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Congenital Diaphragmatic Hernia and Pulmonary Hypoplasia: New Insights From Developmental Biology and Genetics

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Congenital diaphragmatic hernia (CDH), a common birth defect affecting as many as 1/3000–1/4000 live born infants has long been recognized as a major congenital anomaly, with drawings and written descriptions dating back to 1700 [Irish et al., 1996]. During the past 50 years, surgical and medical management techniques have greatly improved but so has our understanding of the complexities of CDH including the facts that CDH:(a) does *not* designate a single or specific type of diaphragmatic defect; (b) almost always co-occurs with serious and often life-threatening abnormalities of pulmonary airway and vascular development; (c) is accompanied by additional major malformations in as many as half of cases; and (d) appears to be etiologically heterogeneous. These factors contribute to the wide discrepancy in outcomes. While some survivors are healthy and have normal development, others either die or have long-term morbidity. The mortality of CDH is persistently high and approaches 50% when all cases of CDH are considered [Stege et al., 2003; Colvin et al., 2005]. The particularly deleterious impact of cardiovascular malformations (CVMs) which commonly co-occur with CDH is well-described in the article by Lin et al.; the authors also provide management guidelines pertaining to clinical and genetic aspects of this potentially life-threatening combination of major malformations. Improved outcomes for individuals with either isolated CDH (e.g., CDH is the only major malformation), or those with complex CDH (e.g., CDH plus one or more additional malformations) will most likely come after greater understanding of basic biology and of pathophysiology have been achieved.

There is increasing evidence that mutations in genes belonging to one or more important developmental pathways contribute to CDH and its accompanying defects. Current knowledge of the genes and pathways associated with CDH and lung development in both humans and model organisms are presented in this issue. For example, *Fog2*, *Gata4*, and *COUP-TFII* likely function in the same developmental pathway, and all three of these genes have been implicated in diaphragm development in mouse models. In humans, a *FOG2* mutation has been associated with a posterior diaphragmatic defect while *GATA4* and *COUP-TFII* are strong CDH candidate genes based on their locations at cytogenetic hot spots [Ackerman et al., 2005; Klaassens et al., 2005; Slavotinek et al., 2005; You et al., 2005; Ackerman et al., 2006; Jay et al., 2006]. Numerous perturbations in the retinoic acid pathways are associated with CDH; these include mutations in genes that are part of, or interact with members of, this pathway (such as *RXR*, *RAR*, *STRA6*, *COUP-TFII*, *GATA4*, and *FOG2*) [Mendelsohn et al., 1994; Mascrez et al., 1998; Malpel et al., 2000; Clabby et al., 2003; Scribner et al., 2006; Pasutto et al., 2007] as well as teratogenic exposures such as vitamin A deficiency and the herbicide nitrofen [Andersen, 1941; Kluth et al., 1990]. Although not proven, these findings suggest that genetic abnormalities in the vitamin A pathway may contribute to the most common form of CDH, namely isolated “Bochdalek” hernia and even more intriguingly raise the possibility of a role for preventative supplementation.

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However, these articles also point out the likely genetic heterogeneity underlying CDH and indicate that a variety of newer techniques (including molecular cytogenetics, gene sequencing, SNP arrays, and increasingly sophisticated analyses of genetic/teratogenic model organisms) will be required to dissect this complex birth defect. Standard G-banding chromosome studies identify aneuploidy in ~10% of all CDH cases. Array-based comparative genomic hybridization (aCGH) at the 1 Mb resolution has already identified small CDH-associated deletions below the resolution of standard karyotyping [Slavotinek et al., 2005; Kantarci et al., 2006], and it is likely that new higher resolutions platforms will detect additional even smaller areas of anueploidy serving to narrow critical regions that, in turn, will pinpoint genes key for normal diaphragm development.

Since pulmonary hypoplasia and pulmonary hypertension are the major determinants of survival in the neonatal period and predictors of long-term morbidity, several articles (Dr. Kinane, Dr. Khan and co-authors, and Dr. Keller) in this issue pay particular attention to normal and abnormal lung development, to pulmonary vascular development, and to potential pulmonary rescue therapies. The data presented therein make it abundantly clear that CDH-associated pulmonary hypoplasia is not SOLELY due to mechanical compression of the developing lung from herniation of abdominal contents into the chest cavity. Rather, intrinsic pulmonary developmental arrest accounts for at least some degree of the pulmonary hypoplasia seen at birth. Evidence for this hypothesis, coined the “two hit hypothesis,” is based on the discovery that both pulmonary development and diaphragm development are *primarily* affected by the chemical nitrofen [Keijzer et al., 2000]. There is now genetic evidence supporting this hypothesis (as discussed in the article in this issue by Ackerman and Greer) in that several genes have been identified which play an important role in the development of the lung and of the diaphragm, such as *Fog2* and *Gata4*. At this time, the percentage of CDH patients with primary defects of both lung and diaphragm development versus the percentage of patients with only secondary pulmonary hypoplasia, due to lung compression and abnormal diaphragmatic function, remains unknown. It is also likely, that some of the co-morbidity that occurs with CDH in the gastrointestinal system such as gastroesophageal reflux and dysmotility is not simply secondary to anatomic aberrations caused by herniation. Development of the esophagus and stomach occurs in close proximity to the lung, heart, and diaphragm, and some transcriptional regulatory pathways required for the development of these organs likely also play a role in upper gastrointestinal development [Muratore et al., 2001; Jacobsen et al., 2005; Jay et al., 2006].

The exciting new therapeutic possibility of tracheal occlusion (TO) is discussed in great detail by Khan and co-authors. Although TO appears to promote pulmonary development in model organisms by mechano-transduction (e.g., stretch-induced) acceleration of Type I pneumocyte cell division, the authors cautiously point out not only the lack of long-term outcome studies but also the immediate effect of diminished Type II pneumocytes with consequent diminished surfactant production. Identification of TO-induced genetic changes may ultimately lead to gene therapy targets that can stimulate lung development in utero or shortly after birth. The rationale, implementation, and outcomes for TO in the human population are discussed in the article by Dr. Keller.

GOOD GENETIC STUDIES WILL REQUIRE CAREFUL PHENOTYPING

At this time, it is uncertain how many different types of diaphragmatic defects occur in humans or whether certain phenotypes are developmentally related. As part of our ongoing research studies, we review surgical reports and autopsy records from many different medical centers and see that a wide range of hernia phenotypes are categorized, or “lumped” together, as “Bochdalek” hernias. There is also no consensus on describing diaphragmatic defects that are covered with a membrane (and often the description isomitted). Likewise, the description of

eventration defects overlaps with that of sac hernia, as both the size of the amuscularized diaphragmatic defect and the extent of herniation are extremely variable.

Since many published reports as well as operative or autopsy reports pertaining to diaphragmatic defects lack sufficient phenotypic information, we have developed a descriptive and pictorial schematic that attempts to capture the full diversity of human diaphragm defects (see Fig. 1, adapted from *Anatomy of the Human Body* with permission from Bartleby.com, Inc.) [Gray, 1918]. This is currently being tested by pediatric surgeons and pathologists at several institutions, and we encourage others to use it and to provide feedback to us about how it might be improved. We anticipate that widespread use of a standard approach to CDH classification will improve the quality of information in the medical literature, and ultimately will be used to develop genotype–phenotype correlations.

CDH remains a serious, even devastating birth defect, not only for the affected individual and their family, but also for society at large due to the enormous costs associated with providing care [Robbins et al., 2007]. We are fortunate to be working in a time where technological and scientific advances justify increased research attention toward this birth defect, since the goal of deciphering the pathogenesis and genetic mechanisms responsible for diaphragmatic defects and associated pulmonary hypoplasia seems obtainable. The articles in this issue of the Seminar series provide a comprehensive review of the current knowledge in this field. More importantly, we hope that their contents will stimulate further progress, not only in achieving a greater understanding of the spectrum of human CDH but in developing better therapies, especially for the accompanying pulmonary hypoplasia, that will lead to improved outcomes for patients with CDH.

Biographies

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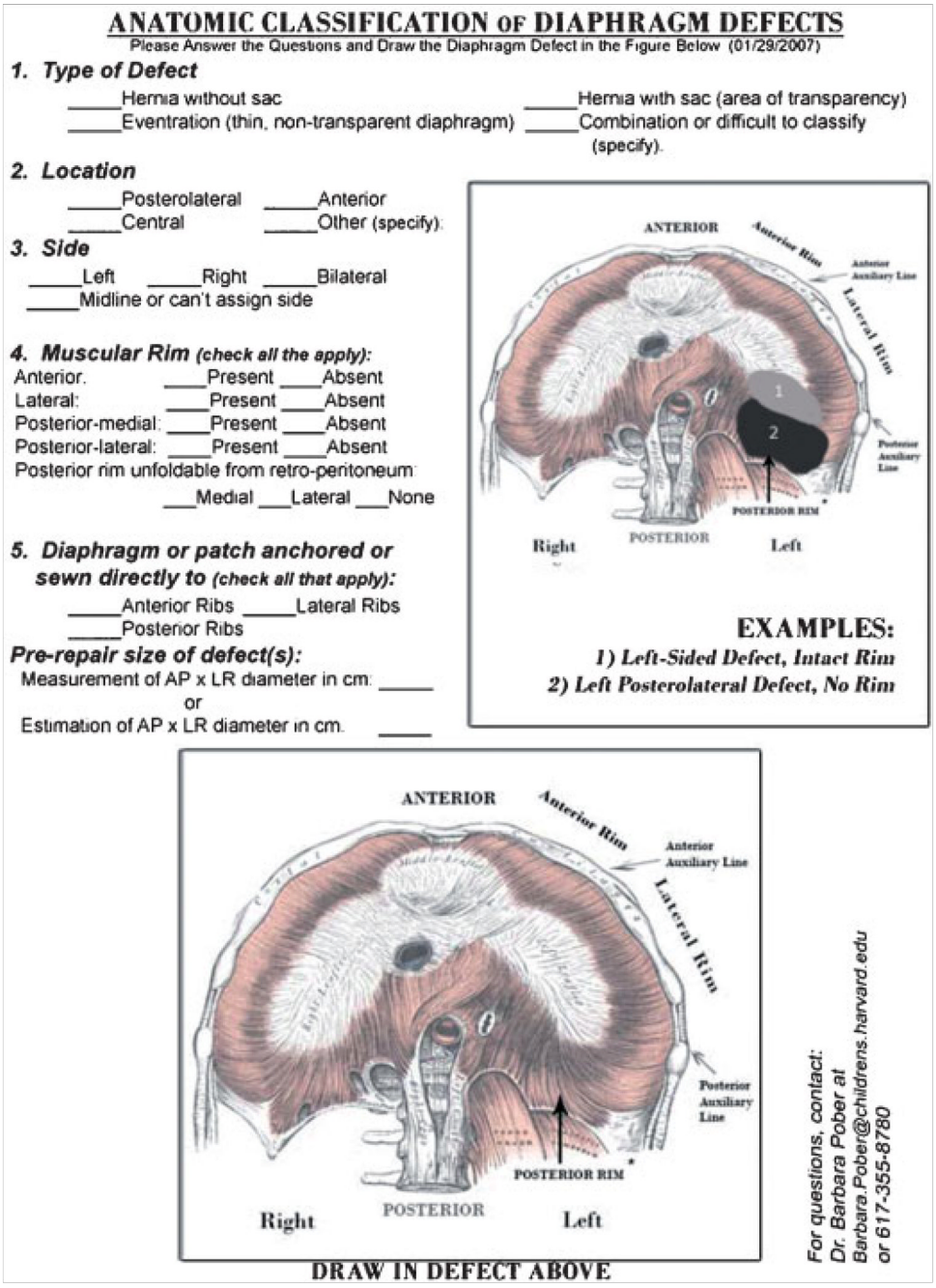

Figure 1.

Pictorial classification of Diaphragmatic Defects. We ask that surgeons and pathologists test this schematic by "drawing in" the exact location of the diaphragm defects and completing the requested information. Completed forms as well as comments to improve this schematic should be sent to us at the contact information provided.