Symposium Summary

Gene-Environment Interactions in Rare Diseases that Include Common Birth Defects

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Rare syndromes often feature specific types of birth defects that frequently are major diagnostic clues to the presence of a given disorder. Despite this specificity, not everyone with the same syndrome is equally or comparably affected, and not everyone with a specific birth defect manifests the same syndrome or is affected with all the features of a particular syndrome. A symposium sponsored by the National Institutes of Health Office of Rare Diseases, and the National Toxicology Program Center for the Evaluation of Risks to Human Reproduction attempted to explore how much of this variability is due to genetic factors and how much is due to environmental factors. The specific types of birth defects examined included cardiovascular defects, holoprosencephaly, clefts of the lip and/or palate, neural tube defects, and diaphragmatic hernias. *Birth Defects Research (Part A) 73:865–867, 2005.* © 2005 Wiley-Liss, Inc.

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Dr. John Belmont from the Baylor College of Medicine (Houston, TX) began the symposium with a discussion of genes that are involved in congenital cardiovascular malformations (CCVMs), which are among the most common of all medically significant birth defects in the United States (5–7/1000 live births). Economic costs are also important. Of the 11 most expensive birth defects identified in the California Birth Defects Registry, 7 are cardiac malformations. Numerous environmental and genetic factors have been implicated in causing CCVMs. Known environmental agents include congenital rubella infection, coxsackie B virus infection, maternal diabetes, ethanol, retinoids, and anticonvulsants. Cytogenetic abnormalities cause 12–14% of CCVMs, and submicroscopic deletion syndromes, including submicroscopic deletions such as velocardiofacial and Williams syndromes, are also clinically important. Single genes responsible for syndromic CCVMs have also been identified, e.g., Noonan (*PTPN11*), Holt-Oram (*TBX5*), and Char (*TFA2B*) syndromes. Nonsyndromic or isolated CCVMs caused by mutations in *NKX2.5*, *GATA4*, and *ZIC3* show how abnormalities in transcriptional control of gene expression lead to simple and complex heart defects (Ware et al., 2004). Zic3 acts upstream of Nodal and is required for maintenance of Nodal expression at the node (Purandare et al., 2002). Zic-family proteins are known to interact with the Shh-signaling pathway. The recent identification of nodal vesicular parcels (NVPs) may provide a critical link (Tanaka et al., 2005). NVPs are microscopic membrane-bound particles that carry both Shh and retinoic acid. NVPs are swept leftward by the gyratory motion of lrd-positive monocilia at the node, a process that is required for induction of left-sided Nodal expression. The recent identification of *CHD7*, an important gene that causes CHARGE syndrome, illustrates a new research paradigm in which high-resolution microarray methods may enable the detection of gene defects when linkage analysis is not feasible (Vissers et al., 2004). Belmont discussed recent results from U.S. patients

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with CHARGE that indicate that more than half of children with the condition have a mutation in *CHD7*.

However, together these conditions account for <20% of CCVMs, and the causative factors in most cases are unknown. Classification of CCVMs based on the likely developmental mechanisms that are disturbed during cardiac morphogenesis seems like a good way to start. A plausible strategy for investigating the etiology of CCVMs is to classify patients by the likely embryological mechanism involved, and then search for genes that contribute to susceptibility in that subgroup (Belmont et al., 2004). Current information about the genes required for normal cardiac development can be used to find some of the genes that are responsible for common CCVMs. The mechanisms that underlie teratogenic malformations are similar to those that operate in the genetic causes of CCVMs (Mark et al., 2004).

Dr. Max Muenke from the National Human Genome Research Institute (Bethesda, MD) continued this discussion of gene-environment interactions with a paper on holoprosencephaly (HPE), which is the most common anomaly of the developing forebrain in humans. The incidence of HPE is 1:10,000–20,000 live births, and it is even more common in early embryogenesis, at 1:250. HPE is characterized by variable phenotypic expression within the same family, ranging from unaffected carriers to severely affected patients with a single cerebral ventricle and cyclopia (Edison and Muenke, 2003). Although environmental factors, such as maternal diabetes, are known to be associated with HPE, genetic causes are also well documented and include nonrandom chromosome anomalies in HPE, familial occurrence of HPE, and known syndromes or associations with HPE. To date, mostly loss-of-function and occasionally gain-of-function mutations have been identified in genes such as Sonic hedgehog (SHH), Nodal, and other signaling pathways. These include not only SHH, DISPATCHED, PATCHED, and GLI2, but also TGIF, ZIC2, SIX3, and others (Roessler and Muenke, 2003; Roessler et al., 2003). More recently, low maternal cholesterol was associated with HPE, as was gestational use of cholesterol-lowering (statin) drugs (Edison and Muenke, 2004a; Edison and Muenke, 2004b). It is likely that "multiple hits" (i.e., environmental influences and genetic factors or mutations in 2 genes of the same or different signaling pathway) lead to extreme variability of the phenotypic expression, as seen in holoprosencephaly.

Dr. Sonja Rasmussen from the Centers for Disease Control and Prevention (Atlanta, GA) discussed the contribution of rare syndromes toward our understanding of clefts. Orofacial clefts are among the most common birth defects in humans, with cleft palate (CP) occurring in ~1 in 2000 infants, and cleft lip (CL) with or without CP (CL/P) occurring in 1 in 1000 infants. Both genetic (single-gene conditions and chromosome abnormalities) and environmental (teratogenic) causes for orofacial clefts have been identified, but the causes in most cases remain unknown. Epidemiological studies have shown that more than half of infants with CP, and at least 30% of those with CL/P have additional defects, and more than 400 syndromes with orofacial clefts have been identified. Although each of these syndromes is rare, their study has contributed to our understanding of the etiology of orofacial clefts. For example, the study of single-gene conditions led to the identification of genes and pathways that were later found to be involved in the etiology of nonsyndromic clefts. Specific examples of rare syndromes associated with orofacial clefts, and their impact on our understanding of their etiology, include ectrodactyly, ectodermal dysplasia, and clefting (EEC) syndrome and *p63*, CL/P with ectodermal dysplasia and *PVRL1*, orofacial clefting with tooth agenesis and *MSX1*, CP with ankyloglossia and *TBX22*, and Van der Woude syndrome and *IRF6* (Lidral and Murray, 2004). In other cases, chromosome variations have shed light on genes that may be involved in clefting, such as *ACOD4* on 4q21, and CL (Beiraghi et al., 2004). Teratogens associated with clefting include smoking, alcohol, diphenylhydantoin, trimethadione, retinoids, aminopterin, methotrexate, and hyperthermia (Cohen, 2002), and studies of these teratogens and the syndromes associated with them have also provided insights into the etiology of orofacial clefts (Sulik et al., 1981; Diehl and Erickson, 1997; Shaw and Lammer, 1999).

Interactions between common exposures and susceptibility genes may also play important roles in the pathogenesis of orofacial clefting.

Dr. Barbara Pober from the Massachusetts General Hospital for Children and Boston Children's Hospital (Boston, MA) discussed congenital diaphragmatic hernias (CDHs), a common group of birth defects that involve the structural integrity of the diaphragm and are often associated with lethal pulmonary hypoplasia. CDH affects ~1 in 3000 births. The majority of cases occur as isolated defects or in association with other birth defects that do not constitute a recognized syndrome. The etiologies of human CDHs are largely unknown but appear to be quite heterogeneous. There are several lines of evidence that suggest genetic causation, including 1) single gene disorders associated with CDHs; 2) identification of de novo mutations in 2 genes—*FOG2* (Ackerman et al., in press) and *WT-1* (Devriendt et al., 1995)—as the cause of human CDHs; 3) recurring small chromosome abnormalities associated with CDHs, with the recent detection of a CDH-critical region in 15q26.2 (Klaassens et al., 2005); 4) the existence of multiplex families with CDHs (Wolff, 1980); and 5) genetic mutations in mice associated with diaphragm defects, such as *Slit3*, RAR/RXR, *Wt-1*, and *Fog2* (Yuan et al., 2003; Ackerman et al., in press).

Teratogenic causes of human CDHs have not been identified to date. In animals, 2 models—nitrofen administration and vitamin A deficiency—have been studied extensively for their role in diaphragm and lung development. Nitrofen (2,4-dichlorophenyl 4-nitrophenyl ether) is an herbicide that produces CDHs in exposed rodent fetuses. Its mechanism of teratogenic action is not fully known, but nitrofen may work by inhibiting retinal dehydrogenase (RALDH2), which is an enzyme in the pathway for converting vitamin A to its bioactive metabolite, retinoic acid (Greer et al., 2003). Of note, nitrofen teratogenicity can be rescued by administration of vitamin A or retinoic acid.

Environmental disturbances in the retinol pathway, such as nutritional vitamin A deficiency or exposure to teratogens that suppress retinoic acid formation, may be risk factors for the development of CDHs. Likewise, genetic mutations that lead to enzyme or receptor deficiencies in the vitamin A pathway may be independent risk factors for CDHs. It seems possible that the combined presence of both environmental and genetic disturbances in the vitamin A pathway could act in concert to greatly increase the

risk for developing CDHs. Current research is exploring this pathway, and mutations that affect the vitamin A pathway are prime candidate genes for causing CDHs, especially in cases in which the phenotype resembles vitamin A deficiency in experimental animals (Macayran et al., 2002).

Finally, Dr. Gary Shaw of the California Birth Defects Monitoring Program (Berkeley, CA) discussed the complex interactions that exist between genes and environmental factors from an epidemiological perspective. The etiologies for most congenital anomalies remain unknown, whether these anomalies occur in isolation or in conjunction with other anomalies. Some known teratogens appear to produce multiple congenital anomaly phenotypes, and a number of investigators have noted that infants with 2 or more congenital anomalies may be productive to study because multiple anomalies in the same child may be indicators of exogenous teratogens. Thus, by investigating rare defects that occur in combination, researchers may be able to identify etiologic clues that can be further investigated as causes for more common single defects that were part of the original rare multiple-malformation syndrome. Alternatively, one could start from the premise that a given anomaly may have multiple causes. One way to tease apart such multiple potential causes is to investigate interactions between genes and exposures, genes and genes, and exposures and exposures. Examples of efforts that have been made in this area include 1) interactions between a single nucleotide polymorphism (SNP) in *RFC1* that reduces folate transport and increases the risk for conotruncal cardiac defects (Shaw et al., 2003a), 2) interactions between multiple environmental factors that influence the glycemic control (e.g., prepregnancy obesity, intake of carbohydrates/glycemic index, and diabetes) (Shaw et al., 2003b), 3) SNPs in *GSTT1* and *GSTM1* and maternal smoking (Lammer et al., in press), and 4) a *NOS3* SNP combined with maternal smoking and decreased periconceptional folate intake (Shaw et al., in press). Of interest, many of these combination effects reduce a large group of cases to only a few informative affected individuals, even when the exposure or the frequency of the gene variant is relatively common. Both philosophical approaches are necessary to narrow in on unexplained etiologies in birth defects research.

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