this program. BE was detected in 7% of the participating patients at a median age of 32 years – 4-times higher than in the general population[54] – which confirmed the need for surveillance as advocated in the current international guidelines.[55] Four new cases of BE were detected during surveillance, while initial endoscopic screening was normal in two of them. Moreover, we diagnosed two new cases of esophageal carcinoma in patients who were not under surveillance. These observations underline the importance of standardized endoscopic surveillance for all adults born with EA.

Currently, the interval in our surveillance program is three or five years, depending on the patient's age. Based on the findings in Chapter 8, we have adjusted the intervals of our surveillance program (see Figure 1 of Chapter 8). We consider it justified to start screening at the age of 20 years (instead of 17 years) with a surveillance interval of 10 years (instead of 5 years) up to the age of 40 years. After the age of 40 years, we still advise intervals of 5 years due to the observed increased incidence of both esophageal squamous cell carcinoma and esophageal adenocarcinoma in adults with EA from the age of 40 years.[7] Furthermore, to detect dysplasia and early esophageal squamous cell carcinoma, we recommend to perform chromoendoscopy with Lugol's staining in patients ≥30 years (instead of ≥25 years) and in patients who have been previously curatively treated for esophageal cancer.

Nevertheless, we should bear in mind the balance between the harm and benefit of these repeated endoscopies. As we learned from the EA focus groups in Chapter 7, the invitation

for a surveillance endoscopy commonly exacerbates their psychological distress. This phenomenon can also be found in BE patients who are under surveillance, and show elevated anxiety levels prior to the surveillance endoscopy.[56] Based on findings from the focus groups, our research group currently investigates psychological distress around the surveillance endoscopies in adults with EA. Potential side effects and complications of an upper endoscopy include a sore throat, feeling bloated, bleeding or a perforation of the esophagus. Both the psychological distress and complication rate should be included in a harm-benefit analysis.

Another factor is the cost-effectiveness. With respect to our earlier statement about the significance of value-based health care for chronic conditions, we cannot ignore the costs that frequent endoscopies entail. The direct costs of an upper endoscopy are €471, or €848 when including the appointments at the outpatient clinic.[57] Indirect costs include work absence and travel expenses for the patient. The threshold for an intervention to be considered cost-effective is $100,000 per quality-adjusted life years (QALY) in the United States, and $50,000 per QALY in most other countries.[58] In the Netherlands, the willingness-to-pay threshold depends on the burden of disease – a calculation of multiple factors, ranging from 0.1-1.0 – and varies from €20,000-€80,000 per QALY.[59] A review on the cost-effectiveness of screening and surveillance of BE patients supported repeated endoscopies.[60] However, the screening studies embedded in this review only included older patients, or patients in whom BE was already present.[61, 62] Cost-effectiveness analyses of screening and surveillance in adults born with EA are currently lacking.

Third, each patient's personal (epi)genetic risk factors should be taken into account. In Chapter 3, we tried to identify EA patients at risk for developing BE. It appeared that those who had developed BE had a higher polygenic risk score than patients with BE who were not born with EA, which suggests a genetic susceptibility. Moreover, the increased induction of inflammatory processes upon acid exposure hints at an increased susceptibility to GER too. Nevertheless, the polygenic risk score was only slightly elevated and calculated from multiple existing single nucleotide polymorphisms that have been associated with BE or esophageal carcinoma in previous literature, though not with the combination of BE and EA specifically. The same applies to clinical risk factors used to select people from the general population for endoscopic screening, such as male sex, age ≥50 years, Caucasian race, presence of central obesity (waist circumference >102 cm or waist-hip ratio >0.9), history of tobacco smoking, a family history of BE or esophageal carcinoma, hiatus hernia and GER[63-65]). Though we found an association between age and history of GER with BE development in patients with EA specifically in Chapter 8, the association was only slightly significant. To confirm both the (epi) genetic and the clinical risk factors, studies in large cohorts of patients – such as the CLEAR consortium – are needed.

Altogether, the results from this thesis could form a next step towards a tailor-made surveillance strategy. Future research should address the different aspects of the harm-benefit balance in-depth. Ideally, this would lead to an algorithm that includes all these factors, through which to decide which patients should or should not be selected for endoscopic surveillance. In theory, the benefit of preventing the development of esophageal carcinoma should outweigh the potential harm of psychological distress, side effects, risk of complications and costs. The balance could be influenced by personal risk factors, the presence of which reflects a general predisposition, but can shift the fulcrum to the left, making endoscopic screening even more important in that specific patient.

Although endoscopic surveillance is important in the follow-up of adults born with EA, they may benefit from a more patient-centered and multidisciplinary approach. PROMs are particularly helpful in identifying, prioritizing and monitoring health problems, and enhance shared decision-making and patient-professional communication.[66] To complement the multidisciplinary approach, we developed the 'Specific Quality of life in Esophageal atresia Adults' (SQEA) questionnaire (Chapter 12). As we discussed in Chapter 12, the SQEA questionnaire captures all domains of the International Classification of Functioning, Disability and Health – a framework of the World Health Organization to classify the consequences of conditions (see Figure 2).[67] This instrument is suitable as a signaling tool, enabling clinicians to recognize patients that require intervention. Implementing the SQEA questionnaire in follow-up creates the possibility of longitudinal standardized assessment of QoL of EA adults. This will provide insight in the health care problems these patients encounter in daily life, on all domains (Chapter 7).

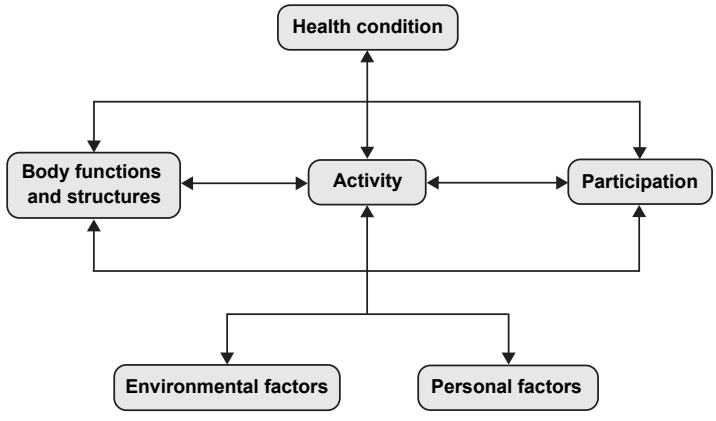

Figure 2. The World Health Organization International Classification of Functioning, Disability and Health[67]

Moreover, as mentioned earlier, in 2019 a screening program for pulmonary abnormalities was added. Many adults with EA suffer from respiratory complaints, such as a barky cough, recurrent lower respiratory tract infections, wheezing or shortness of breath.[68-70] As respiratory symptoms have occurred since early childhood, adults born with EA may perceive these symptoms as normal. Insufficient treatment may lead to pulmonary abnormalities diagnosed at a later stage. In addition, general practitioners often do not recognize pulmonary complaints as a long-term comorbidity of EA (Chapter 7). Frequent recurrent pneumonia, due to aspiration of GER or sputum retention due to inefficient clearance by tracheomalacia, can cause irreversible damage to the lung parenchyma, and lead to bronchiectasis (Appendix).[69]

In our hospital, pulmonologists are involved from the beginning. During the first admission of a neonate, they are consulted in case of severe choking incidents, atelectasis or lower respiratory tract infections. Throughout the child's childhood, they are consulted if a child experiences two or more lower respiratory tract infections in the first four years of life, or more than once a year. At the standardized follow-up moment at the age of 5 years old, they are consulted in case of chronic cough, dyspnea d'effort, impaired exercise tolerance or persistent tachydyspnea. At the standardized follow-up moment at the age of 8 years old, each child undergoes maximum exercise capacity testing, lung function testing and a chest computer tomography (CT) scan. In addition, the pediatric pulmonologist examines the child, irrespective of potential pulmonary complaints. A bronchoscopy is performed only on indication.

The adult pulmonary screening program includes lung function testing and a chest CT scan prior to a consultation with a pulmonologist. In some cases, further investigation is needed, for example assessment of bronchial responsiveness to rule out asthma. Depending on the test results, patient history and physical examination, patients are taught how to evacuate sputum, or are treated with antibiotics or inhalation medication. The interval between check-ups is set on the basis of the findings, in consultation with the patient. To our knowledge, this is currently the only prospectively and routinely offered pulmonary screening program for adults born with EA worldwide. All findings are documented and in time will give us insight in the long-term pulmonary comorbidities of EA.

Nevertheless, more is needed to accomplish a multidisciplinary approach. Chapter 7 mapped the medical and psychosocial needs of adults born with EA. Besides gastrointestinal and pulmonary problems, patients face problems with mental health, social and economic participation, and the coordination of the different follow-up appointments in the hospital. Low-threshold referral to a psychologist should be made possible in case of any signs of post-traumatic stress disorder, such as re-experiencing traumatic events, avoiding certain situations, or fear for hospital visits or medical procedures.[71] The focus group interviews revealed that some female patients throughout the entire pregnancy had worried about

passing on EA to the child. A clinical geneticist should be involved to counsel patients with an active child wish at fertile age, preferably before pregnancy. In our institution, genetic counselling is offered to patients at 18 years of age, as part of the transition to adult health care. However, since most adolescents do not have an active child wish at that time, genetic counselling is also integrated in preconception care, offered by the gynecologist to future parents. Preconception care in the general population in the form of individual consultations is advocated by the Dutch Health Council.[72] This is even more important for adults born with EA who suffer from concomitant cardiac of pulmonary problems. Patients who struggle with social and economic participation benefit from a joint effort of the medical staff and the patient support association to offer coping mechanisms. Providing proper information material and helping patients to explain their condition to, for example, friends and coworkers is essential.

This list of different specialties and collaborations underlines the need for a patient-centered, multidisciplinary approach. In all stages of life, a dedicated case manager should be appointed, who can also facilitate the transition of pediatric to adult health care. A tailor-made follow-up program that limits the burden of multiple hospital visits is important.

Recommendations:

- Standardized endoscopic surveillance should be offered to adults born with esophageal atresia, starting at the age of 20 years with a surveillance interval of 10 years up in patients <40 years old and of 5 years in patients ≥40 years old.
- Future research should investigate the harm-benefit balance of endoscopic surveillance, including psychological distress and complications of the endoscopy, and a cost-effectiveness analysis. This could potentially lead to an algorithm that can select patients for surveillance.
- All adults born with esophageal atresia should be offered pulmonary screening.
- A multidisciplinary approach is needed in the follow-up of adults born with esophageal atresia, including a gastroenterologist, pulmonologist, psychologist, clinical geneticist, gynecologist and the patient support association.
- The condition-specific 'Specific Quality of life in Esophageal atresia Adults' (SQEA) questionnaire is suitable as a signaling tool. Implementation in follow-up creates the possibility for longitudinal standardized assessment of the quality of life of adults born with esophageal atresia.

The added value of condition-specific PROMs

As we mentioned earlier in this chapter, condition-specific instruments are more sensitive to the clinical characteristics of a disease.[45] However, one of the downsides of using condition-specific instruments is that different conditions or diverse populations cannot be compared. Therefore, most studies asses a combination of specific and generic instruments. Moreover, it seems that the added value of responsiveness differs per condition and in some cases is even negligible.[73-76] Optimizing health care implies reduction of the burden for the patient as well. Earlier, we discussed the prioritization of treatment of comorbidities – taking into account what matters to the patient – but one could state that this also applies to the assessment patients fill out.

The strong correlation we found between the condition-specific EA-QOL© questionnaire and the generic PedsQL questionnaire (Chapter 11) revived this discussion. For the SQEA questionnaire we found a strong correlation with the Gastrointestinal Quality of Life Index (GIQLI) questionnaire and the St. George Respiratory Questionnaire (SGRQ) questionnaire, which cover gastrointestinal and pulmonary symptoms, but a weaker correlation with the RAND-36, which focusses more on general health and participation in daily life (Chapter 12). Recently, the usefulness of PROMs for chronic diseases was questioned.[77] The authors of that opinion paper considered PROMs time consuming and non-contributing to the doctor's visit. However, when accurately anticipating on the outcomes of a PROM, especially condition-specific instruments can be most useful as a signaling tool. Combining multiple aspects of life in one questionnaire lowers the burden for the patient, but provides the health care professional with the needed information to intervene if necessary. A similar method (KLIK: '*Kwaliteit van leven in kaart*') that uses PROMs to render consultations more efficient, has proved helpful for many patient populations.[78]

Recommendations:

- Condition-specific PROMs can be useful as a signaling tool to maximize the efficiency of a consultation with a health care professional.

The challenge of cultural differences in rare diseases

We already briefly discussed the consequences of cultural differences on QoL in different countries. These differences, together with the heterogeneity and small sample sizes in rare diseases such as EA, are important to bear in mind when working with condition-specific PROMs. In Chapter 11, we mentioned the possibility of computer adaptive testing for administering PROMs. Computer adaptive testing would create the possibility to customize a questionnaire to an individual's clinical situation by using skip patterns.[79]. It has been designed to dynamically administer items from a calibrated bank, based on a subject's prior responses, in order to provide the best information.[80] Item banks for physical, mental and

social health in adult and pediatric populations already exist.[81] When this could be established for condition-specific items as well, this would not only allow instruments to be personalized to an individual patient, but also create possibilities to combine generic and condition-specific items. Items that tend to be often selected could form a baseline questionnaire, with supplemental questions if a patient reports certain complaints.[80] Ideally, this way the instrument would fit perfectly to the patient, but comparison of items between conditions of populations would remain possible. Combining the validation results obtained in different countries into an item-response theory model, with the addition of missing topics, could be the first step.

Apart from QoL, cultural differences are a challenging issue on its own in rare diseases. Formulating guidelines for the standardization of treatment, is dependent of international collaboration. For research purposes in rare diseases, too, large patients cohorts are needed, which can only be collected on an international level. The European Rare Disease Research Coordination and Support Action consortium (ERICA) is the overarching organization of all European reference networks.[82] Their aim is to create a platform that integrates the research and innovation capacity of all the reference networks. These international networks have made cultural differences more explicit. In fact, these differences are not only apparent between countries, but also within a nation due to centralization of health care. There is a key role for reference networks such as ERNICA to unravel these cross-cultural differences.[83] A starting point could be to cross-culturally evaluate the results of validation studies of the same PROM in multiple countries, for example the EA-QOL© questionnaire.

Recommendations:

- A potential solution to overcome cultural differences in PROMs could be computer adaptive testing. Future research should focus on creating an item bank by combining the validation results obtained in different countries into an item-response theory model.
- International networks should investigate cultural differences on a larger level through a multidisciplinary approach. A starting point could be to cross-culturally evaluate the results of validation studies of the same PROM in multiple countries.

International collaboration is key

Given the above, it goes without saying that international collaboration is essential to optimize health care of patients with EA, both in daily practice as for research. International networks are fundamental in rare diseases, and are well established for EA. Among others, the CLEAR consortium in the United States and Canada, and the Genetic Risk for Esophageal Atresia

(GREAT) consortium in Germany are well acknowledged internationally for their research efforts.[20, 84]

In 2013, the International Network on Esophageal Atresia (INoEA) was formed.[85] Starting as a working group of pediatric gastroenterologist and pediatric surgeons from the European Society for Pediatric Gastroenterology Hepatology and Nutrition (ESPGHAN) and the North American Society for Pediatric Gastroenterology, Hepatology and Nutrition (NASPGHAN), they formulated guidelines for the treatment of children with EA.[55] Since then, INoEA has organized international meetings every three years with the purpose to promote scientific knowledge and improved care in the field of EA. The survey in Chapter 4 included the participation of INoEA members.

Another international collaborative initiative is the European federation of the Esophageal ATresia Global Support Groups (EAT).[86] Patient and family support associations are herein unified. They work closely together with INoEA, with the aim to share knowledge, experience and resources.

In the Netherlands, the university hospitals in which patients with EA are treated have initiated the Dutch Consortium of Esophageal Atresia (DCEA) in 2016. Members of the Dutch patient support association '*Vereniging voor Ouderen en Kinderen met een Slokdarmafsluiting*' (VOKS) are represented in the DCEA.[87] The validation of the EA-QOL© and SQEA questionnaires (Chapters 11 and 12) was performed by the DCEA study group.

In 2016, the European Commission launched twenty-five European reference networks for a number of rare diseases. ERNICA focusses on congenital gastrointestinal diseases.[88] One of the five working groups within ERNICA focusses on the esophagus, with special attention to EA. In this network, patient support associations, too, are represented at an international level. ERNICA organizes consensus conferences on the management of patients with EA. So far, three consensus papers have been published on the diagnostics and operative management of EA, on long gap EA, and on the follow-up of these patients.[89-91]

So far, health care recommendations and guidelines are most often based on expert opinions and consensus meetings. Randomized controlled trials are rarely performed in this population, on account of which first level evidence for treatment and follow-up is scarce. With the STEPS-EA trial (Chapter 6), we started a unique multicenter project within ERNICA.

Recommendations:

- International collaboration is essential in rare diseases, both for clinical practice as to obtain high-level evidence in research.
- International patient cohorts should more often be combined to enlarge the sample size, enabling first-level research, which the current guidelines strongly demand.

Future perspectives

The research presented in this thesis contributes to the full multidisciplinary spectrum of health care for patients born with EA. It ranges from genetic profiling to endoscopic surveillance in adults and the burden of disease, combining the perspectives of professionals and patients or parents. All things considered, the small sample sizes in rare diseases remain a topic of attention. Most studies included a relatively large number of patients considering the disease prevalence, due to multicenter collaboration.

Despite the life-long scope of this thesis, not all health care needs throughout life could be covered. As shown in Figure 1, some topics have not been addressed, leaving a knowledge gap for future research. In addition to the general recommendations described throughout this chapter, the results reported in this thesis have raised new questions. The following recommendations can be made for future research:

- Future research on the origin of EA should focus on multifactorial models, including both genetic mechanical and environmental factors. Given the amount of previous research – many studies with small cohorts – this would only be useful in large cohorts that should be compiled within international networks.
- The management of anastomotic strictures should be standardized. A consensus meeting would allow the recommendations in this thesis to be adopted in clinical guidelines. Future research should evaluate the outcomes on a large scale, and make adjustments based on new insights if necessary.
- Prioritizing treatment of comorbidities in relation to the burden of disease from a child's perspective is in line with the trend of value-based health care. We should bear in mind, however, that children are still at a developing age and might not always oversee the consequences of certain comorbidities. Future research should investigate the added value of this approach in children born with EA, for example through cost-effectiveness analyses.
- International comparison of validation studies of the condition-specific EA-QOL© questionnaire in multiple countries should be performed to evaluate cultural differences between countries.

- The adjusted endoscopic surveillance intervals have recently been implemented in our clinic. In five to ten years, the yield of the surveillance program should again be evaluated, comparing the different intervals. If necessary, the program should be adjusted further.
- Ideally, the endoscopic surveillance program should be internationally rolled out. International networks would be a good set-up, allowing combining outcomes.
- The harm and benefit of the endoscopic surveillance program should be considered. In this respect, future research should map the psychological distress around endoscopy and any side effects and complications of the endoscopy, and include a cost-effectiveness analysis.
- In the future, ideally an algorithm can be generated including patient-specific risk factors as well, through which can be decided which patients should or should not be selected for endoscopic surveillance.
- After implementation of the condition-specific SQEA questionnaire in the follow up of adults born with EA, the longitudinal QoL of these patients should be evaluated.

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CHAPTER 14

Summary

The research described in this thesis aims to improve health care for patients born with esophageal atresia (EA). By enlightening both new genetic and clinical insights, this thesis enhances the management of this anatomical congenital anomaly. Moreover, we present new insights in the quality of life of these patients that may contribute to evaluation of management. In this chapter, the main findings and conclusions have been summarized.

Studying patients with a combination of foregut-derived anomalies, could lead to a better understanding of the etiology of EA. In **Chapter 2**, we investigated the genetic variation of 15 patients with both EA and infantile hypertrophic pyloric stenosis (IHPS) whose parents were unaffected, using exome sequencing and SNP genotyping combined with mouse transcriptome data of the developing foregut. Multiple rare inherited variants were detected in EA or IHPS disease genes, or in genes important in foregut development at the proper time points, but none of these could individually explain the etiology of these congenital anomalies. We proposed two multifactorial models to explain the combination of anomalies: a burden model and a slippery slope model. In both models, there is a balance between multiple high impact genetic, mechanical and environmental risk factors and protective mechanisms (see Figure 3 of Chapter 2). So, in the burden model the protective mechanisms outweigh the risk factors in healthy people, whereas in affected persons the risk factors have a more important impact (or influence) than the protective mechanisms. This may lead to one of more affected organ systems – or even intrauterine death. In a slippery slope model, the balance between risk factors and protective mechanisms is very difficult to disturb. But once the balance disrupted, mostly multiple organ systems are affected instead of just one.

In **Chapter 3**, we aimed to unravel the increased susceptibility of patients with EA to develop Barrett's esophagus (BE), since the prevalence of BE in patients with EA is four times higher (6.6% versus 1.6%) than in the general population, and onsets at a much younger mean age (34 versus 60 years). We compared risk loci for BE development and transcriptomes of 19 patients born with EA who have developed BE (EA/BE), 44 patients born with EA without BE (EA only), 10 patients with BE who were not born with EA (BE only) and 730 unaffected European controls. EA/BE patients had a higher polygenic risk score than BE only patients (3.24 versus 2.63), which indicated the contribution of risk loci to the increased prevalence of BE in patients with EA. Furthermore, pathways involved in inflammatory, stress response and oncological processes were disturbed when comparing EA/BE patients with BE only patients. These alterations were confirmed in in-vitro experiments in fibroblasts of patients with EA upon acid exposure. Our results suggest that both genetic susceptibility and an increased induction of inflammatory processes contribute to the earlier age of onset of BE in patients with EA.

Chapter 4 describes the results of an international survey about endoscopic dilatation treatment of pediatric anastomotic strictures. We analyzed the responses of 115 centers

from 32 countries worldwide. Most centers (68%) preferred to dilate an esophageal stricture by balloon dilatation with hydrostatic pressure. Seventeen percent of the centers preferred semi-rigid dilatation (known as bougienage), and 15% applied both techniques.

In 103 centers (90% of total), dilatation was performed by a pediatric gastroenterologist, and in 48 centers (42% of total) by a pediatric surgeon. Furthermore, in some centers dilatation was performed by an adult gastroenterologist (n=24) or adult surgeon (n=12). In the majority of centers, physicians of multiple disciplines were involved in the dilatation procedures. Overall there was a large variation in the endoscopic dilatation treatment, confirming the lack of consensus. Given the importance of harmonizing the treatment of children with rare diseases, we provided several recommendations to standardize the management of anastomotic strictures in children with EA:

- Selective approach: only perform dilatation procedures in symptomatic patients
- Standardize the dilatation technique within a center
- Insufflation of 60 seconds per diameter when using balloon dilatation
- Only insufflate the balloon with fluids: use a dilatation system that supports hydrostatic pressure
- Availability of fluoroscopy, in case of problematic guidewire insertion
- Expertise: a center which performs <10 dilatations in EA patients per year should refer these patients to an expert center

These recommendations were made based on literature, common practice and the current consensus in the field. However, many subjects are subject to further research.

In **Chapter 5** we presented our initial experience with intralesional steroid injections prior to endoscopic dilatation in six children with anastomotic strictures after corrective surgery for EA. In five of them, the stricture was resolved. No postoperative complications or symptoms of adrenal suppression were reported. We recommend to determine the effectiveness and safety of this treatment in a randomized controlled trial, which was further explored in Chapter 6.

Chapter 6 describes the study protocol for the STEPS-EA trial: an international, multicenter, randomized controlled trial on intralesional STEroid Injections to Prevent Refractory Strictures in patients with EA. The set-up of the study is discussed, including the three main objectives and outcome parameters:

1. Effectiveness: the effect of an intralesional steroid injection prior to balloon dilatation on the luminal esophageal diameter (esophagram) and the severity of proxy-reported dysphagia (questionnaire)

2. Safety: the systemic effects of a one-time intralesional steroid injection (physical growth by length and weight, and cortisol levels in hair samples)
3. Cost-effectiveness: the incremental costs to prevent a refractory stricture, taking into account both medical (chart review) and non-medical (questionnaire) costs

Health care needs of adults born with EA are different from those of children. **Chapter 7** describes the medical and psychosocial needs of adults born with EA and their family members, revealed through semi-structured focus group interviews with 15 adult patients with EA and 13 of their family members. The interviews were transcribed verbatim (word-for-word) and processed using computerized thematic analysis. This means that all transcripts were reviewed and marked with codes that cover basic elements like 'eating' or 'scars'. In total, 74 codes were identified, which could be classified into 20 overarching themes. The most important findings included the impact of gastrointestinal and pulmonary problems on patients' daily life, long-term emotional distress of patients and parents, and the need of a standardized multidisciplinary follow-up program during both child- and adulthood. We formulate health care recommendations suitable for daily practice of clinicians who treat adults with EA, e.g. counselling on esophageal dysmotility or post-traumatic stress disorders, and offering timely referral to a psychologist if necessary.

Endoscopic surveillance for BE in adults with EA is advocated, and in 2013 a standardized endoscopic screening and surveillance program for all patients ≥17 years old was initiated in our hospital. In **Chapter 8**, we assessed the yield of this surveillance program in 271 patients, who underwent a total of 391 endoscopies. BE was found in 7% of the patients at a median age of 32 years. The youngest patient with a clinically relevant BE was 20 years. Before the start of this surveillance program, our research group had described four cases of esophageal carcinoma in our cohort. Since then, during surveillance, four patients developed BE (no dysplastic or neoplastic progression), one 45-year-old woman with a colon interposition developed an adenoma with high-grade dysplasia, and two patients (55 and 66 years old, not under surveillance) were diagnosed with esophageal carcinoma. One of them had been curatively treated for esophageal carcinoma 13 years earlier. In this chapter, we provide recommendations to optimize the surveillance strategy. Our findings justify to start endoscopic screening at the age of 20 years with a surveillance interval of 10 years up to the age of 40 years and an interval of 5 years afterwards. Endoscopic surveillance seems also warranted in patients with EA who needed a bowel interposition and in those who survived esophageal carcinoma.

A patient's psychosocial well-being can be affected by the burden of disease, which can be evaluated through several concepts: generic health status, generic quality of life (QoL) and condition-specific QoL. Chapter 9 and 10 investigated generic instruments to measures these concepts, whereas Chapter 11 and 12 investigated condition-specific instruments

In **Chapter 9**, we longitudinally evaluated the health status (HS) and quality of life (QoL) of 110 children with EA at 8 and/or 12 years old using two generic patient-reported outcome measures (PROMs). We compared self-reports and parent proxy-reports with normative sex-specific data of healthy Dutch children. At 8 years, self-reported HS was below normal for both boys and girls. At 8 and 12 years, self-reported QoL was below normal for girls. Proxy-reported HS was normal at both ages, whereas proxy-reported QoL was below normal at 12 years for girls. Over time, HS improved while QoL tended to decline. This discrepancy confirms that HS and QoL are two different concepts, which should be measured concurrently during follow-up.

In **Chapter 10**, we assessed the relationship between of PROMs for HS and QoL with clinical outcomes, measured with standardized assessments of health and daily functioning. We performed multiple linear regression analyses between self-reported HS or QoL and different clinical outcome domains in 93 children born with EA, 114 children born with congenital diaphragmatic hernia and 13 children who had required neonatal surgery for congenital lung malformations. Though overall clinical outcome was favourable, 86% of all 220 children had below normal scores for at least one outcome domain. In children born with EA, lower cognition was significantly associated with lower HS. We concluded that generic PROMs and clinical outcome evaluations evaluate different aspects of a child's wellbeing. Therefore, we recommended to combine all aspects in a holistic follow-up approach aimed at optimizing value-based health care for these children.

It can be questioned whether generic PROMs are suitable for patients with rare diseases. In **Chapter 11**, we translated into Dutch a Swedish condition-specific PROM (EA-QOL© questionnaire) for the assessment of health-related QoL of children born with EA and evaluated its psychometric properties. First, cognitive debriefing interviews were held with 19 parents and 10 children born with EA, aged 2-7 (n=9) and 8-17 (n=10) years old. Subsequently, a field test was conducted in 247 children with EA in the same age categories. Despite good reliability and validity results, feasibility was poor due to a large number of missing values, which could be credited to the set-up of the questionnaire. Based on the feedback we received during the cognitive debriefing interviews, we instructed participants to omit questions that were deemed not applicable. Both cultural differences that affect the perception of a patient's problems and differences in long-term morbidities (e.g. physical growth or feeding difficulties) contribute to the heterogeneity of EA. Heterogeneity of health perception and of clinical course poses a well-known challenge for using PROMs in rare diseases. A potential solution for this could be computer-adaptive testing through which a questionnaire can be better customized to the individual patient. Cross-cultural evaluation of the validation results obtained in multiple countries should further explore this.

Chapter 12 describes the development of a condition-specific PROM – the SQEA ('Specific Quality of life in Esophageal atresia Adults') questionnaire – to measure health-related QoL in adults born with EA. The different phases of the developmental process are discussed. A pilot questionnaire was generated after thematic analysis of the focus group interviews described in Chapter 7, and filled out by 42 participants. The results of the pilot test led to item reduction, after which a concept questionnaire was formed, that was evaluated for validity and reliability in 447 participants. Overall, the SQEA questionnaire showed satisfactory feasibility, reliability and validity. Though associations with clinical outcomes need further investigation, it shows discriminative ability to detect the burden of disease. Therefore, the SQEA questionnaire is not only a valid tool to assess the health-related QoL in EA adults, but also an interesting instrument to use as a signalling tool in clinical practice, enabling clinicians to recognize more severely affected EA patients.

The General Discussion in **Chapter 13** addresses all research described in this thesis, in a broader perspective. It discusses the health care needs throughout life, from a professional and a patient or parent perspective. We make several recommendations for each stage of life, both for clinical practice and future research.

CHAPTER 15

Nederlandse samenvatting

Het onderzoek wat in dit proefschrift wordt beschreven heeft als streven de gezondheidszorg voor patiënten geboren met een slokdarmatresie te optimaliseren. De behandeling van deze aangeboren afwijking kan worden verbeterd door de nieuwe inzichten in genetische en klinische factoren die dit onderzoek heeft opgeleverd. Bovendien worden nieuwe inzichten in de kwaliteit van leven gepresenteerd, wat kan bijdragen aan de evaluatie van de behandeling. In dit hoofdstuk worden de belangrijkste bevindingen en conclusies samengevat.

Slokdarmatresie (slokdarmafsluiting) is een zeldzame aangeboren afwijking die voorkomt bij 1 op de 4000 levendgeborenen. Per jaar worden er in Nederland ongeveer 40 kinderen geboren met slokdarmatresie. De meeste van deze kinderen hebben tevens een verbinding tussen de slokdarm en de trachea (luchtpijp), een zogeheten tracheo-oesofageale fistel.

Sommige patiënten hebben afwijkingen aan meerdere organen die afkomstig zijn van de voordarm. Door deze patiënten intensiever te bestuderen, zouden we meer kunnen leren over het ontstaan van slokdarmatresie. In **Hoofdstuk 2** hebben we daarom de genetische variatie bestudeerd van 15 patiënten met zowel een slokdarmatresie als een pylorushypertrofie, van wie de ouders niet zijn aangedaan. We hebben de resultaten van *whole exome sequencing* en SNP genotypering van deze patiënten gecombineerd met transcriptoom data van de zich ontwikkelende voordarm van muizen. We hebben verschillende zeldzame, overgeërfde varianten gevonden in ziektegenen voor slokdarmatresie of pylorushypertrofie, of in genen die belangrijk zijn voor de voordarmontwikkeling op de juiste tijdspunten. Geen van deze varianten kon echter op zichzelf het ontstaan van slokdarmatresie verklaren. We stellen twee multifactoriële modellen voor die deze combinatie van afwijkingen zouden kunnen verklaren: een *burden model* en een *slippery slope model*. In beide modellen is er een evenwicht tussen meerdere genetische, mechanische en omgevingsfactoren en beschermende mechanismen (zie Figuur 3 in Hoofdstuk 2). In een *burden model* heeft ieder mens bepaalde risicofactoren, die bij gezonde mensen minder zwaar wegen dan de beschermende mechanismen. Bij patiënten met afwijkingen, wegen de risicofactoren zwaarder dan de beschermende mechanismen, wat leidt tot een of meerdere aangedane orgaansystemen, of zelfs tot intra-uteriene dood. In een *slippery slope model* is dit evenwicht tussen risicofactoren en beschermende mechanismen heel moeilijk te verstoren. Echter, indien de balans eenmaal is verstoord, zijn er meestal meerdere orgaansystemen aangetast in plaats van slechts één.

In **Hoofdstuk 3** hebben we getracht om een verklaring te vinden voor het feit dat bij patiënten geboren met een slokdarmatresie een Barrett slokdarm (ontsteking van de slokdarm) vier keer vaker voorkomt dan in de algemene bevolking (6.6% versus 1.6%), en ontstaat op veel jongere leeftijd (34 versus 60 jaar). We hebben risicogenen voor het ontwikkelen van een Barrett slokdarm en transcriptomen vergeleken tussen slokdarmatresiepatiënten met een Barrett slokdarm (n=19), slokdarmatresiepatiënten zonder een Barrett slokdarm (n=44), patiënten met een Barrett slokdarm zonder slokdarmatresie (n=10) en niet-aangedane Europese

controles (n=730). We vonden een hogere polygene risicoscore bij slokdarmatresiepatiënten met Barrett slokdarm in vergelijking met patiënten met een Barrett slokdarm zonder slokdarmatresie (3.24 versus 2.63), wat wijst op de bijdrage van risicogenen bij de verhoogde prevalentie van Barrett slokdarm onder slokdarmatresiepatiënten. Bovendien waren signaalwegen die betrokken zijn bij ontstekingsprocessen, stressreacties en oncologische processen verstoord bij slokdarmatresiepatiënten met een Barrett slokdarm in vergelijking met patiënten met een Barrett slokdarm zonder slokdarmatresie in de voorgeschiedenis. De veranderingen in deze signaalwegen werden bevestigd middels in-vitro experimenten in fibroblasten van slokdarmatresiepatiënten na blootstelling aan zuur. Onze resultaten suggereren daarom dat de jongere leeftijd waarop slokdarmatresiepatiënten een Barrett slokdarm ontwikkelen te maken heeft met een verhoogde genetische gevoeligheid en een versterkte ontstekingsreactie.

Hoofdstuk 4 beschrijft de resultaten van een internationale enquête naar de behandeling van anastomotische slokdarmstricturen bij kinderen door middel van endoscopische dilatatie. We hebben de reacties geanalyseerd van 115 medische centra uit 32 landen wereldwijd. Bij de meeste centra (68%) ging de voorkeur uit naar ballondilatatie met hydrostatische druk. Zeventien procent van de centra behandelt de strictuur bij voorkeur middels bougienage, en 15% gebruikt beide technieken.

In 103 centra (90% van het totaal) verrichte een kinder-maag-darm-lever-arts de dilataties, en in 48 centra (42% van het totaal) een kinderchirurg. In sommige centra verrichtte een maag-darm-leverarts (n=24) of een chirurg (n=12) de dilataties. In de meeste centra werden de dilataties verricht door verschillende specialisten. Over het algemeen varieerde de behandeling sterk, wat het huidige gebrek aan consensus over de optimale behandeling bevestigt. Het is belangrijk om de behandeling van kinderen met een zeldzame aandoening onderling overeen te stemmen, zodat kinderen overal hetzelfde behandeld worden. Derhalve doen wij in dit hoofdstuk verschillende aanbevelingen om de behandeling van anastomotische stricturen bij kinderen geboren met een slokdarmatresie te standaardiseren:

- Selectieve aanpak: dilateer stricturen alleen als een kind klachten heeft
- Standaardiseer de dilatatietechniek binnen één centrum
- Indien ballondilatatie wordt toegepast, insuffleer de ballon gedurende 60 seconden per diameter
- Insuffleer de ballon alleen met vloeistoffen: gebruik een dilatatiesysteem dat hydrostatische druk ondersteunt
- Mogelijkheid tot doorlichting, voor het geval dat er problemen zijn met het opvoeren van de voerdraad
- Expertise: indien een centrum <10 dilataties bij kinderen met slokdarmatresie per jaar uitvoert, moet er worden doorverwezen naar een expertcentrum

In **Hoofdstuk 5** hebben we onze eerste ervaringen gepresenteerd met het injecteren van intralesionale steroïden voorafgaand aan endoscopische dilatatie bij zes kinderen met een anastomotische strictuur na operatieve correctie van de slokdarmatresie. Bij vijf van hen werd met deze combinatie de strictuur opgeheven. Er werden geen postoperatieve complicaties of symptomen van bijnierschorsinsufficiëntie gemeld. Om een definitieve conclusie te trekken over de effectiviteit en veiligheid van deze behandeling raden we een gerandomiseerde gecontroleerde studie aan.

Hoofdstuk 6 omvat het onderzoeksprotocol voor de STEPS-EA studie: een internationale, multicenter, gerandomiseerde gecontroleerde studie naar intralesionale steroïdinjecties voorgaand aan de dilatatie ter voorkoming van refractaire stricturen van de anastomose bij kinderen geboren met een slokdarmatresie. De opzet van het onderzoek wordt besproken, inclusief de drie hoofddoelen en bijbehorende uitkomstparameters:

1. Effectiviteit: het effect van een intralesionale steroïdinjectie voorgaand aan de dilatatie op de luminale slokdarmdiameter (slikfoto) en de mate van oudergerapporteerde dysfagie (vragenlijst)
2. Veiligheid: de systemische effecten van een eenmalige intralesionale steroïdinjectie (groei door middel van lengte en gewicht, en cortisolspiegels in het haar)
3. Kosteneffectiviteit: de bijkomende kosten om een refractaire strictuur te voorkomen, waarbij we rekening houden met zowel medische (dossierevaluatie) als niet-medische (vragenlijst) kosten

De zorgbehoeften van volwassenen geboren met een slokdarmatresie verschillen van die van kinderen. **Hoofdstuk 7** beschrijft welke medische en psychosociale behoeften van volwassenen geboren met een slokdarmatresie en hun familieleden aan het licht zijn gekomen tijdens semigestructureerde focusgroepinterviews met 15 volwassen slokdarmatresiepatiënten en 13 van hun familieleden. De interviews zijn woord-voor-woord uitgeschreven en verwerkt middels thematische analyse. Dit betekent dat alle transcripten zijn gemarkeerd met codes zoals ‘eten’ of ‘littekens’. In totaal zijn 74 codes geïdentificeerd, ingedeeld in 20 overkoepelende thema’s. De belangrijkste bevindingen waren onder meer dat gastro-intestinale en pulmonale problemen impact hebben op het dagelijks leven van patiënten, dat patiënten en ouders last hebben van langdurige emotionele stress, en dat deelnemen aan een gestandaardiseerd multidisciplinair follow-up programma tijdens zowel de kindertijd als op volwassen leeftijd wenselijk is. We hebben een aantal aanbevelingen geformuleerd die zorgverleners van volwassenen geboren met een slokdarmatresie kunnen gebruiken in de dagelijkse praktijk, bijvoorbeeld om uitleg te geven over dismotiliteit van de slokdarm, of om alert te zijn op posttraumatisch stresssyndroom en indien nodig te verwijzen naar een psycholoog.

Endoscopische surveillance voor Barrett slokdarm wordt aanbevolen voor volwassen slokdarmatresiepatiënten vanaf 17 jaar en ouder. Sinds 2013 is er in ons ziekenhuis een gestandaardiseerd screenings- en surveillanceprogramma. In **Hoofdstuk 8** hebben we onderzocht wat de uitkomsten van dit surveillanceprogramma zijn bij 271 patiënten, die in totaal 391 endoscopieën ondergingen. Barrett slokdarm werd gevonden bij 7% van deze patiënten, op een mediane leeftijd van 32 jaar. De jongste patiënt met een klinisch relevante Barrett slokdarm was 20 jaar. Al voor de start van het surveillanceprogramma had onze onderzoeksgroep vier gevallen van slokdarmkanker in ons cohort beschreven. Sindsdien hebben vier patiënten onder surveillance een Barrett slokdarm (zonder dysplastische of neoplastische progressie) ontwikkeld, is er bij een 45-jarige vrouw met een coloninterponaat een adenoom met hooggradige dysplasie geconstateerd en is er bij twee patiënten (55 en 66 jaar oud, die niet onder surveillance waren) slokdarmcarcinoom gediagnosticeerd. Een van hen was 13 jaar geleden curatief behandeld voor slokdarmcarcinoom. We doen in dit hoofdstuk aanbevelingen om de surveillancestrategie te optimaliseren. Op basis van onze bevindingen achten wij het verantwoord om te starten met screening op de leeftijd van 20 jaar, met een interval van 10 jaar tot de leeftijd van 40 jaar en een interval van 5 jaar nadien. Endoscopische surveillance lijkt ook gerechtvaardigd bij patiënten met een coloninterponaat of bij patiënten die een slokdarmcarcinoom hebben overleefd.

De ziektelast die een patiënt ervaart kan zijn of haar psychosociaal welzijn beïnvloeden. In **Hoofdstuk 9** hebben we longitudinaal, op 8- en/of 12-jarige leeftijd, met behulp van twee generieke vragenlijsten de gezondheidsstatus en kwaliteit van leven geëvalueerd van 110 kinderen geboren met een slokdarmatresie. Hun zelfrapportages en ouderrapportages hebben we vergeleken met geslachtspecifieke normdata van gezonde Nederlandse kinderen. Op 8-jarige leeftijd was de zelfgerapporteerde gezondheidsstatus voor zowel jongens als meisjes lager dan normaal. Op de leeftijd van 8 en 12 jaar was de zelfgerapporteerde kwaliteit van leven voor meisjes lager dan normaal. De oudergerapporteerde gezondheidsstatus was normaal op beide leeftijden, terwijl de oudergerapporteerde kwaliteit van leven voor 12-jarige meisjes lager was dan normaal. Tussen 8 en 12 jaar verbeterde de gezondheidsstatus, terwijl de kwaliteit van leven af leek te nemen. Deze discrepantie bevestigt dat gezondheidsstatus en kwaliteit van leven twee verschillende concepten zijn, die gelijktijdig dienen te worden gemeten tijdens follow-up.

In **Hoofdstuk 10** hebben we de relatie onderzocht tussen de uitkomsten van generieke vragenlijsten voor gezondheidsstatus en kwaliteit van leven en klinische uitkomsten, gemeten met gestandaardiseerde beoordelingen van gezondheid en dagelijks functioneren. Regressieanalyses tussen zelfgerapporteerde gezondheidsstatus of kwaliteit van leven en verschillende domeinen met klinische uitkomsten zijn uitgevoerd voor in totaal 220 kinderen: 93 kinderen geboren met een slokdarmatresie, 114 kinderen geboren met een hernia diafragmatica en 13 kinderen met aangeboren longafwijkingen. Hoewel de klinische

uitkomsten over het algemeen gunstig waren, scoorde 86% van de 220 kinderen onder de norm voor ten minste één uitkomstdomein. Bij kinderen geboren met een slokdarmatresie was een lagere cognitie significant geassocieerd met een lagere gezondheidsstatus. We concludeerden dat vragenlijsten en klinische uitkomsten verschillende aspecten van het welzijn van een kind meten. We raden daarom aan om al deze aspecten te combineren in een holistische benadering tijdens follow-up, wat essentieel is voor het optimaliseren van waardegedreven zorg.

Men zou zich kunnen afvragen of generieke vragenlijsten geschikt zijn voor patiënten met zeldzame aandoeningen. In **Hoofdstuk 11** hebben we een ziektespecifieke vragenlijst (EA-QOL© vragenlijst) uit Zweden vertaald waarmee de kwaliteit van leven van kinderen geboren met een slokdarmatresie kan worden gemeten. De psychometrische eigenschappen van deze vertaalde vragenlijst hebben we onderzocht in de Nederlandse bevolking. Allereerst zijn cognitieve debriefing interviews gehouden met 19 ouders en 10 kinderen, in de leeftijd van 2-7 jaar (n=9) of 8-17 jaar (n=10) oud. Vervolgens is de vragenlijst onderzocht bij 247 kinderen geboren met een slokdarmatresie in dezelfde leeftijdscategorieën. Ondanks dat de betrouwbaarheid en validiteit van de vragenlijst in orde bleek, was de geschiktheid matig vanwege een groot aantal ontbrekende antwoorden. Dit kan worden toegeschreven aan de studieopzet. Op basis van de feedback die wij kregen op de vertaalde vragenlijst tijdens de cognitieve debriefing interviews hebben we deelnemers geïnstrueerd om een vraag over te slaan indien deze niet van toepassing was. Culturele verschillen kunnen van invloed zijn op hoe iemand potentiële problemen ervaart. Daarnaast kan de ernst van lange termijn comorbiditeiten, zoals groei of voedingsproblemen, verschillen tussen patiënten. Deze aspecten dragen bij aan de heterogeniteit van slokdarmatresie. Heterogeniteit van gezondheidsperceptie en van het klinisch beloop van een aandoening is een bekend probleem bij vragenlijsten voor patiënten met zeldzame afwijkingen. Een mogelijke oplossing zou computerondersteund toetsen kunnen zijn, waarmee een vragenlijst beter kan worden aangepast op de individuele patiënt. Door middel van cross-culturele evaluatie van de validatie resultaten die zijn gevonden in verschillende landen zou dit verder kunnen worden onderzocht.

Hoofdstuk 12 beschrijft de ontwikkeling van een ziektespecifieke vragenlijst voor de kwaliteit van leven van volwassen slokdarmatresiepatiënten: de SQEA ('Specific Quality of life in Esophageal atresia Adults') vragenlijst. De verschillende fasen van het ontwikkelingsproces worden besproken. Een pilotvragenlijst werd samengesteld na thematische analyse van de focusgroep interviews beschreven in Hoofdstuk 7, en ingevuld door 42 deelnemers. De resultaten hiervan resulteerden in itemreductie, waarna een conceptvragenlijst werd samengesteld. De conceptvragenlijst werd geëvalueerd op betrouwbaarheid en validiteit bij 447 patiënten. Over het algemeen toonde de SQEA vragenlijst een adequate haalbaarheid, betrouwbaarheid en validiteit. Hoewel de associatie met klinisch uitkomsten nog verder

onderzoek behoeft, toont de vragenlijst een onderscheidend vermogen in het detecteren van de ziektelast. Daarom is de SQEA vragenlijst niet alleen een valide instrument om de kwaliteit van leven van volwassenen geboren met een slokdarmatresie te meten, maar ook een interessant instrument om te gebruiken als signaleringsinstrument in de klinische praktijk, waardoor ernstiger aangedane patiënten eerder kunnen worden herkend.

De algemene discussie in **Hoofdstuk 13** behandelt al het onderzoek beschreven in dit proefschrift in een breder perspectief. De zorgbehoeften gedurende het gehele leven worden besproken van uit een professioneel en een patiënt- of ouderperspectief. We doen verschillende aanbevelingen voor iedere levensfase, zowel voor de klinische praktijk als voor toekomstig onderzoek.

APPENDICES

KLINISCHE LES

Slokdarm- en longafwijkingen op volwassen leeftijd

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ABSTRACT

Long-term consequences of esophageal atresia; esophageal and lung abnormalities

Esophageal atresia is a rare congenital anomaly. Due to increased survival rates, the population of adults born with this malformation is growing. These patients turn out to have an increased risk to develop Barrett's esophagus, esophageal carcinoma or lung abnormalities like bronchiectasis. This is illustrated by three cases: a 42-year-old man with an irresectable esophageal squamous cell carcinoma; a 23-year-old man with a Barrett's esophagus without any reflux complaints; and a 51-year-old women with a reflux esophagitis and extensive bronchiectasis due to a combination of gastroesophageal reflux with chronic aspiration and a reduced sputum clearance because of a history of tracheomalacia. It is important for healthcare providers to be aware of these risks and the possible absence of symptoms, in order to detect abnormalities at an early stage and improve quality of life of these patients.

KERNPUNTEN

- De prevalentie van slokdarmatresie is 1 op de 4000 levendgeborenen.
- Naar schatting zijn er in Nederland ruim 1000 volwassenen met operatief gecorrigeerde slokdarmatresie.
- De prevalentie van een Barrett-slokdarm onder volwassen patiënten met slokdarmatresie is viermaal zo hoog als in de algemene populatie.
- De prevalentie van een plaveiselcelcarcinoom van de slokdarm onder volwassen patiënten met slokdarmatresie is ruim 100 maal hoger dan in de algemene populatie.
- Zowel Barrett-slokdarm als slokdarmkanker komen op een jongere leeftijd voor dan in de algemene populatie.
- Recidiverende luchtweginfecties – al dan niet het gevolg van langdurige aspiratie bij gastro-oesofageale reflux of een verminderde sputumklaring door tracheomalacie – kunnen leiden tot irreversibele longschade in de vorm van bronchiëctasieën.
- In alle umc's in Nederland houdt een mdl-arts zich bezig met de zorg voor volwassen patiënten met slokdarmatresie in het kader van een landelijk screenings- en surveillanceprogramma.

Dames en Heren,

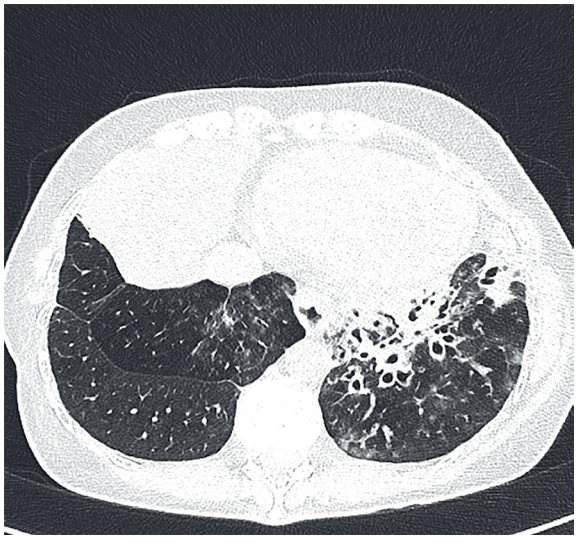
De overlevingskansen van pasgeborenen met slokdarmatresie zijn de afgelopen decennia sterk toegenomen, waardoor er tegenwoordig in Nederland een groeiende groep volwassenen is met operatief gecorrigeerde slokdarmatresie. Deze patiënten hebben een verhoogd risico op slokdarmafwijkingen, zoals een Barrett-slokdarm en een slokdarmcarcinoom, en longafwijkingen, zoals bronchiëctasieën. Omdat zij al vanaf de kinderleeftijd klachten hebben, beschouwen zij passageklachten, gastro-oesofageale refluxklachten en longklachten vaak niet als abnormaal. In dit artikel beschrijven wij 3 patiënten met operatief gecorrigeerde slokdarmatresie die op de volwassen leeftijd slokdarm- of longafwijkingen hadden.

Patiënt A werd geboren met een 'long gap'-slokdarmatresie, anusatresie, sacrale agenesie en een geringe lumbale scoliose. Na operatieve correctie van de anusatresie, werd op de leeftijd van 9 maanden de slokdarm operatief gecorrigeerd met een 'end-to-end'-anastomose. Op 37-jarige leeftijd meldde hij zich met passageklachten bij de mdl-arts. Hij gebruikte geen medicatie, rookte een half pakje sigaretten per dag en nuttigde alcohol in het weekend. De klachten werden veroorzaakt door een naadstenose, waarna de slokdarm meerdere malen werd gedilateerd. Histopathologisch onderzoek van slokdarmbiopten die tijdens de dilataties waren afgenomen, toonde inflammatie, maar geen tekenen van een maligniteit. In de jaren daarna bleven de passageklachten aanwezig. Op 42-jarige leeftijd onderging patiënt een gastroscopie vanwege toenemende dysfagie. Er bleek sprake te zijn van een irresectabel circulair plaveiselcelcarcinoom met lymfekliermetastasen (T4N2M0). De tumor reageerde goed op inductiechemotherapie, waarna patiënt chemoradiotherapie (maximale dosis: 50,4 Gy) kreeg met curatieve intentie. Ruim 10 jaar na de behandeling waren er geen tekenen van een recidief of metastasering.

Patiënt B, een 23-jarige man, reageerde op een oproep van de kinderchirurg om zijn slokdarm te laten controleren. Hij was geboren met slokdarmatresie met een tracheo-oesofageale fistel, die kort na de geboorte operatief gecorrigeerd werd. In zijn eerste levensjaar onderging hij tevens een Nissen-fundoplicatie (vanwege zeer ernstige gastro-oesofageale reflux) en een pyloromyotomie (vanwege pylorushypertrofie). Ook werd de slokdarm meerdere malen gedilateerd vanwege een naadstenose. Patiënt gaf aan dat hij alles kon eten en geen passage- of refluxklachten had. Hij gebruikte geen medicatie, rookte niet en had een stabiel, gezond gewicht. Bij gastroduodenoscopie werd echter een Barrett-slokdarm vastgesteld (C2M6 volgens de Prague C&M-criteria),[1] zonder dysplasie. Patiënt kreeg een maagzuurremmer voorgeschreven. Patiënt zal over 3 jaar worden opgeroepen voor gastroscopische controle van de Barrett-slokdarm.

Patiënt C, een 51-jarige vrouw, kreeg eveneens een oproep van haar vroegere kinderchirurg om haar slokdarm te laten controleren. Naar aanleiding van deze oproep had zij op eigen initiatief ook een afspraak gemaakt bij de longarts, omdat haar conditie was verslechterend. Patiënte

was geboren met slokdarmatresie met een tracheo-oesofageale fistel en tracheomalacie. Kort na de geboorte was de slokdarmatresie operatief gecorrigeerd. Als kind had zij veel last gehad van recidiverende pneumonie, waarvoor zij in totaal 14 keer opgenomen was geweest in het ziekenhuis. Na haar 18e verjaardag had zij geen controles meer gehad. Patiënte vertelde aan de longarts dat zij vaak longklachten had, waaronder overmatig sputum en benauwdheid, en koorts. Ze gebruikte geen medicatie en ze had in de afgelopen jaren geen antibiotica gebruikt. Ze was minder gaan sporten vanwege kortademigheid en vermoeidheid. Patiënte kon alles eten en had geen passage- of refluxklachten. Ze had nooit gerookt en dronk maximaal 2 glazen alcohol per week. Longfunctieonderzoek toonde een obstructief én restrictief gestoorde longfunctie, zonder reversibiliteit. Op een CT-scan van de thorax waren uitgebreide bronchiëctasieën en infiltratieve afwijkingen zichtbaar in de linker onderkwab (zie Figuur 1). Bij gastroscopie werd refluxoesofagitis vastgesteld (graad A volgens de Los Angeles-criteria),[2] die vermoedelijk het gevolg was van langdurige aspiratie bij gastro-oesofageale reflux in combinatie met een verminderde sputumklaring door tracheomalacie. Patiënte kreeg een maagzuurremmer voorgeschreven, evenals vernevelingen met hypertoon zout om het sputum te mobiliseren en een onderhoudsbehandeling met azitromycine. Patiënten zal frequent op controle komen bij de longarts en over 3 jaar opnieuw een gastroscopie ondergaan, geheel volgens het protocol van het surveillanceprogramma voor volwassenen met slokdarmatresie.[3]



Figuur 1. Transversale hogeresolutie-CT-scan van de thorax van patiënt C met bronchiëctasieën in de linkeronderkwab. De CT-scan toont ook een tree-in-bud fenomeen (kleine vertakte structuren met lokale verdikkingen), wat kenmerkend is voor ontsteking in de kleinere luchtwegen.

Beschouwing

Slokdarmatresie is een zeldzame aangeboren afwijking die voorkomt bij 1 op de 4000 levendgeborenen.[4] Per jaar worden er in Nederland ongeveer 40 kinderen geboren met slokdarmatresie. Naar schatting zijn er in Nederland ruim 1000 volwassenen met operatief gecorrigeerde slokdarmatresie. Uit recente literatuur blijkt dat volwassenen met slokdarmatresie nog diverse klachten hebben. Zo ervaart de helft van hen passageklachten, heeft een derde van de patiënten last van zuurbranden en heeft meer dan de helft recidiverende luchtweginfecties.[3, 5]

Slokdarmafwijkingen

Een groot deel van de patiënten met slokdarmatresie heeft levenslang gastro-oesofageale reflux. Niet bij iedere patiënt uit die reflux zich in klachten van zuurbranden, zoals bij patiënt C het geval was. Hoewel volwassenen zonder slokdarmatresie ook gastro-oesofageale reflux kunnen hebben zonder klachten van zuurbranden te hebben, is het mogelijk dat patiënten met slokdarmatresie hun klachten anders ervaren. Klachten die langdurig of zelfs al vanaf de kinderleeftijd aanwezig zijn, worden mogelijk minder intens waargenomen door de patiënt.

In de internationale literatuur wordt beschreven dat patiënten met slokdarmatresie een verhoogd risico hebben op slokdarmafwijkingen, zoals een Barrett-slokdarm en een slokdarmcarcinoom. Recentelijk is dit ook beschreven voor de Nederlandse populatie.[3] Opvallend is dat niet alleen een Barrett-slokdarm – een voorstadium van het adenocarcinoom van de slokdarm – vaker voorkomt bij patiënten met slokdarmatresie, maar ook het plaveiselcelcarcinoom.[6] Van de ongeveer 290 patiënten hadden 4 patiënten op een mediane leeftijd van 46,5 jaar een (slok)darmcarcinoom ontwikkeld: 1 patiënt met een adenocarcinoom van het coloninterponaat en 3 patiënten met een plaveiselcelcarcinoom van de slokdarm, van wie 1 patiënt een tweede plaveiselcelcarcinoom van de proximale slokdarm ontwikkelde, 15 jaar na een in opzet curatieve behandeling van de eerste tumor.

Patiënten die geboren zijn met slokdarmatresie hebben vaak al vanaf de kinderleeftijd een gestoorde voedselpassage (dysfagie), die zij niet als afwijkend beschouwen. Slokdarmtumoren worden bij patiënten met slokdarmatresie veelal pas in een vergevorderd stadium worden gediagnosticeerd, vermoedelijk omdat zij zich laat melden met klachten, zoals bij patiënt A het geval was. Daarom wordt in de huidige Europese richtlijn aanbevolen om volwassenen met slokdarmatresie elke 5-10 jaar routinematig te screenen op slokdarmafwijkingen met endoscopie, en om daarbij biopten af te nemen in 4 kwadranten op de gastro-oesofageale overgang en ter hoogte van de anastomose.[3, 7, 8]

Longafwijkingen

Recidiverende luchtweginfecties zijn een bekend probleem bij kinderen met slokdarmatresie, maar ook op de volwassen leeftijd treden nog frequent luchtwegproblemen op.

Veelvoorkomende luchtwegklachten zijn: (blaffend) hoesten, luchtweginfecties, een piepende ademhaling en benauwdheid. Bij longfunctieonderzoek kunnen een luchtwegobstructie, een verlaagde totale longcapaciteit, of een combinatie hiervan, gevonden worden. In het Erasmus MC worden deze longfunctieafwijkingen momenteel nader geanalyseerd om te kunnen differentiëren tussen verschillende oorzaken.[5, 9, 10] Vanwege het chronische karakter van de luchtwegklachten zullen patiënten niet snel zelf aan de bel trekken, waardoor longafwijkingen pas laat worden gediagnosticeerd, zoals bij patiënt C het geval was. Recidiverende luchtweginfecties – al dan niet het gevolg van langdurige aspiratie bij gastro-oesofageale reflux of een verminderde sputumklaring door tracheomalacie – kunnen leiden tot irreversibele longschade in de vorm van bronchiëctasieën.

Screening en surveillance

Slokdarmafwijkingen

In 2013 is in het Erasmus MC begonnen met een screenings- en surveillanceprogramma voor de eigen populatie van volwassen patiënten met slokdarmatresie. Vanwege het verhoogde risico op slokdarmafwijkingen ondergaan patiënten < 30 jaar elke 5 jaar een gastroscopie, en patiënten ≥ 30 jaar elke 3 jaar.[3] Momenteel nemen bijna 300 patiënten deel aan dit programma.

Uit ons onderzoek blijkt dat de prevalentie van een Barrett-slokdarm onder deze patiënten 4 maal zo hoog was als in de algemene populatie (6.6% vs. 1.3-1.6%) en dat zij een Barrett-slokdarm ontwikkelden op een mediane leeftijd van 32 jaar. Ook bleek dat slokdarmkanker vaker voorkwam bij volwassenen met slokdarmatresie, vergeleken met leeftijdsgenoten zonder slokdarmatresie. De prevalentie van het plaveiselcelcarcinoom van de slokdarm was ruim 100 maal hoger dan in de algemene populatie (0.7% vs. 0.006%).[3] Ook vonden wij dat een antirefluxoperatie, zoals een Nissen-fundoplicatie, niet beschermt tegen intestinale metaplasie in de slokdarm op de volwassen leeftijd. De correlatie tussen gastro-oesofageale refluxklachten enerzijds en endoscopische en histopathologische bevindingen anderzijds was zwak.[3] Bij patiënten zonder slokdarmatresie is dysfagie mogelijk een alarmsymptoom van een slokdarmtumor, maar in onze patiëntenpopulatie was dysfagie een slechte voorspeller voor de aanwezigheid van een carcinoom.

In 2019 is het screenings- en surveillanceprogramma voor volwassenen met slokdarmatresie in samenwerking met het Dutch Consortium for Esophageal Atresia in heel Nederland uitgerold. In alle umc's houdt een mdl-arts zich bezig met de zorg voor deze patiënten. Het programma bereikt echter niet alle volwassenen met slokdarmatresie, omdat alleen de eigen, bekende patiënten op vaste momenten een uitnodiging krijgen.

Longafwijkingen

In 2019 is in het Erasmus MC begonnen met een gestandaardiseerde screening op longafwijkingen bij volwassenen met slokdarmatresie. Voorafgaand aan het eerste consult wordt longfunctieonderzoek en CT-onderzoek van de thorax uitgevoerd. Tijdens het eerste consult wordt op basis van de onderzoeksresultaten, de anamnese en het lichamelijk onderzoek besloten of nader aanvullend onderzoek nodig is, bijvoorbeeld een histamineprovocatietest om astma uit te sluiten. Afhankelijk van de bevindingen krijgen patiënten technieken aangeleerd om sputum op te hoesten, worden zij behandeld met bijvoorbeeld vernevelingen, antibiotica of inhalatiemedicatie, en worden vervolgafspraken gemaakt.

Wat had er anders gekund?

Bij patiënt A werd enkele jaren vóór aanvang van het screenings- en surveillanceprogramma een irresectabele slokdarmtumor vastgesteld, 5 jaar nadat hij zich bij de mdl-arts had gemeld met passageklachten. Mogelijk had de tumor in een eerder stadium ontdekt kunnen worden, als surveillance had plaatsgevonden.

Dames en Heren, patiënten met slokdarmatresie hebben een verhoogd risico op slokdarm- en longafwijkingen op relatief jonge leeftijd. Bewustwording van deze risico's en alertheid op klachten dragen bij aan een vroege detectie van ziekte en daarmee aan een betere kwaliteit van leven.

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PHD PORTFOLIO

Name	Chantal ten Kate
Department	Pediatric Surgery and Intensive Care
PhD period	2017-2021
Promotor	Prof. dr. R.M.H. Wijnen
	Prof. dr. M.C.W. Spaander
Copromotors	Dr. H. IJsselstijn
	Dr. E. Brosens

	Year	**Workload** (ECTS)
General courses		
Systematic Literature Retrieval in PubMed and Other Databases & EndNote	2017	1.0 ECTS
Integrity in Scientific Research	2017	0.3 ECTS
Biostatistical methods I: Basic Principles	2017	5.7 ECTS
BROK (Basiscursus Regelgeving Klinisch Onderzoek)	2017	1.5 ECTS
Photoshop and Illustrator CS6	2018	0.3 ECTS
Open Clinica, LimeSurvey and GemsTracker	2017-2018	1.0 ECTS
CPO Course	2019	0.3 ECTS
Schientific English Writing	2019	3.0 ECTS
Specific courses		
Basic Genetics Course	2017	3.0 ECTS
Course on Molecular Diagnostics XI	2017	1.0 ECTS
Epigenetic Regulation in Health and Disease	2018	0.8 ECTS
CLC Genomics Workbench 12 and Ingenuity Variant Analysis (IVA) & Ingenuity Pathway Analysis (IPA)	2019	0.6 ECTS
Safely working in the laboratory	2019	0.3 ECTS
Clinical courses		
Basis Life Support (BLS) & Pediatric Basis Life Support (PBLS)	2017	0.3 ECTS
Teaching courses		
“Omgaan met groepen voor tutoren”	2017	0.3 ECTS
“Coachen van toekomstige Erasmusartsen: Basis”	2018	0.3 ECTS
Teach the Teacher I	2018	0.6 ECTS
Deel van Basiskwalificatie Onderwijs (BKO)	2018	1.0 ECTS
Symposia and workshops		
Member’s day VOKS	2017-2019	0.9 ECTS
Annual MGC Symposium	2018	0.3 ECTS
Erasmus MC PhD Day	2017	0.3 ECTS
MGC Workshop Texel (oral n=1)	2018	1.0 ECTS
Sophia Research Day (oral n=1)	2018-2019	1.3 ECTS
National conferences		
Digestive Disease Days NVGE, Veldhoven (oral n=1)	2018	1.0 ECTS
Digestive Disease Days NVGE, online (webinar n=1)	2020	1.0 ECTS
Digestive Disease Days NVGE, online (webinar n=1)	2021	1.0 ECTS

PhD portfolio (*continued*)	**Year**	**Workload** (ECTS)
International conferences		
ESPGHAN 51st Annual Meeting, Genève (poster n=1)	2018	1.0 ECTS
EUPSA 19th Annual Congress, Paris (oral n=1, poster n=2)	2018	2.0 ECTS
ESPGHAN 52st Annual Meeting, Glasgow (poster n=2)	2019	1.0 ECTS
EUPSA 20th Annual Congress, Belgrado (poster n=3)	2019	1.0 ECTS
International Conference on Esophageal Atresia, Rome (oral n=3)	2019	3.0 ECTS
1st International Pediatric Chest Conference, Rotterdam	2019	0.6 ECTS
Teaching activities		
Tutor 1st year medical students ('tutoraat')	2017-2019	3.0 ECTS
Coaching medical students 1st – 3rd year (n=7)	2018-2020	1.5 ECTS
Supervising medical research students (n=3)	2017-2021	1.0 ECTS
Supervising master thesis Hilde Koese	2021	1.0 ECTS
Organizational activities		
Evening symposium for adult patients with esophageal atresia	2019	1.0 ECTS
Other		
Development information brochure for adult patients with esophageal atresia in collaboration with VOKS	2019	1.0 ECTS
Research meeting Department of Pediatric Surgery (oral n=3), monthly	2017-2021	1.0 ECTS
Research meeting Department of Surgery (oral n=2), monthly	2017-2021	1.0 ECTS
Research meeting CHIL (oral n=1), monthly	2017-2021	1.0 ECTS
Research meeting Department of Clinical Genetics (oral n=3), weekly	2017-2021	1.0 ECTS
Research meeting Gastrointestinal Genetics research group prof. Hofstra, weekly	2017-2021	1.0 ECTS

ECTS = European Credit Transfer and Accumulation System
1 EC represents 28 hours

ABOUT THE AUTHOR

Chantal Annabel ten Kate was born on January 4th 1992 in 's-Gravenzande, the Netherlands, were she grew up with her parents and younger sister. In 2010, she completed her gymnasium degree at Interconfessionele Scholengroep Westland Gasthuislaan in 's-Gravenzande, after which she started her medical training at the University of Rotterdam. During medical school, she participated as a student researcher in multiple studies and worked as a side job at the Orthopedium in Delft.

Throughout medical school her interest in both research and surgery grew. After obtaining her medical degree in 2017, Chantal started as a PhD candidate at the Department of Pediatric Surgery at the Sophia Children's Hospital in Rotterdam (prof. dr. R.M.W. Wijnen). She combined her research with her clinical work as a resident not in training. Her research focused on optimizing health care for patients born with esophageal atresia, and was in collaboration with the Departments of Gastroenterology and Hepatology (prof. dr. M.C.W. Spaander) and Clinical Genetics at the Erasmus MC in Rotterdam.

In July 2021, Chantal started as a resident not in training at the Department of Surgery at the Ikazia Hospital in Rotterdam (dr. P.T. den Hoed and dr. W.J. Vles). Subsequently, in July 2022, she started her surgical residency training in Rotterdam (dr. S.M. Lagarde) at the Franciscus Gasthuis (dr. M.M. Poelman).

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