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Gene/environment interactions in craniosynostosis: A brief review

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Abstract

It is suggested that craniosynostosis is caused by a heterogeneous set of effects including gene mutations, teratogenic exposure during critical periods of development, and gene/environment interactions. Distinguishing between sufficient, additive, and interactive effects is important to the study of gene/environment interactions and allows for segregation of environmental exposures effecting susceptible populations. Through the identification of sufficient and interactive effects efforts in prevention of craniosynostosis may be successful. Here we provide a brief review focusing on defining these categorized exposures and relevant literature that has interrogated gene-environment interactions for craniosynostosis.

I. Craniosynostosis

The cranial suture is defined as the fibrous joint between the bony plates of the skull that allows for neuro-expansion during development. Functionally, the suture may dampen biomechanical stress upon the calvarial bones once neuro-cranial expansion is complete. Beginning about the 2nd decade of life bony fusion of the cranial sutures often begin. When the cranial suture undergoes bony infiltration (synostosis) prior to the completion of brain growth, deformation of the cranium and associated anomalies termed craniosynostosis can occur. Craniosynostosis occurs in 1 in every 1800–2500 live births (1) and affects males versus females at a ratio of 2:1. Often the co-morbidities associated with craniosynostosis or identification of aberrant growth trajectory allow for diagnosis. Many of the co-morbidities associated with craniosynostosis, including ocular proptosis and increased intracranial pressure, pose a threat to normal neurological development. Neurosurgery is often indicated in cases of craniosynostosis allowing for the “release” of the suture and hopeful reversion towards normal growth trajectories. Surgical intervention varies due to the heterogeneous nature of craniosynostosis but can include strip suturectomies, posterior expansion, fronto-orbital advancements, or partial and complete calvariectomies (1–3).

The fused suture drives alterations in morphological development associated with craniosynostosis. Normally the calvarium expands perpendicular to the patent sutures (Virchow’s law), however when premature fusion occurs, compensatory growth occurs in

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dimensions not restricted by fusion. The most common suture involved in clinical cases of craniosynostosis is the sagittal which, depending on timing of fusion, can lead to a scaphocephalic (elongated in the antero-posterior dimension) phenotype (1,4). The coronal is the second most commonly involved suture which when bilaterally affected can drive brachycranic (widening) of the cranium, or anterior plagiocephaly (asymmetry) when unilaterally compromised. Premature fusion of the metopic and lambdoid sutures are more rare accounting for only 15–20% of clinical cases.

Craniosynostosis is associated with more than 180 syndromes many of which present with limb abnormalities in addition to restricted cranial growth. The most commonly identified syndromes include those resulting from mutations in *FGFr* and *TWIST* genes. Syndromic craniosynostosis can be associated with multiple suture (e.g., Apert syndrome, Pfeiffer syndrome, Crouzon syndrome, Antley-Bixler syndrome), or single suture fusion (e.g., Saethre-Chotzen syndrome, Muenke syndrome) (1,3). However, greater than 85% of all craniosynostosis cases are classified as isolated non-syndromic occurrences where no genetic information is identified. From a clinical perspective, although non-syndromic cases are often less severe, clinical protocols are similar. Neurosurgical intervention is still indicated in cases of non-syndromic craniosynostosis; only strategies for genetic counseling differ.

In addition to the genetic factors associated with craniosynostosis, there have been several environmental exposures (e.g. teratogens) associated with craniosynostosis identified from case and surveillance studies. These exposures include maternal thyroid disorders (5,6), cigarette smoking (7–9), alcohol use (4,10), and maternal use of anti-depression drugs (11,12) although the identification of these exposures as causative is not conclusive. Overall it seems likely that these teratogens and others are sufficient to cause craniosynostosis. However, there are also likely genetic polymorphisms that exist allowing for a more complicated interpretation of the causation of craniosynostosis. Genetic factors (e.g., *FGFr* mutations), environmental factors (teratogen exposures), and gene/environment interactions, a concept we will develop below, have all been identified as causative for craniosynostosis.

II. Defining Gene/Environment Interactions

The literature can be unclear on how exactly to define a gene/environment effect, but is consistent in defining the necessity of polymorphisms (a variant occurring in greater than 1% of the population) or mutations to allow for appropriate genetic variability to be acted upon by an external factor (13–16). It is this variability that dictates where one genetic status responds differently to a stimulus (Figure 1). This concept should be distinguished from environmental only effects where a teratogen is sufficient to cause an anomaly. Segregation may also occur due to exposure versus non-exposure, e.g. hypervitaminosis A causing cleft lip/palate (16–18). Gene/environment interactions should also be distinguished from additive effects where a condition likely caused by a mutation is exacerbated phenotypically by an exposure. Thus, although gene mutations associated with disease states are amenable to gene/environment interactions it is likely that many more genetic polymorphisms are at work.

There are some well-defined gene/environment interactions for craniofacial development with respect to anomalies. This is not a surprise as research has long indicated environmental contribution or developmental plasticity of the craniofacial skeleton. For example, biomechanics and environment including temperature and diet effect final craniofacial form (19–23). One clear example of gene/environment effects is genetic variability in *TGFa* acted upon by cigarette smoking in causation of cleft lip/palate. Data suggests use of cigarettes acts upon variant *TGFa* alleles, which encode for a molecule important for proliferation and differentiation within tissues during both primary and secondary palate formation. Alteration of this homeostatic relationship can result in cleft lip/palate (8,16–18).

There are likely many more interactions between genetic polymorphisms and environmental exposures than may be detected. Craniosynostosis is heterogeneous in presentation, including age of presentation and severity (optimal age of repair is <1 year), and thus it is not as amenable to identification of gene/environment interaction as cleft lip/palate of which a majority of cases are identified at or before birth. The disjointed timeline between potential environmental or teratogenic exposure and identification of craniosynostosis may introduce further error (i.e. maternal/paternal self-report) to elucidation of these relationships. Craniosynostosis is also more rare than orofacial clefting leading to difficulty in large scale analyses such as genome wide associations (GWAS), discussed below.

Identification of gene/environment interaction relies on molecular epidemiology of DNA sequencing including GWAS, identification of candidate single nucleotide polymorphisms (SNPs), and linkage disequilibrium. A trait can be studied using GWAS, where common allele variants are interrogated for segregation of disease or phenotype, either through investigation of patients with or without disease, or phenotypic variants within disease states. Deletions, insertions, and single nucleotide polymorphisms are all amenable to analysis which is most powerful when a trait is normally distributed. This is to be contrasted with linkage studies which are amenable to heterogeneous genetic presentation and analysis of different alleles as well as allele states (13,14).

Linkage disequilibrium, non-random association of alleles at different loci, suggests an association between variation found at a genetic locus and phenotypic expression of a trait, usually within the context of a disease state. As linkage disequilibrium relies on patterns of inheritance it is susceptible to the selected populations of study as well as rate of mutation and genetic drift within that population. Overall, SNPs found in coding regions are particularly susceptible to segregating differences, which results in different levels of susceptibility of disease and occurrence with environmental challenge (13,14). Cohort studies and genetic database sets are the most powerful tools to enhance identification of susceptible alleles, but case control studies are also used. Environmental variables that segregate by geographical location or condition are more easily recognized due to identification of cohort and proper controls. It is through these studies utilizing molecular epidemiology that better diagnosis and prevention of diseases, including craniosynostosis, can occur.

III. Examples from Human Condition

The United States Center for Disease Control Birth Defects Prevention Study has suggested that a focus of research and dissemination on craniosynostosis should be gene/environment interactions (24). Despite this call there are few examples in the clinical literature focusing on this topic. On example is a report on a familial case of *FGFr3* mutation at Pro250Arg (Muenke syndrome), which revealed what was suggested to be a gene/environment interaction. The described environmental factor was maternal diabetes which appears to have exacerbated the paternally inherited craniosynostosis disorder. In this case, the child presented with laterality disorder and hepatoblastoma, conditions not previously associated with mutations at this locus. There was however, no interactive effects with respect to the craniosynostostic phenotype, which was described as presenting as typical Muenke syndrome. These associated effects were most likely additive, not interactive as the phenotype was severe likely due to a two-hit phenomenon. The gene mutation was sufficient to cause craniosynostosis and the hepatoblastoma was associated with maternal diabetes exposure (25). There is another recent report focusing on environmental variability of fetal constraint and a novel mutation in *FGFr2* (Ala315Ser). It is interesting that this locus is on a gene well known to cause craniosynostosis and the patient presented as Crouzon-like. This locus has however, not previously been identified in human craniosynostosis cases or syndromes and thus may represent a gene/environment interaction, in this case fetal constraint being the environmental variable (breach) (26).

Research has indicated several genetic loci amenable to further study identified by GWAS that were not associated with traditional *FGFr* or *Twist* mutations. (14). For example, a study of non-syndromic cases of sagittal craniosynostosis found several susceptible loci including one down stream of *BMP2*, a gene which encodes for a potent bone development protein, and one within the gene *BBS9*. As neither of these genes has a known segregating population within craniosynostotic patients or is associated with a syndrome, these candidates may be amenable to gene/environment interactions. Research should continue to move forward to distinguish between additive versus gene/environment effects particularly for single suture isolated non-syndromic craniosynostosis cases.

Research has also focused on utilizing mechanistic targets of effect (mRNA expression) to identify likely gene markers or pathway in lieu of pure genetic markers that segregate in craniosynostosis cases. For example, calvarial osteoblast cells were cultured from single suture craniosynostosis cases and results identified *SFRP4*, *FGF7*, and *VCAM1* as having segregating expression levels associated with craniosynostosis. These results are suggestive of *FGF/IGF WNT* signaling importance in non-syndromic craniosynostosis (27). These and associated genes (upstream) are likely candidates for gene polymorphism associated with disease. The identification of these genes also suggests that although the genes associated with syndromic craniosynostosis are less likely candidates for gene/environment interactions, the associated molecular pathways are rational targets for study.

IV. Examples of Modeling

Many preclinical models of human craniosynostosis syndromes exist; most of which are *in vivo* (transgenic) and *in vitro* (derived from transgenic or *ex vivo* transfected cells) murine models. Additional models of study concerning cranial suture biology and craniosynostosis may represent non-syndromic (although clearly genetic) craniosynostosis (e.g. lagomorph model) (1–3,28,29) or challenges to the normal developing mammalian skull by effecting genes and proteins important for suture patency, and finally the study of normal ontogenetic fusion (e.g. the murine posterior interfrontal suture) (30–34). It is difficult to model gene/environment interactions pre-clinically as there are exceedingly abundant possibilities for environmental variables and teratogens to act on polymorphisms that allow for susceptibility to craniosynostosis. Teratogenic studies alone are informative, but do not address interactive effects. Further, the addition of a teratogens within a genetic model of craniosynostosis have several drawbacks including the strong possibility of no effect as craniosynostosis is already predicted to occur (i.e. *Twist 1* +/− mouse) (35). However, these studies in both the wild-type mammalian skull and genetic models of craniosynostosis may provide sufficient information on molecular pathways of effect (downstream of the gene) specific to an exposure. Further, as data becomes available from GWAS or linkage studies these preclinical investigations can proceed with greater likelihood of success.

V. Future Directions of Research

It is likely that within the context of gene/environment interaction there are thresholds levels at which teratogens act upon unknown genetic polymorphisms (Figure 2). Thus, testing these interactions either by amelioration of the contribution of that gene or loci alteration will likely prove to be quite an expensive and frustrating venture. If segregating populations are suggested from GWAS or linkage studies, then these may be the most useful directions for future analyses because of their ability to link genetic variants to a disease state. Overall, teratogenic studies are much easier from an implementation perspective, both for clinical identification and preclinical modeling. If identified teratogens are established to alter growth and development in a wild type pre-clinical model, those data may prove useful for loci specific testing via cellular transfection studies *in vitro* or *in vivo* modeling to inform the clinical practice of counseling, identification, and prevention.

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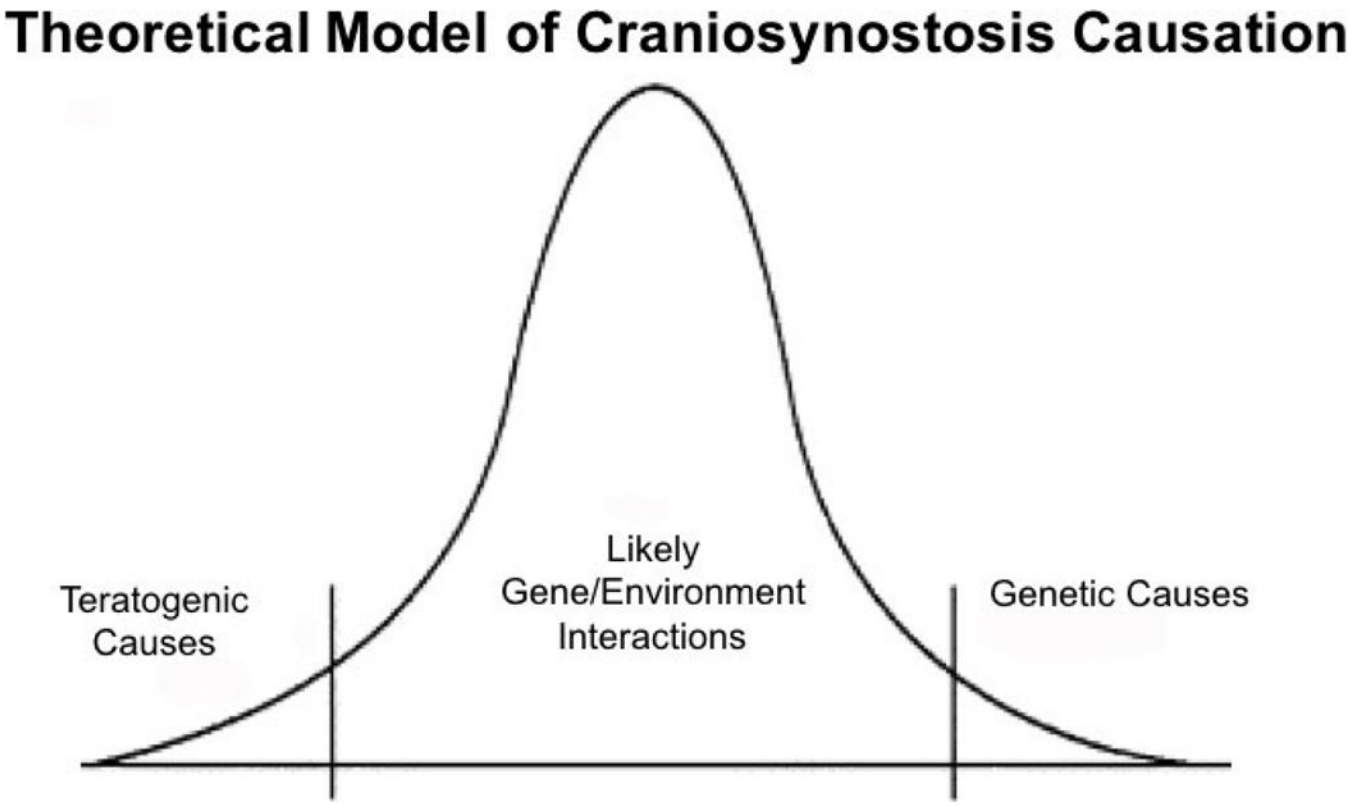

Figure 1. Theoretical Model of Craniosynostosis Causastion

Gaussian curve represents cases of craniosynostosis of which the majority are likely caused by gene/environment interactions.

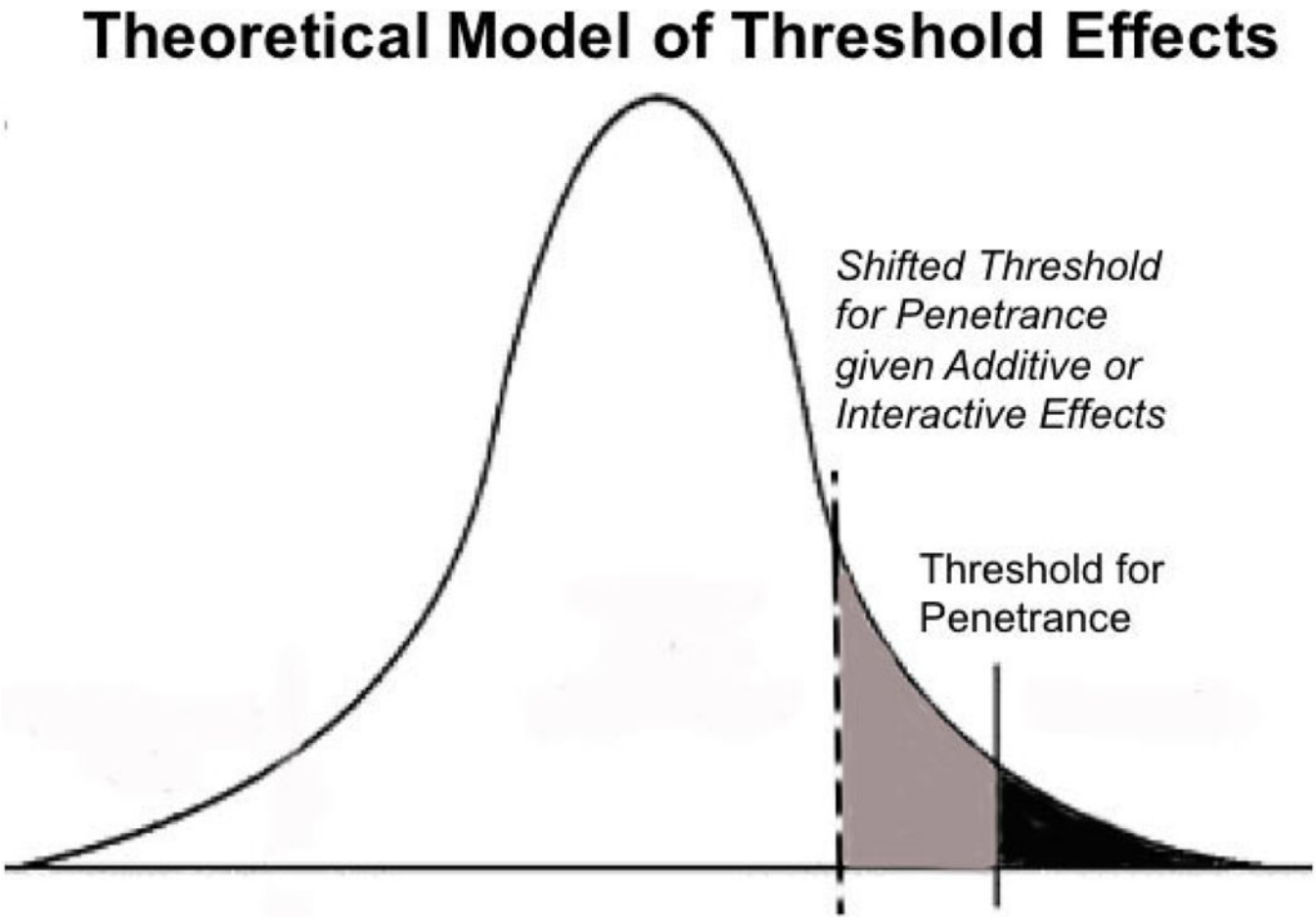

Figure 2. Theoretical Model of Threshold Effects

Gaussian curve highlighting a shifted threshold for penetrance of craniosynostosis in the general population due to additive or interactive effects of genes and environmental factors.