Closing the Genetic Gap in Orofacial Clefts

**FACIAL STRUCTURES** such as our nose, lips and jaw bones are formed from cranial neural crest (CNC) cells, a group of pluripotent cells that delaminate from the neural tube during embryogenesis. Derangement of this process results in cleft lip and palate, the most common structural congenital malformations in humans. Researchers at Massachusetts General Hospital for Children (MGHfC) are looking into the specific genetic miscues leading to these craniofacial anomalies. Understanding the pathways and genetics involved in CNC malformation represents the next step forward in cleft diagnosis. A revolutionary advance would be to go beyond improved genetic diagnosis and identify targeted compounds to potentially mitigate orofacial clefts before they form, explains Chien-Wei (Eric) Liao, MD, PhD, who leads the Craniofacial Developmental Biology Laboratory at MGHfC. Dr. Liao is also the co-director of the MGHfC Cleft Lip and Craniofacial Clinic, a multidisciplinary clinic treating patients with craniofacial disorders.

**Zebrafish Models of Human Clefts**

To investigate the role specific pathways and genes may play in cleft pathogenesis, Dr. Liao developed a functional genomics model using the zebrafish, an animal sharing developmental similarities to human palate formation. Because zebrafish develop quickly and are optically transparent at larval stages, researchers can observe structural anomalies as they develop. Using this model, Dr. Liao reproduced the pathology of a severe form of human orofacial cleft (oblique facial clefts) in the zebrafish. Specifically, he and his colleagues demonstrated that disrupting specc1lb, a homolog to the human SPECC1L gene known to cause clefts in humans, contributes to oblique facial clefts in zebrafish. This was the first time an animal model was used to mimic a human oblique facial cleft abnormality.

**Compounds Involved in Orofacial Development**

The team at MGHfC also used chemical screens to map the development of CNC cells. Again using zebrafish, Dr. Liao found that when the animal was exposed to the nitric oxide synthase inhibitor 1-(2-[trifluoromethyl] phenyl) imidazole, or TRIM, it developed clefts as CNC maturation, migration and differentiation were impaired. This work proves that nitric oxide signaling and histone acetylation are necessary contributors that regulate CNC differentiation and convergence during development. The implications of this research are significant, as they point to the possible discovery of compounds that can be...
**Treatment and Screening in Cryptorchidism**

ALTHOUGH CRYPTORCHIDISM is the most common congenital abnormality of the genitourinary tract, lingering uncertainties remain regarding the appropriate management and long-term consequences of an undescended testicle (UDT). Jack S. Elder, MD, chief, Division of Pediatric Urology at MassGeneral Hospital for Children, has been investigating clinical scenarios regarding cryptorchidism to clarify outcomes, screening and treatment strategies.

When an infant is referred with UDT, the testes should be located, as they cannot be palpated in 10 percent to 15 percent of cases. Nearly 40 percent of nonpalpable testes are present high in the inguinal canal or in the abdomen. The remainder are atrophic testes, a secondary consequence of in utero spermatic cord torsion. Ultrasound examination prior to surgical referral has been a common diagnostic modality, but has not been found to be beneficial in diagnosing either of those conditions. Instead, laparoscopy is the diagnostic tool of choice to locate a UDT.

**Cancer and Fertility Risks in UDT**

Cryptorchidism carries long-term risks, and long-term screening should follow treatment. Surgical orchidopexy by age 10 to 12 years, in which the testis is moved into the scrotum and sutured there, reduces the risk of developing testicular cancer by a factor of two to six but the surgical procedure does not eliminate it, according to the research from Dr. Elder’s center.1 Even after orchidopexy, individuals with a history of cryptorchidism carry a testicular cancer risk of one in 80 (compared with one in 400 in the general population) with usual onset between ages 15 and 45 years. Because the risk remains despite surgical intervention, adolescents with a history of UDT should be instructed in self-examination.

Risks to male fertility are also a primary concern. In UDT, the process of germ cell sperm development is inhibited, leading to a dearth of stem cells for post-pubertal spermatogenesis.2 When a testis remains in the undescended position, germ cell transformation does not occur, significantly reducing its reproductive potential. Orchidopexy between six and 12 months of age is recommended to maximize future fertility. Consequently, male infants should be screened for UDT at birth, at every well-child visit, and annually throughout the prepubertal period.

Although UDT is considered congenital, some males may develop a retractile or ascending testis during early childhood. A retractile testis can become a true ascending (undescended) testis in one-third of cases.3 Annual monitoring is required to assess the position of retractile testes. An ascending testis that develops after birth requires orchidopexy by age 10 years to maximize fertility and reduce the risk of testicular cancer.

Estrogen Replacement in Anorexia Nervosa

**YOUNG FEMALES WITH** anorexia nervosa (AN) suffer many adaptive, physiological changes, including endocrine changes such as low estrogen levels, low levels of insulin-like growth factor 1 (IGF-1), higher levels of cortisol and alterations in other hormones. These hormonal changes can have deleterious effects on bone development and peak bone mass—the maximum amount of bone an individual will have during her/his lifetime—putting girls at increased risk for bone deficits later in life.

Although estrogen deficiency is a significant contributor to low bone density in adolescents with AN, studies have shown that oral doses of estrogen are ineffective in increasing bone density in this population.

A team at MassGeneral Hospital for Children and Massachusetts General Hospital, led by Madhusmita Misra, MD, chief of the Division of Pediatric Endocrinology, and Anne Klibanski, MD, chief of the Neuroendocrine Unit, explored this estrogen deficiency and strategies for estrogen replacement in AN. Oral delivery of estrogen may have reduced efficacy because it undergoes hepatic first-pass metabolism, which leads to a decrease in IGF-1, a hormone that contributes greatly to bone formation during puberty. Levels of IGF-1 are already low in patients with AN, and oral estrogen may be a further suppressive factor.

The team studied the transdermal delivery of estrogen for AN patients, a method that bypasses first-pass metabolism in the liver. They also looked at dosages tailored to maturity, based on previous studies suggesting that very low doses of oral estrogen, which mimic the small rises in estrogen during early puberty, showed less inhibition of IGF-1.

The study randomized girls with AN ages 12–18 to either estrogen or placebo and followed them for an 18-month period. They grouped the girls according to bone age: If their bones were still growing, they were given very small, incremental doses of oral estrogen to mimic the early increases in estrogen during puberty. Girls who had completed bone growth received the transdermal estrogen patch at a full replacement dose to match what their bodies would normally produce, with cyclic progesterone.

Girls who received physiologic estrogen replacement, whether low-dose oral or transdermal, had a significant increase in bone density, which approximated levels seen in normal-weight controls without AN. There were no significant side effects and no difference in weight gain between girls who received physiologic estrogen in either form and girls given placebos.

The team also found that participants who received the transdermal patch showed significant improvements in anxiety scores over the study duration—in particular, trait anxiety decreased. Rodent studies had previously shown that estrogen is protective against anxiety. Additionally, girls with AN who did not receive estrogen and who gained weight over the follow-up period showed an increase in state anxiety and body dissatisfaction, which is a core consideration in treatment. In contrast, girls who received estrogen and gained weight did not show this increase in state anxiety or body dissatisfaction, suggesting that physiologic estrogen replacement might not only help bone density but assist the psychological process of recovery from AN as well.

**DIFFERENCES IN BONE DENSITY** changes between AN girls receiving placebo (purple), AN girls receiving physiologic estrogen replacement (blue), and normal-weight controls without AN (orange). The AN girls receiving physiologic estrogen replacement showed an increase in bone density similar to that of normal-weight girls without AN, while the girls with AN who received a placebo showed a decrease in bone density over the 18-month study (*p<0.05).

**PHYSIOLOGIC ESTROGEN REPLACEMENT** led to a significant decrease in anxiety trait (but not state) scores, as measured by the the State-Trait Anxiety Inventory for Children (STAIC). AN-E+ represents girls with AN who received estrogen. AN-E- represents girls with AN who received a placebo (*p<0.05).


developed for pharmacologic manipulation of craniofacial development, in zebrafish and possibly in future human trials.

The lab continues to pursue this line of inquiry, focusing on the design of novel chemical screens to identify compounds that can actively disrupt abnormal CNC during development. The goal is to use the chemical screen in combination with the zebrafish model to produce a rapid, reliable model system of cleft malformations that will facilitate large-scale screens for drug discovery. With such a catalogue of chemical agents that are significant to this malformation, teams could work to uncover existing or new medicines that, when applied in utero, may halt or reverse cleft development.

Dr. Liao and his team are pursuing functional genomics research with funding support from the National Institutes of Health, which uses prenatal DNA sequencing to identify patients with congenital anomalies and to identify the genes involved in early human development. An end goal for the research would be for physicians to detect a predisposition to cleft in a baby’s DNA as early as six to eight weeks into a pregnancy, and to have effective pharmacological agents that promote proper palate development.
