2018

Maternal health-related causes of cranial neural crest cell migration dysregulation, and their common clinical effects

BOSTON UNIVERSITY

SCHOOL OF MEDICINE

Thesis

MATERNAL HEALTH-RELATED CAUSES OF CRANIAL NEURAL CREST CELL MIGRATION DYSREGULATION, AND THEIR COMMON CLINICAL EFFECTS

by

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B.S., University of California Davis, 2016

Submitted in partial fulfillment of the

requirements for the degree of

Master of Science

2018

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ABSTRACT

Neural crest cells arise during neurulation, a process that occurs during the third week of embryogenesis. These diverse cells then divide into various subtypes including cranial neural crest cells and cardiac neural crest cells. Each of these subtypes gives rise to a wide range of features throughout the fetus. While these cells are extremely diverse, they are also incredibly sensitive to their surrounding environment. Many maternal conditions affect neural crest cell division and migration, but maternal alcohol consumption and hyperglycemia due to gestational diabetes will be discussed in detail, with special attention paid to tissues that derive from cranial neural crest cells.

While the initial mechanisms of the pathology vary for both of these conditions, what is remarkable is that they ultimately cause effects in similar ways. Both mechanisms lead to the creation of reactive oxygen species, which in turn trigger apoptotic pathways. Neural crest cell death causes a variety of congenital anomalies in fetuses, including craniofacial defects and cardiac outflow tract defects. Treatment options that have been researched in both conditions also vary, but are based on similar principles. Antioxidant therapies reduce the production of reactive oxygen species, thus reducing the severity of the anomalies affecting the fetus during development.

Both maternal alcohol consumption and gestational diabetes are important public health concerns, and their management is of utmost priority in society. By decreasing the rates of women who consume alcohol during pregnancy, and managing gestational diabetes in those at highest risk, the rates of fetal congenital defects could be decreased.

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LIST OF ABBREVIATIONS

ADA ..... American Diabetes Association

ATM-Chk2..... Ataxia-telan-giectasia mutated-checkpoint 2

ATR-Chk1 ..... ATR-Rad3-related-checkpoint 1

CarNCC ..... Cardiac Neural Crest Cells

CDC..... Center for Disease Control

CNCC ..... Cranial Neural Crest Cells

CIL ..... Contact Inhibition of Locomotion

EMT..... Epithelial-to-Mesenchymal Transition

FGF..... Fibroblast Growth Factor

GD ..... Gestational Day

GDM..... Gestational Diabetes Mellitus

NCC..... Neural Crest Cells

PCOS ..... Polycystic Ovary Syndrome

PDGF..... Platelet Derived Growth Factor

Ptc..... Patched

SHBG ..... Sex Hormone Binding Globulin

Shh..... Sonic Hedgehog

SNCC..... Sacral Neural Crest Cells

SOD..... Superoxide Dismutase

TNCC ..... Trunk Neural Crest Cells

VNCC..... Vagal Neural Crest Cells

WHO..World Health Organization

INTRODUCTION

A Brief Summary of Embryology

To understand neural crest cells (NCC) and their pathologies, it is important to have a basic understanding of embryology. Following fertilization, the zygote undergoes multiple steps of cleavage. By three days post-fertilization, the embryo has reached the stage of a morula, containing 16 cells. Inner cells of the morula become the inner cell mass, or the actual embryo, and the outer cells form the trophoblast. After further divisions, the inner cell mass divides into the embryoblast. By day eight, the trophoblast develops into the inner cytotrophoblast and the outer syncytiotrophoblast. The embryoblast differentiates into the hypoblast and epiblast. The synctiotrophoblast is responsible for embedding and nourishment of the embryo, and eventually becomes the fetal portion of the placenta, while the inner layers continue differentiating to form the embryo itself (Sadler, 2012, Chapter 4).

The third week of development is particularly significant, as it is the point at which gastrulation, the process of creating three germ layers, occurs. Once the primitive streak forms in the epiblast, cells migrate toward the primitive streak. At the primitive streak, cells undergo invagination. This process creates three germ layers – the endoderm, mesoderm and ectoderm. This is also the time at which the notochord forms from mesoderm in the cranial region. It should be noted that the ectoderm above the notochord differentiates into the neural plate at this time. Notably, the third week is an important stage as the mesoderm further differentiates into segments, or somitomeres (Sadler, 2012, Chapter 5). Each somite later differentiates into 3 components: the sclerotome, myotome

and dermatome via a complex signaling process involving proteins such as *sonic hedgehog* and Wnt (Sadler, 2012, Chapter 6).

The end of the third week also leads to the process of neurulation, when the edges of the neural plate fold up to create neural folds and a neural groove (Sadler, 2012, Chapter 5). Neurulation happens in two stages – primary and secondary neurulation. Once the neural plate has folded into a tube, primary neurulation is comprised of the rostral part of the neural tube becoming the brain and the caudal part becoming the spinal cord. Secondary neurulation then proceeds in mammals. In this process, cells at the very caudal end aggregate into a caudal eminence, becoming the medullary cord. This cord eventually gives rise to the neural tube via secondary neurulation (ten Donkelaar, Bekker, Renier, Hori, & Shiota, 2014). While the neural tube forms, cells at the lateral border of the neural folds begin to separate and migrate away. These are the future NCC (Sadler, 2012, Chapter 5). Figure 1 provides a visual demonstration of this process.

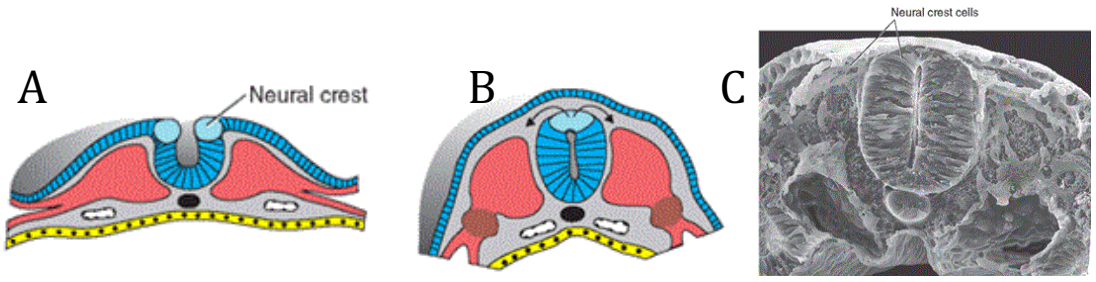

Figure 1. Neurulation and neural crest cell separation. (A) Folding of neural plate into neural tube during neurulation. (B) Once neural tube has folded, most lateral cells begin to separate and migrate away to form neural crest cells. (C) Electron microscopy of processes of neurulation and neural crest cell separation (Sadler, 2012).

Migration Patterns of Neural Crest Cells and Subtypes of Cells

As NCCs separate from the rest of the neural tube, they undergo an epithelial-to-mesenchymal transition (EMT). This process is characterized by two main steps. The first occurs when epithelial cells lose their cellular polarity, and the second when they undergo detachment from neighboring cells through the downregulation of E-cadherins (Thiery, 2003). While this process is not unique to NCCs, it is most relevant for these purposes.

EMTs in NCCs are driven by fibroblast growth factor (FGF), Wnt, Notch, and retinoic signaling pathways (Thiery, Acloque, Huang, & Nieto, 2009). E-cadherin expression is regulated by zinc-finger transcription repression (Thiery, 2003), and is tightly associated with *Snail* transcription factors. *Snail* transcription factors have been found to repress the expression of E-cadherins, allowing for NCC to dissociate and migrate away from the neural tube. *Snail* was found to interact with the E-pal element of the E-cadherin promoter in a mouse model, and a corresponding element in humans (Cano et al., 2000). The mechanism of EMT will be discussed in greater detail in the following section.

NCCs can be classified into several cell types that give rise to unique features in the embryo. These include trunk neural crest cells (TNCC), vagal neural crest cell (VNCC) and sacral neural crest cells (SNCC), cranial neural crest cells (CNCC), and cardiac neural crest cells (CarNCC). While it is not a comprehensive list, Table 1 describes some of the features that arise from each of these cell types. As this paper will focus on CNCC pathologies, special attention will be paid to tissues that arise from those cells.

Table 1. Neural Crest Cell Subtypes and a Selection of Derived Tissue Types. This table lists each subtype of neural crest cell and a selection of the tissues derived from these cell types (Hall, 2009).

NCC Subtype	Derived Tissue Types
Trunk neural crest cells (TNCC)	Merkel cells, Dorsal root ganglia, Chromaffin cells in adrenal medulla
Vagal neural crest cells (VNCC) and Sacral neural crest cells (SNCC)	Peripheral nervous system, Autonomic and parasympathetic nervous system, Schwann and glial cells, Enteric ganglia
Cranial neural crest cells (CNCC)	Mesenchyme, Connective tissue, Craniofacial cartilage and bone, Dentin, Parafollicular cells of the thyroid gland, Cornea, Ciliary muscle and muscles for eye attachment, Inner ear, Sensory ganglia of cranial nerves V, VI, IX, X
Cardiac neural crest cells (CarNCC)	Connective tissue for great vessels of heart, Heart septa, Smooth muscle of great arteries

Epithelial-to-Mesenchymal Transition

EMT is a multistep process, with the first being the loss of cell polarity and the second being the loss of cell-to-cell adhesion through the downregulation of E-cadherins. It is an important process in many developmental stages, but it most relevant to this paper in the context of NCC differentiation and migration.

E-cadherin molecules make up the epithelial junctional complex to form tight junctions (Ikenouchi, Matsuda, Furuse, & Tsukita, 2003). The presence of tight junctions designates an apical and basolateral membrane to a cell, giving it polarity. Tight junctions are also necessary for preventing the inappropriate diffusion of molecules across membranes (Shin, Fogg, & Margolis, 2006).

EMT allows NCCs to separate from the rest of the contiguous tissue and migrate. Thus, EMT is dependent on the downregulation of E-cadherins. This downregulation is a carefully regulated process. The transcription factor *Snail* represses E-cadherin expression by binding to the E-cadherin promoter. As a zinc-finger transcription factor, *Snail* binds to the E-boxes in the E-cadherin promoter region. It is also known to regulate claudin and occludin synthesis through the same mechanism (Ikenouchi, Matsuda, Furuse, & Tsukita, 2003).

Like *Snail*, another zinc-finger transcription factor involved in EMT is *Slug*. *Slug's* regulation is what provides NCCs with their specialization and migratory ability. *Slug* has been found to only be expressed in migrating NCCs, and is not found in the cell before it is ready to begin its migratory process (Savagner, Karavanova, Perantoni, Thiery, & Yamada, 1998).

Neural Crest Cell Differentiation and Migration

CNCCs give rise to various structures in the head and neck region, including sensory and autonomic ganglia as well as facial bone structures. Prior to migrating to their final site, and differentiating into CNCCs, NCCs must migrate from the neural tube. The neural tube is subdivided into rhombomeres, which dictate cell lineage and final differentiation. CNCC migration is unlike that of TNCCs, which is associated with molecules such as fibronecin, tenascin, and various other glycoproteins (Thiery, Duband, & Delouvée, 1982). Instead, rhombomeres segregate based on segmental specification. Most of these cells then migrate through the rostral half of the dorsal sclerotome and exit

at the level of their specified rhombomere. This allows for differentiation of cells and the creation of the specified structure (Lumsden, Sprawson, & Graham, 1991). Notably, CNCCs begin migration as sheets of cells, for the first 3-5 hours of migration. They then begin to lose intercellular adhesion and migrate individually (Alfandari, Cousin, & Marsden, 2010). On the other hand, TNCCs migrate as individual cells from the beginning (Czarnobaj, Bagnall, Bamforth, & Milos, 2014). Figure 2 will demonstrate the seven rhombomeres of CNCCs and their derived structures in the head and neck region.

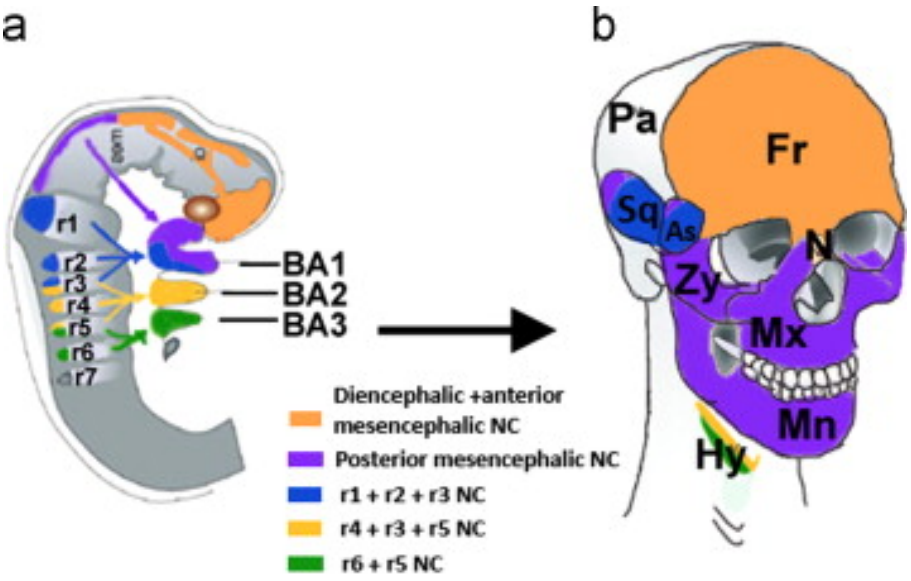

Figure 2. Segmental specification of rhombomeres to form structures derived from cranial neural crest cells. The neural crest cells of the embryo can be divided into seven rhombomeres, each of which migrates and develops into specific structures in the head neck (Gong, 2014).

Cranial Neural Crest Cell Differentiation

Once NCCs have undergone EMT, they are free to migrate to their specified sites. Some NCCs specifically become CNCCs that later go on to form cranial features. This differentiation is mediated by *Sonic hedgehog (Shh)* signaling, shown by one group to be required for CNCC participation in jaw development. In *Shh* knockout cells, CNCCs underwent apoptosis and did not form the appropriate structures (Brito, Teillet, & Le Douarin, 2006). This has since been confirmed in multiple studies showing the necessity of *Shh* signaling in the development of various structures derived from CNCCs such as the first pharyngeal arch (Yamagishi et al., 2006) as well as other craniofacial structures (Ahlgren & Bronner-Fraser, 1999).

Shh is a protein that plays a critical role in cell patterning at various stages of embryological development. Among other processes, *Shh* has been shown to drive sclerotome formation from somites. *Shh* protein acts through the *Patched (Ptc)* transmembrane receptor (Ahlgren & Bronner-Fraser, 1999). *Ptc* homologues were first identified in Drosophila, and then in mice. In mice, it was noticed that the expression of each homologue was specific to the tissue type, indicating specialization of function (Motoyama et al., 1998).

Development of Tissues Derived from CNCCs and CNCC Migration

Unlike *Shh* which has a role in the patterning process, *Wnt* signaling was thought to solely regulate cellular expansion by acting as a mitogen (Ikeya, Lee, Johnson, McMahon, & Takada, 1997). However, it has more recently been shown that *Wnt*

signaling can actually also specify NCC fate. It is needed for processes such as contact inhibition of locomotion (CIL) and collective chemotaxis, indicating its importance in NCC migration (Maj et al., 2016).

CIL is a process through which cells use contact signaling to change direction of migration. During EMT, expression of cadherins switches from E-cadherin to N-cadherin, such that chemokines can provide direction for cell migration (Bahm et al., 2017). As neurulation progresses, cadherin expression slowly shifts from E-cadherins, to N-cadherins. By the time the neural tube closes, E-cadherin expression is negligible, with N-cadherins being primarily expressed (Nakagawa & Takeichi, 1995). In mouse models, N-cadherin knockout mice showed abnormal somite migration. However, unlike *in vitro* models, some migration did occur, suggesting that other adhesion molecules were able to take over. One such suggested molecule is cadherin-6 (Radice et al., 1997). Chemotaxis is mediated by the platelet-derived growth factor (PDGF) receptor tyrosine kinase pathway that has been shown to be necessary in NCC proliferation, migration and EMT (Bahm et al., 2017). Studies in Xenopus have further shown that expression of cadherin-11 is required as the cell-cell adhesion molecule for CIL and collective migration. When the adhesive function of cadherin-11 was blocked in experiments, improper migration resulted. When cadherin-11 was overexpressed, migration of CNCCs was inhibited altogether (Becker, Mayor, & Kashef, 2014).

In vitro studies of NCC migration show that NCC populations invade mesoderm and ectoderm layers, but do not invade other NCC populations that are migrating in the opposite direction. In fact, when NCC populations encounter other populations of NCCs,

they penetrate deeper into mesodermal tissue. The same group confirmed these results *in vivo* and found similar results, further supporting the concept of CIL (Carmona-Fontaine et al., 2008).

NCC migration is dependent on actin filament interactions with the cytoskeleton. The gene *capzb* was initially identified through whole-genome sequencing in a patient with micrognathia, cleft palate, and hypotonia – all features associated with dysregulation of CNCC. Once the gene was identified, the group studied the protein in a zebrafish model. This gene encodes an actin-capping protein that binds to the growing end of F-actin and caps it in order to regulate cell locomotion. In *capzb* knockout individuals, CNCC migration was not possible and a phenotype similar to that of the patient in which the gene was identified resulted (Mukherjee et al., 2016). In a prior study by a different group, it was found that CNCCs undergo apoptosis when the actin-cytoskeleton interaction is interrupted (Hinoue Atsushi et al., 2005).

The process of induction to form specialized cells such as osteoblasts or chondroblasts has also been studied in great detail. Mandibular and maxillary bone, two craniofacial bones derived from CNCCs, form when mandibular and maxillary epithelia signals mandibular and maxillary mesenchyme and induces it to form osteoblasts. These osteoblasts then undergo intramembranous ossification to form structures (Hill, Yuasa, Schoenecker, & Goudy, 2014). In addition to craniofacial bones, it should be noted that CNCCs give rise to many other structures, such as some cells and tissues of the tooth including dental papilla and odontoblasts, through similar signaling pathways like *Wnt1* (Chai et al., 2000).

Importance of Understanding the Effects of Maternal Health on the Fetus

Teratogens are endogenous or exogenous compounds that can cause developmental issues in the embryo, and have significant effects. Examples of teratogens that will be discussed in this work are alcohol and maternal hyperglycemia. Fetal development is a tightly regulated process that is time-sensitive and embryogenesis is defined by various critical periods. For example, fetal exposure to ethanol on gestational day (GD) 7 to 7.5 caused apoptosis in the neuroectoderm, as most embryos undergo gastrulation by this stage. However, by GD 8, apoptosis is seen specifically in the neuroectoderm, and by GD 8.5, specifically in surface ectoderm (Dunty, Chen, Zucker, Dehart, & Sulik, 2001). At very early stages, cell death of an entire germ layer would cause massive consequences to the phenotype. In the case of fetal alcohol exposure, fetuses that were exposed to ethanol at gastrulation or neurulation underwent apoptosis of entire cranial NCC populations, whereas fetuses that were exposed to ethanol at somitic stages, as little as eight days post-conception, only underwent apoptosis in caudal cell populations, resulting in less severe damage (Cartwright & Smith, 2006).

Another teratogen known to cause CNCC defects is maternal hyperglycemic conditions. It has been shown that excessive hyperglycemia during gestation prevents CNCC from proliferating (Wang et al., 2016). In a clinical setting, maternal hyperglycemia can be managed. As suggested previously, proper maternal education during pregnancy can prevent severe fetal effects caused by this condition.

Understanding the ways in which maternal behavior affects the development of the fetus is essential from a public health standpoint.

While maternal alcohol consumption and maternal hyperglycemia begin through separate mechanisms, they both ultimately trigger NCC apoptosis, and cause similar effects in the fetus. Understanding the similarities between these conditions and the fetal effects they cause is important from a clinical perspective, as they provide an understanding of high-risk pregnancies. This literature review will examine current studies related to fetal effects on CNCC-derived structures as a result of exposure to alcohol and maternal hyperglycemia *in utero*.

Specific Aims and Objectives

- Examine the mechanisms of cranial neural crest cell migration and their dysregulation
- Explore the embryology and clinical effects of specific fetal syndromes related to cranial neural crest cell migration dysregulation
- Correlate syndromes of cranial neural crest cell migration defects with maternal health related causes
- Discuss public health implications of maternal behavior and health during pregnancy

MATERNAL ALCOHOL CONSUMPTION

Fetal Alcohol Syndrome

One teratogen that will be examined in this paper is maternal alcohol consumption. Fetal alcohol syndrome, a collection of phenotypes associated with maternal alcohol consumption during gestation, has been studied in great detail. Alcohol is a low molecular weight drug, and is able to pass from maternal circulation to the fetus via the placenta at levels such that alcohol concentrations are roughly equal in maternal and fetal blood. The first step of alcohol metabolism is the breakdown of alcohol to acetaldehyde by alcohol dehydrogenase, in the liver. Acetaldehyde is cytotoxic, mutagenic and interferes with microtubular structure during mitosis, causing chromosomal abnormalities. While alcohol has been shown to have direct negative effects on embryological development, the development of fetal alcohol syndrome is thought to be primarily mediated by acetaldehyde (Kumar, 1982).

Gestational exposure to ethanol is known to cause craniofacial deformities in the fetus, presumably by affecting CNCC development and migration (Zhang et al., 2017). Effects of alcohol on various cell types have been compared in multiple studies. Some of the earliest research established that neural tissue and craniofacial bones are most severely affected by maternal alcohol consumption (Clarren, Alvord, Sumi, Streissguth, & Smith, 1978). More recent groups have delved into alcohol's effects on these structures, and have focused on using them as diagnostic tools for fetal alcohol syndrome (Shen et al., 2013). Further research has shown that class IV alcohol dehydrogenase is

localized to CNCCs in the craniofacial region after neural tube closure. This has important implications in why maternal alcohol consumption so severely affects craniofacial bone (Ang, Deltour, Hayamizu, Žgombic-Knight, & Duester, 1996).

Craniofacial bones are formed by intramembranous ossification as CNCC-derived cells migrate inward and differentiate into osteoblasts (Zhang et al., 2017). To begin the process of craniofacial bone development, CNCCs migrate ventrolaterally until they make contact with pharyngeal ectoderm and endoderm. Once this occurs, CNCCs proliferate and form branchial arches. CNCCs from rhombomeres 1, 2 and 3 migrate into the first branchial arch, where they receive signals to regain polarity. This polarity orders the cells from maxillary to mandibular prominences (Huang & Thesleff, 2013). Without the inward migration of these cells, craniofacial bones are unable to form, leading to observable defects in facial features. It should also be noted that fetal alcohol syndrome includes a collection of other symptoms including, but not limited to, microcephaly, cardiac abnormalities, neurodevelopmental impairment, short stature, and seizures (Jones & Smith, 1975). While these symptoms have extremely important effects on patients' lifestyles, most pertinent to this discussion are the effects on craniofacial structures, due to the involvement of CNCCs. Figure 3 demonstrates common craniofacial abnormalities that have been associated with fetal alcohol syndrome.

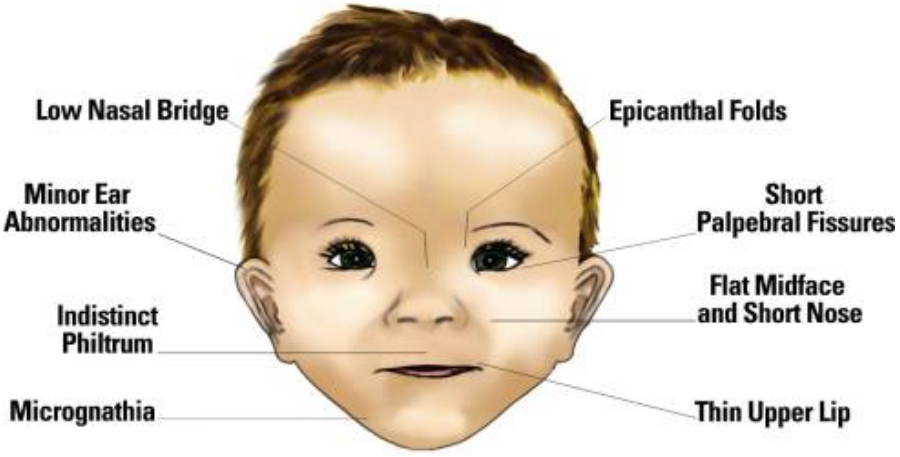

Figure 3. Craniofacial features characteristic of fetal alcohol syndrome. This image shows a variety of craniofacial deformities that are commonly indicative of fetal alcohol syndrome (Warren, Hewitt, & Thomas, 2011).

Early studies of the effects of acetaldehyde on fetal development show acetaldehyde to be a teratogen, causing growth defects in the very preliminary days of gestation (day 10). Acetaldehyde was shown to cause failure of neural tube closure, thus leading to many of the issues associated with fetal alcohol syndrome. It has been shown to have dose-dependent effects (O'Shea & Kaufman, 1979), although not all fetuses exposed to alcohol *in utero* develop fetal alcohol syndrome (Popova, Lange, Probst, Gmel, & Rehm, 2017). The intricacies of whether or not a fetus will develop congenital defects after exposure to alcohol *in utero* are not yet completely understood, leaving this open for future studies to examine. An understanding of the mechanisms that trigger effects in some fetuses and not others would be a valuable tool for clinicians.

Alcohol's Effects on Cranial Neural Crest Cell Migration and Pathogenesis of Fetal Alcohol Syndrome

A recent study examined alcohol's effects on craniofacial structures through its direct dysregulation of CNCC migration through the dysregulation of EMT. With ethanol treatment, CNCCs upregulate cadherin 6B, a molecule that is usually downregulated in normal CNCC migration. The downregulation of cadherin 6B is normally necessary for CNCC EMT. Other adhesion molecules found to be up-regulated include laminins and N-cadherins. The upregulation of these molecules further prevents the conversion of CNCCs into migratory cells. The regulation of N-cadherin and cadherin 6B expression is through the transcription factor *Slug* (Zhang et al., 2017). In *in vitro* studies, ventral migration was also found to be inhibited, with CNCCs seen to be amassing dorsally, when exposed to alcohol (Shi et al., 2014). Figure 4 describes several pathways that are disrupted by alcohol, leading to abnormalities in NCC development.

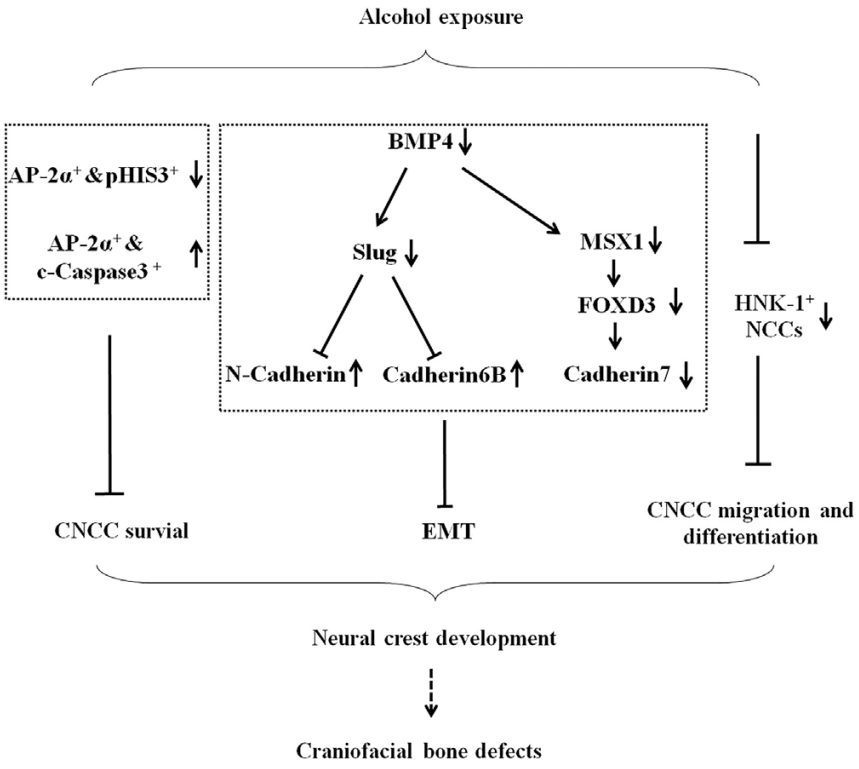

Figure 4. Summary of ethanol's effects on cranial neural crest cell development and migration. This image demonstrates the various pathways through which alcohol exposure disrupts neural crest cell development, thus causing craniofacial bone defects (Zhang et al., 2017).

Perhaps most importantly in the development of the craniofacial morphology characteristic of fetal alcohol syndrome, ethanol has been found to push CNCCs toward apoptosis. Ethanol causes oxidative stress in CNCCs, eventually triggering the apoptotic pathway. It has been found to decrease Bcl-2 levels, an anti-apoptotic protein. Additionally, it has been shown to increase p53 expression (Wentzel & Eriksson, 2009). The mechanism through which apoptosis is triggered has been studied in great detail. Chronic alcohol users have been found to have increased iron uptake at the intestinal

mucosa (Duane, Raja, Simpson, & Peters, 1992). While this addresses increased iron levels in the maternal tissue, it has also been shown that alcohol consumption during gestation increases fetal iron levels as well (Chen & Sulik, 2000). Furthermore, ethanol metabolism, and the subsequent depletion of NADH stores causes iron to be freed from intracellular stores, further increasing blood iron concentration. Due to oxidative stress, iron then leads to the formation of hydroxyl radicals, which induces apoptosis once cellular integrity is compromised (Nordmann, Ribière, & Rouach, 1992).

Folic Acid Deficiency and its Effects on Cranial Neural Crest Cell Apoptosis

CNCC dysregulation in fetal alcohol syndrome has also been shown to be linked to increased levels of maternal serum homocysteine (hyperhomocysteinemia), due to folate deficiency. Adequate folic acid levels have been shown to be crucial in pregnancy, for many reasons including folate's importance in hematopoiesis (Henry et al., 2017). Folate deficiencies have been found to cause megaloblastic anemia in pregnancy, leading to congenital birth defects (Chanarin, Rothman, Ward, & Perry, 1968). During pregnancy, folate requirements are elevated, and adequate folate levels are essential to the prevention of neural tube defects (Christensen & Rosenblatt, 1995). Deficiencies in folic acid and vitamin B12 prevent adequate activity of methionine synthase, resulting in elevated serum levels of homocysteine and deficiencies in methionine (Kirke et al., 1993). Via the placenta, homocysteine can enter amniotic fluid from the mother (Steegers-Theunissen et al., 1995). Because NCCs are unable to properly handle oxidative stress, the generation of reactive oxygen species by ethanol and acetaldehyde

cause apoptosis in CNCCs. Homocysteine is readily oxidized to homocystine in the blood, generating hydrogen peroxide (H_2O_2), superoxide ions (O_2^-) and other ROS. These compounds trigger apoptosis in many cell types including endothelial cells (Starkebaum & Harlan, 1986), and NCCs (Knott, Hartridge, Brown, Mansell, & Sandy, 2003).

Prevalence, Prevention, Screening and Diagnosis of Fetal Alcohol Syndrome

The relationship between maternal alcohol consumption during gestation and the incidence of fetal alcohol syndrome has been clearly demonstrated for years. In a study conducted by the Center for Disease Control (CDC) in 2012, it was found that 51.5% of non-pregnant women between the ages of 18-44 (including those women who are of child-bearing age) used alcohol within 30 days of being surveyed. More importantly, about 7.6% of pregnant women in the same age range used alcohol in that period. Amongst those pregnant women, the highest report of alcohol use was in women aged 35-44 years at 14.3%, white females at 8.3%, college graduates at 10%, and employed women at 9.6%. Notably, this study found that pregnant and non-pregnant binge drinkers consumed alcohol at the same frequency and intensity. The CDC continues efforts to incorporate education into clinical practice and community interventions in order to further bring these numbers down. The CDC has created questionnaires for use by family practitioners and obstetricians in their regular screenings among women of childbearing age. It has also pushed for the creation of public health policies that increase alcohol excise taxes in an effort to reduce alcohol consumption, and therefore misuse (Center for Disease Control and Prevention, 2012). Through a meta-analysis examining data from

studies conducted all over the world, one team was able to conclude that about one in 67 women who consumes alcohol while pregnant gives birth to a child with fetal alcohol syndrome (Popova et al., 2017). While the consumption of alcohol during pregnancy does not lead to fetal alcohol syndrome in every case, the implications remain extremely serious.

Currently in use are two screening questionnaires – TWEAK and T-ACE (Russell, 1994). In one study, these questionnaires, plus two more that are commonly used in traditional alcoholism screening, the MAST and CAGE, were compared for their efficacy in predicting whether mothers were at risk of drinking alcohol during pregnancy. The study found that TWEAK, a combination of T-ACE, MAST and CAGE, was effective, but that T-ACE was most effective when paired with MAST and CAGE screening tools (Russell et al., 1996). TWEAK measures five items and each item is described as such:

T – "Tolerance – How many drinks can you hold?"

W – "Worried - Have close friends or relatives worried or complained about your drinking in the past year?"

E – "Eye opener – Do you sometimes take a drink in the morning when you first get up?"

A – "Amnesia – Has a friend or family member ever told you about things you said or did while you were drinking that you could not remember?"

K(C) – "Cut down – Do you sometimes feel the need to cut down on your drinking?" (Russell, 1994).

Because symptoms can occur along a wide range, diagnosis of fetal alcohol syndrome can sometimes be difficult. However, proper diagnosis is important for many reasons, including proper patient care, and collection of proper statistics to calculate prevalence. Thus, in 2000, a group in Washington created the 4-Digit Diagnostic Code. This classification system includes four components – growth component, facial phenotype component, brain damage/dysfunction component and gestational alcohol exposure component. In each of these components, the patient can receive a score of 1 to 4, resulting in a combined score ranging anywhere from 1111 to 4444. The results of this score places a patient within the fetal alcohol syndrome and related fetal alcohol spectrum disorders scale (Astley & Clarren, 2000). Figure 5 is a demonstration of a hypothetical scoring code grid that is used by clinicians.

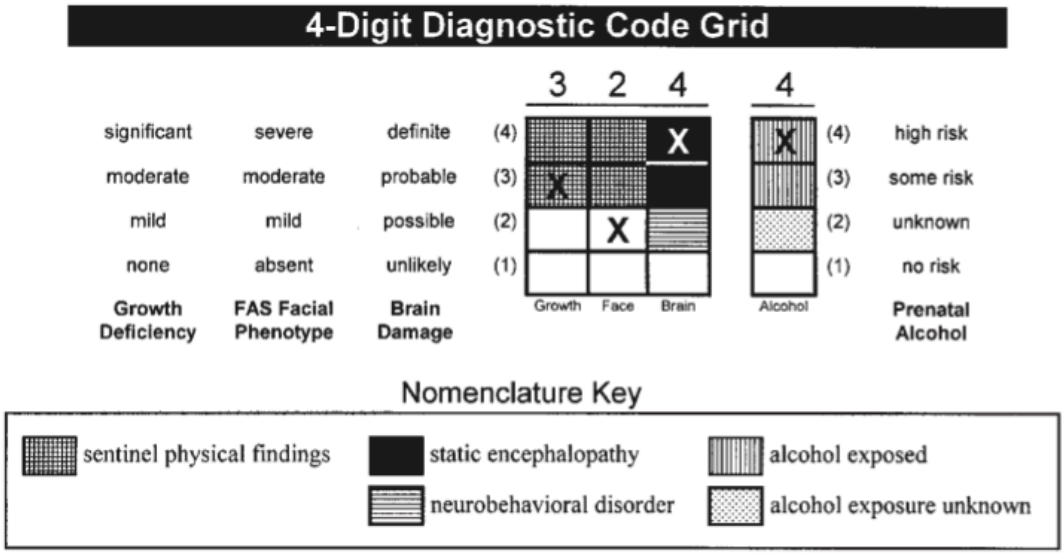

Figure 5. Example of 4-Digit Diagnostic Code. This figure provides example of the way in which a score is calculated in each of the four categories to give an overall composite score, leading to a diagnosis (Astley & Clarren, 2000).

While less common from a clinical perspective, other methods of diagnosis have been suggested as well. For example, the use of maternal blood markers to detect whether or not the fetus has been exposed to teratogenic levels of alcohol could be useful in determining risk (Stoler et al., 1998). A more common prenatal method of diagnosing fetal alcohol syndrome is through ultrasound. Detection of facial abnormalities and reduced frontal cortex size in fetuses with prenatal alcohol exposure can be an indication of fetal alcohol syndrome (Montag et al., 2016). Early detection and subsequent proper diagnosis are essential to ensuring proper treatment. Children with fetal alcohol syndrome are faced with a lifetime of issues including neurodevelopmental impairment and a variety of physical abnormalities (Jones & Smith, 1975). Thus, early detection of these conditions allows medical professionals to treat patients accordingly. Additionally, a proper understanding of the prevalence of fetal alcohol syndrome in various communities can allow professionals to create policy and public health interventions that are directed at decreasing alcohol exposure among women of childbearing age.

Potential Treatments

If a primary cause of CNCC apoptosis due to alcohol exposure is oxidative stress, potential treatments should ideally aim to prevent this. One study suggests that the compound sulforaphane, found abundantly in broccoli and similar vegetables, provides antioxidant benefits. Sulforaphane has been found to act through the Nrf2 signaling pathway that is normally involved in ethanol-induced apoptosis (Chen, Liu, & Chen, 2013). A transcription factor that regulates enzymes such as superoxide dismutase (SOD)

and catalase involved in the antioxidant response, and protects cells against damage and apoptosis, Nrf2 is upregulated by reactive oxygen species (Nguyen, Sherratt, Nioi, Yang, & Pickett, 2005). According to the study on sulforaphane, suppressing the Nrf2 signaling pathway suppressed sulforaphane's protective effects on CNCCs, further proving the necessity of this mechanism. Thus, consuming vegetables such as broccoli, especially in pregnant women with a history of chronic alcohol abuse, can provide a protective mechanism against the damage caused by reactive oxygen species that leads to the symptoms of fetal alcohol syndrome (Chen et al., 2013). In a research setting, adding SOD, an antioxidant enzyme, also decreased the production of reactive oxygen species and decreased the incidence of fetal defects (Wentzel & Eriksson, 2009).

Another important finding in the mechanism of CNCC dysregulation in fetal alcohol syndrome is maternal hyperhomocysteinemia due to folate deficiency. 5-methyltetrahydrofolate has been found to decrease homocysteine levels, and prevent many of its detrimental effects such as CNCC apoptosis dysregulation of migration (Shi et al., 2014). Additionally, exogenous folic acid and vitamin B12 supplementation in pregnant women has been found to decrease homocysteine levels (Xu et al., 2006).

Children born with fetal alcohol syndrome also commonly experience neurodevelopmental abnormalities, leading to behavioral issues and learning delays. Executive function, or the ability to plan, regulate actions, and monitor one's own behavior (Chan, Shum, Toulopoulou, & Chen, 2008), is affected in these children. Thus, these symptoms must also be considered in their management. Studies have found that neurocognitive habilitation programs, such as the Alert program, are in fact effective in

improving executive functioning in children with fetal alcohol syndrome. This program helps children become more cognizant of their own behavior by helping them identify their arousal level, and altering their behavior accordingly. The goal of this program, and others like it, is to provide children with the tools to monitor their own behavior (Wells, Chasnoff, Schmidt, Telford, & Schwartz, 2012).

MATERNAL GESTATIONAL DIABETES

Gestational Pre-Diabetes and Diabetes Mellitus

The World Health Organization (WHO) has classified levels of hyperglycemia during pregnancy into various categories. According to the WHO, mothers who are hyperglycemic during pregnancy should be diagnosed with one of the following – "Diabetes mellitus in pregnancy" or "Gestational diabetes mellitus." Diagnosis should be based on if the patient meets certain values in one or more of these tests – fasting plasma glucose, 2-hour plasma glucose, or random plasma glucose (World Health Organization, 2013). For the purposes of this thesis, only gestational diabetes mellitus (GDM) will be considered.

Incidence of GDM varies between various ethnic groups, with some having a higher risk than others. One group's meta-analysis estimated the number of cases of hyperglycemia in pregnancy based on world region. Table 2 describes the prevalence of hyperglycemia during pregnancy in various geographic regions. (Guariguata, Linnenkamp, Beagley, Whiting, & Cho, 2014).

Table 2. Prevalence of Hyperglycemia in Pregnancy Separated by Geographic Region. Prevalence of hyperglycemia in pregnancy calculated by dividing the cases of hyperglycemia in pregnancy by the number of live births reported in each world region. Adapted from *Global Estimates of the Prevalence of Hyperglycaemia in Pregnancy* (Guariguata et al., 2014)

Region	**Prevalence (Cases of hyperglycemia in pregnancy/Number of live births)**
Africa (Nigeria)	16.0%
Europe	15.2%
Middle East/North Africa	22.3%
North America	13.2%
South America (Argentina, Brazil, Cuba)	13.2%
South Asia	23.1%
Western Pacific (Australia, China, Japan, Malaysia, Singapore, Thailand, Vietnam)	11.8%

Amongst the potential causes of GDM, much attention has been given to maternal weight gain during pregnancy. One study conducted in a population of pregnant women in Wisconsin found that women who entered pregnancy with obesity were more likely to gain unhealthy amounts of weight during pregnancy (Lindberg et al., 2016). In addition to maternal weight, other risk factors include maternal age, a history of spontaneous abortions, family history of diabetes, persistent glucosuria, polycystic ovary syndrome (PCOS) and GDM in a previous pregnancy (Brown, 2016). Table 3 summarizes common risk factors that have been identified in patients with GDM. It also identifies a protective factor that has been shown to reduce the risk of developing GDM.

Table 3. Summary of Risk Factors and Protective Factors Associated with Gestational Diabetes Mellitus. A summary of risk factors and protective factors that have been associated with the occurrence of gestational diabetes mellitus. Adapted from *Epidemiology of gestational diabetes mellitus and its association with Type 2 diabetes* (Ben-Haroush A., Yogev Y., & Hod M., 2004).

Maternal Factors	Advanced maternal age, high parity, pre-pregnancy weight, pregnancy weight gain, BMI 27, short stature, low birth weight, α-thalassemia trait, polycystic ovary syndrome, high intake of saturated fat
Family History	Family history of diabetes, GDM in woman's mother
Previous Obstetric Outcome	Congenital malformation, stillbirth, macrosomia, caesarean section, previous GDM
Pregnancy Factors	Congenital malformation, multiple pregnancy, increased iron stores
Protective Factors	Young age

Pregnancy normally results in decreased insulin sensitivity through multiple mechanisms (Catalano, Tyzbir, Roman, Amini, & Sims, 1991). One such cause is hormonal. In a study conducted in a dog model, elevated levels of progesterone and estradiol, as seen during pregnancy, were studied. It was found that these hormones, along with placental hormones, decreased cells' sensitivity to counterregulatory hormones such as glucagon, epinephrine, norepinephrine, and growth hormone (Batista et al., 2005). Another hormone known to be produced at higher levels during pregnancy is leptin. While leptin production is usually associated with adipose tissue, the hormone has also been found to be produced by the placenta (Masuzaki et al., 1997). Leptin levels have been shown to correlate positively with BMI, and high leptin levels have been

shown to be predictive of development of GDM (Soheilykhah, Mojibian, Rahimi-Saghand, Rashidi, & Hadinedoushan, 2011).

Another mechanism known to decrease insulin sensitivity during pregnancy is inflammation. Pregnancy is pro-inflammatory, with decreased TNF-α levels in early pregnancy and increased production as pregnancy progresses into the third trimester. This change in TNF-α levels is inversely related to insulin sensitivity – as TNF-α levels increase, insulin sensitivity decreases (Kirwan et al., 2002). TNF-α has been found to downregulate IRS-1, an important protein that mediates insulin receptor tyrosine kinase activity (Hotamisligil, Murray, Choy, & Spiegelman, 1994).

While most risk factors associated with GDM are either intrinsic to pregnancy, or are based on environmental factors that surround the mother, GDM has also been found to have genetic components. It has long been understood that maternal glucose metabolism should adapt during pregnancy to adjust for both maternal and fetal needs. How this occurs is better understood through the identification of two particular genes, *BACE2* and *HKDC1,* that were only found to be expressed in pregnant cohorts (Hayes et al., 2013). Figure 6 shows the integration of multiple mechanisms that can eventually lead to GDM.

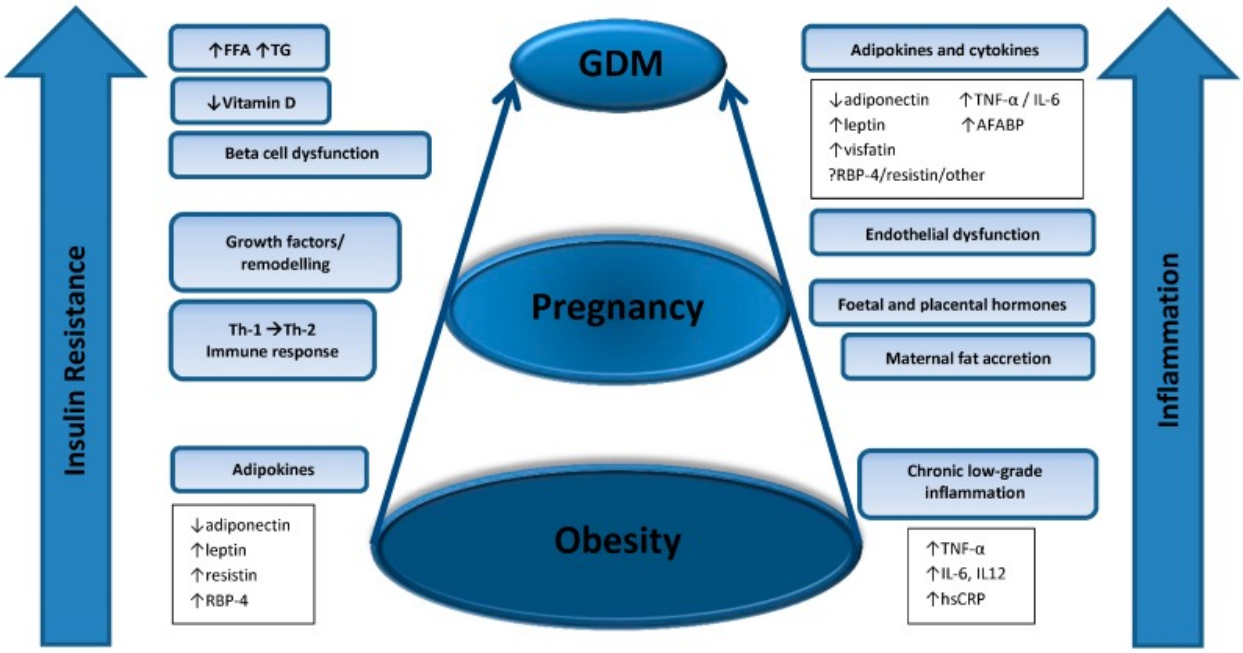

Figure 6. Model of Integration of Mechanisms Leading to GDM. This model integrates the numerous mechanisms that work synergistically to cause GDM in pregnant women (Abell, De Courten, Boyle, & Teede, 2015).

GDM-Induced Hyperglycemia and its Effects on Neural Crest Cells

Initially identified in rats, GLUT1 transporters have been found on the placental barrier in syncytiotrophoblast cells. The combination of gap junctions and glucose transporters allows for glucose to be transported from maternal blood to the fetus via the placenta (Takata, Kasahara, Kasahara, Ezaki, & Hirano, 1994). In diabetic pregnancies, GLUT1 transporter expression in the placenta has been shown to be increased two-fold. This indicates that hyperglycemia in the mother will lead to hyperglycemic conditions for the fetus as well (Gaither, Quraishi, & Illsley, 1999). Hyperglycemic conditions have long been shown to cause fetal macrosomia. Fetuses of GDM pregnancies have been found to be hyperinsulinemic. Glucose crosses the placental barrier and is a secretagogue,

triggering fetal pancreatic β-cells hyperplasia and hypersecretion of insulin. In primate studies, hyperinsulinemia was shown to cause hyperplasia and hypertrophy, particularly of soft tissue organs such as the liver, spleen and heart, leading to macrosomia (Susa & Schwartz, 1985). This has been confirmed in later studies in a fetal rat model. Injection of insulin increased rates of protein synthesis in these tissues, but did not affect rates of protein breakdown (Johnson et al., 1990). More recent studies have examined the effects of fetal hyperinsulinemia to better understand the mechanism of macrosomia. It has been found that hyperinsulinemia in the fetus leads to higher rates of farnesylation of p21-Ras, a key checkpoint in mitosis. Increased farnesylation indicates increased rates of mitosis, promoting growth of these tissues (Thureen et al., 2006).

The mechanism by which GDM affects NCCs has best been described in CarNCCs, even though it is similar in CNCCs as well (Eriksson & Borg, 1993). One of the ways in which maternal hyperglycemia acts as a teratogen in fetuses is via oxidative stress. Increased levels of glucose call for increased mitochondrial activity. This results in higher rates of electron transport chain activity, and other oxidative mechanisms, ultimately leading to an increased production of ROS. The same study to identify this phenomenon was also among the first to associate the production of ROS with incidences of congenital defects. By adding SOD, an antioxidant enzyme, the experimenters were able to decrease the rate of occurrence of the defects, thus implying a correlation between the production of ROS and of the likelihood of the fetus developing a congenital defect (Eriksson & Borg, 1993). Another study found that the two main pathways of DNA damage repair, the Ataxia-telangiectasia mutated-checkpoint 2 (ATM-Chk2) and ATR-

Rad3-related-checkpoint 1 (ATR-Chk1) were more likely to be elevated in the embryo. Also shown to be elevated was the cell-cycle check point p53, under maternal hyperglycemic conditions. Together, these two features indicate that maternal hyperglycemia causes increased amounts of DNA damage in the embryo (Dong et al., 2015).

Interestingly, recent research has revealed some new findings in this field of study. Two separate groups have been able to find a correlation between GDM and upregulation of autophagy in placental tissue as a mechanism of pathogenesis in the fetus (Avagliano et al., 2017). One group went further to correlate the generation of oxidative stress in fetuses of diabetic pregnancies with the increased levels of autophagy (Ji et al., 2017).

Unlike fetal alcohol syndrome, GDM does not always cause a predictable constellation of congenital defects. However, in addition to macrosomia, meta-analysis studies have found a wide range of congenital defects that have been associated with maternal DM and obesity. Of these defects, those that are pertinent to structures derived from NCCs, and in particular CNCCs, include cataract/corneal opacity, orofacial clefts (Moore, Singer, Bradlee, Rothman, & Milunsky, 2000), and cranial nerve malformations (Cederberg, Picard, & Eriksson, 2003). It should be noted that these effects stem from neural tube closure defects (Moore et al., 2000). One group has suggested that fetuses of diabetic pregnancies sometimes express a collection of characteristics similar to those seen in DiGeorge anomaly. This includes low-set external ears, poorly formed Meckel's cartilage, small thyroid and thymus, and the lack of a parathyroid gland. All of these

organs or structures contain cells that are derived from CNCCs. Also of note are cardiac issues in this constellation of features. This group suggests that the incidence of DiGeorge anomaly in diabetic pregnancies can be associated with the diabetes itself (Martin, Gittenberger-De Groot, Wisse, & Erikkson, 2000). Additionally, CarNCCs are also greatly affected, leading to septal closure defects (Moore et al., 2000) and outflow tract defects (Morgan, Relaix, Sandell, & Loeken, 2008).

While the mechanisms through which CNCCs and CarNCCs are affected by GDM are almost identical, much more research has been conducted in the area of CarNCCs and the associated congenital defects (Chan, Cheung, Yung, & Copp, 2004). Therefore, the intricacies of the mechanisms will be explained in the context of CarNCCs here. Hyperglycemia, through the associated oxidative stress, causes apoptosis of CarNCCs, resulting in defects in the outflow tract, the structure through which blood passes in the heart before exiting through the major arteries (Morgan et al., 2008). Like CNCCs, CarNCCs, migrate from the neural tube at the somite stage. Crucial to this migration is the Pax-3 transcription factor (Chan et al., 2004). Pax-3 is also required at the time of neural tube closure during embryological development, as it suppresses p53-induced apoptosis, rather than modifying p53 gene expression (Pani, Horal, & Loeken, 2002). In the embryos of diabetic pregnancies, Pax-3 is underexpressed. The mutant allele, called *Splotch*, contains a single base substitution that renders one of its DNA-binding domains non-functional. Thus, the underexpression of Pax-3 results in higher levels of apoptosis (Phelan, Ito, & Loeken, 1997).

Among the CNCC-derived structures affected by GDM, cranial nerve formation has been studied in diabetic rat models. Hyperglycemic environments were shown to cause cell death in CNCCs, impairing the development of cranial nerves V, VII, VIII, IX and X. This experiment also confirmed that hyperglycemic conditions caused a downregulation of Pax-3, inducing CNCC apoptosis. The experimenters in this study note that they did not examine whether this impairment in development was later recovered during development, as cranial nerves derive from CNCCs and ectodermal placode. Thus, the experimenters conclude that it is possible, at a later stage in development, that the ectodermal placode is able to compensate for the lack of CNCCs (Cederberg et al., 2003).

Gestational Diabetes Screening and Diagnosis

The oral glucose tolerance test has long been in use to screen for GDM in pregnant women. Screening is normally done at 24-28 weeks of gestation. The test is performed after overnight fasting, and is done by administering 75g glucose dissolved in 200-300ml of water orally. Plasma glucose levels are then measured after 2 hours. Based on the plasma glucose levels measured, patients should be diagnosed as having either "diabetes in pregnancy" or "gestational diabetes mellitus." The term "diabetes in pregnancy" is reserved for more severe cases, while the term "gestational diabetes mellitus" can be used to describe patients who meet a lower threshold (World Health Organization, 2013). The values used in diagnosis are given in Table 4.

Table 4. Diagnostic values of gestational diabetes mellitus vs. diabetes in pregnancy. This table summarizes the diagnostic plasma glucose levels to differentiate between severity of hyperglycemia during pregnancy. Adapted from values listed in *Diagnostic Criteria and Classification of Hyperglycaemia First Detected in Pregnancy* (World Health Organization, 2013)

	Gestational diabetes mellitus	**Diabetes in pregnancy**
Fasting plasma glucose	5.1-6.9mmol/l (92-125mg/dl)	≥7.0mmol/l (126mg/dl)
2-hour plasma glucose	≥10.0mmol/l (180mg/dl) following 75g oral glucose dose	≥11.1mmol/l (200mg/dl) following 75g oral glucose dose
Random plasma glucose	8.5-11.0mmol/l (153-199mg/dl) following 75g oral glucose dose	≥11.1mmol/l (200mg/dl) in the presence of diabetes symptoms

Similar testing is performed after the pregnancy ends, to re-evaluate if the case was one of GDM or type II diabetes mellitus. Women with risk factors for GDM should be tested earlier in their pregnancy (World Health Organization, 2013). The importance of the oral glucose tolerance test, and screening in general, has been reiterated in literature. One study found that universally screening all pregnant women, rather than only screening those with known risk factors, resulted in a 2.7% higher incidence of GDM (Griffin et al., 2001), suggesting that many cases are not recognized without screening.

While the oral glucose tolerance test remains the primary clinical method of diagnosis, other methods of testing are being studied in clinical trials. The oral glucose tolerance test has been criticized of being inconvenient for patients, requiring an overnight fast. Instead, many of these methods involve a blood draw, a procedure that is

already commonly done during pregnancy to test for other lab values. One such test includes measuring plasma sex-hormone binding globulin (SHBG) levels. Low levels of SHBG are often found in patients with polycystic ovary disease, and have been found to be associated with a higher risk of developing GDM (Veltman-Verhulst et al., 2010). Because of the inflammatory mechanisms known to cause GDM, inflammatory markers can also be used for diagnosis. TNF-α levels have been found to be elevated in patients 11-13 weeks of gestation who were likely to develop GDM later in pregnancy (Syngelaki, Visser, Krithinakis, Wright, & Nicolaides, 2016). Blood HbA1C levels, commonly used in the diagnosis of diabetes in non-pregnant populations, have not been found to be as effective of an indicator of GDM. In non-pregnant populations, the threshold of HbA1C is 5.7%, whereas in pregnant populations, HbA1C levels above 5.9% may be an indication of GDM (Hughes, Moore, Gullam, Mohamed, & Rowan, 2014).

Treatment, Management and Prevention of Gestational Diabetes Mellitus in Mothers

It has long been understood that women with GDM have a high likelihood of progressing to type 2 DM post-partum. However, studies have found that the risk can be decreased with healthy dietary habits (Tobias et al., 2012). GDM has also been found to increase the risk of cardiovascular disease and other comorbidities, especially in women with a family history of type 2 DM (Carr et al., 2006).

Due to the prevalence of GDM, its treatment options have been studied in great detail. The American Diabetes Association (ADA) has created "Standards of Medical Care in Diabetes," a document that details treatment options in diabetes, and specifically in GDM. In addition to specifying when mothers should be screened, it also details glucose monitoring practices, and management options. Lifestyle management, through dietary habits, physical activity, and weight management are the first line of treatment. Beyond that, pharmaceutical treatment includes sulfonylureas, metformin, and insulin. According to the American Diabetes Association, insulin is the first recommended option, as it does not pass the blood-placenta barrier and affect the fetus (The American Diabetes Association, 2018). However, multiple studies have in fact shown that metformin is as effective, or possibly even more effective, than insulin in reducing serum glucose levels (Beyuo et al., 2015) and does not have any adverse outcomes on the development of the fetus (Tertti, Laine, Ekblad, Rinne, & Rönnemaa, 2014). Studies by the same group have even measured neurodevelopment outcomes of 2-year old children whose mothers were prescribed metformin during pregnancy, and found no adverse effects (Tertti, Eskola, Rönnemaa, & Haataja, 2015). Based on these findings, metformin alone can be used as an alternative to insulin in the treatment of GDM. This is of particular importance in developing countries where cheap and easily accessible therapies are of utmost importance (Ainuddin, Karim, Hasan, & Naqvi, 2015).

Another potential therapy is the supplementation of vitamin D. While not yet completely understood, vitamin D supplementation in women with GDM has been shown to increase glucose tolerance and metabolism. Initially noticed in observational studies

(Rudnicki & Mølsted-Pedersen, 1997), the phenomenon has been further studied since. The mechanism is not yet completely understood, but further studies have shown that co-treatment with vitamin D and omega-3 fatty acids leads to increased levels of serum vitamin D concentrations, which in turn promotes improved glycemic control and decreased serum triglyceride and VLDL-cholesterol levels (Jamilian et al., 2017).

While treatment options are now abundant for patients with GDM, prevention of the disease is still the ideal way to avoid affecting the development of the fetus. Abundant research has been conducted in the field of maternal GDM prevention through lifestyle change. Initially conducted in Finland (Rönö et al., 2014), randomized control trials around the world have found mixed results in the benefits of an exercise regimen in preventing GDM. One study found that moderate-intensity exercise in the second and third trimesters could decrease some of the negative outcomes of GDM in both mother and fetus (Barakat, Pelaez, Lopez, Lucia, & Ruiz, 2013). Another study found no significant benefit in similar patient populations (Stafne et al., 2012).

Treatment and Management of GDM-Induced Effects in the Fetus

It is now well understood that maternal hyperglycemia leads to fetal hyperglycemia, instigating a range of effects in the developing fetus. Management of maternal GDM is the primary method to minimize effects on fetal development. However, in cases that maternal GDM is not controlled, most of the fetal effects have been shown to be caused by the creation of oxidative stress, most likely from the

increased oxidation of glucose. In experiments, antioxidant enzymes such as SOD were shown to decrease the severity of congenital defects (Eriksson & Borg, 1993).

Also shown to decrease the incidence of defects is the injection of arachidonic acid. The treatment with arachidonic acid is of particular interest to this discussion, as it was shown to decrease the incidence of cleft palate (Goldman et al., 1985), a congenital defect known to be associated with CNCCs. However, this is not yet a viable option as therapeutics. Therefore, antioxidant supplementation has been studied for many years. In one study, women with GDM were given varying doses of a combination therapy of vitamin E and vitamin C. Compared to women given the high-dose and untreated women, women given the low-dose showed intermediate rates of occurrence of malformations by preventing apoptosis. Women given the high-dose showed the greatest decrease in rates of occurrence. However, it should be noted that the combination therapy, administering both vitamins in one solution, was not more efficient than administering each of the vitamins alone. This indicates that the doses of each vitamin, rather than the overall molarity of antioxidants given, is most important to determining the level of results seen (Cederberg, Simán, & Eriksson, 2001).

CONCLUSION

Embryology, Migration Patterns and Dysregulation of Cranial Neural Crest Cells

NCCs are a special group of cells that arise with neurulation during the early weeks of embryogenesis. Once NCCs break off from the neural tube, they divide into various subtypes, including CNCCs and CarNCCs. Each of these subtypes gives rise to a variety of structures throughout the fetus. For example, CNCCs give rise to craniofacial bone, parafollicular cells of the thyroid gland, connective tissues such as Meckel's cartilage, the cornea, and some cranial nerves. CarNCCs give rise to the septa of the heart, the outflow tract, and other cardiac structures. The differentiation of these cells and their migration are extremely intricate processes that depend on tightly regulated gene expression mechanisms. Signaling pathways such as *Shh* allow for cells to differentiate appropriately.

Also important to the development of NCC-derived structures is EMT. EMT is another process that is tightly regulated by signaling pathways such as *Wnt*, Notch, and more. It is characterized by two important steps – the loss of cellular polarity, and the detachment from neighboring cells via the downregulation of E-cadherins. EMT is crucial to the migration of NCCs, and is a common step along the pathway of NCC-derived structure formation where problems can occur.

Importantly, in order for gene expression and EMT to occur as expected, cells should be surrounded by ideal conditions. Without an environment in homeostasis, gene expression changes and NCCs undergo apoptosis. Depending on the stage of development at which cells undergo apoptosis, the effects on the fetus will vary. As such,

earlier exposures to teratogenic conditions leads to apoptosis in more primitive structures, leading to more drastic effects in the fully developed fetus.

Comparison of Maternal Alcohol Consumption and Maternal Gestational Diabetes and their Effects on Cranial Neural Crest Cells

While the conditions under which maternal alcohol consumption and maternal GDM affect fetal development are very different, the similarities in the pathogenesis of their congenital defects is remarkably similar. The main mechanism through which CNCC or CarNCC structures are malformed is through apoptosis. NCCs undergo apoptosis when vital genes are no longer expressed properly. The main mechanism through which cells were affected in both cases of maternal alcohol consumption and cases of GDM was through oxidative stress. The generation of reactive oxygen species is extremely harmful to the health of the cell. Without an adequate antioxidant response, cells are unable to handle the stress that free radicals generate, and apoptotic pathways are triggered. In the case of maternal alcohol consumption, Bcl-2 levels dropped and p53 expression increased, leading to cell apoptosis. In the case of GDM, Pax-3 was downregulated, leading to less suppression of p53-induced apoptosis.

While the end mechanism through which cells ultimately underwent apoptosis is similar, what is of most interest is the mechanism by which cells reached that point in each of these pathogenic processes. In cases of maternal alcohol consumption, ethanol metabolism leads to the depletion of NADH, leading to increased release of iron from its stores. Iron, in turn, leads to the generation of free hydroxyl radicals. In the case of

maternal GDM, increased glucose levels lead to increased glucose metabolism, which causes increased amounts of oxidative phosphorylation to occur. As a byproduct, increased levels of reactive oxygen species are generated. In both cases, apoptosis is triggered by the generation of reactive oxygen species, such as free hydroxyl radicals.

Because of the similar pathologies, both conditions are treated in very similar ways. Antioxidants are the primary therapy in decreasing reactive oxygen species, and thus preventing NCC apoptosis. Studies in fetal alcohol syndrome have looked at compounds such as sulforaphane, found in broccoli and similar vegetables. Its mechanism as an antioxidant decreases the formation of reactive oxygen species. Studies in maternal GDM cases have looked at vitamin supplementations, such as vitamin C and E to reduce reactive oxygen species generation and vitamin D with omega-3 fatty acids to reduce serum triglyceride levels.

It is of great interest that maternal alcohol consumption and maternal GDM can result in similar conditions in the fetus. While fetal alcohol syndrome has a collection of symptoms that can be grouped together, congenital defects associated with maternal GDM are not as straightforward. Cardiac defects are extremely common, and one study suggests that the constellation of symptoms known as the DiGeorge anomaly can often be attributed to GDM. Regardless of the name given to the group of symptoms seen in the fetus, what is remarkable is the amount of overlap between the two conditions.

Public Health Implications

Congenital defects in NCC-derived structures can have grave implications in patients' lives. Craniofacial abnormalities can have consequences on the function of the structures, but can also mean a lifetime of social insecurities. Cardiac defects in the outflow tract can lead to severe debilitation, as can abnormalities in thyroid or parathyroid gland structure. However, these congenital defects can largely be prevented through the maintenance of maternal health. As such, keen attention should be paid to keeping mothers healthy when they are conceiving and during gestation (Brown, 2016).

One of the most thorough ways to do so is through adequate prenatal care. Women should ideally have regular access to an obstetrician who can screen for various conditions and manage treatment options if necessary. Prenatal screenings, including those that measure the likelihood of risk drinking during pregnancy, such as TWEAK, and those that are used to diagnose GDM, such as the oral glucose tolerance test, should be done at appropriate points either prior to conceiving (Russell, 1994), or at about 24-28 weeks of gestation (The American Diabetes Association, 2018), respectively. In the case that treatment is needed, regular follow-up with a clinician is important to properly regulating the therapy. However, the reality is that many women, both internationally and domestically, are unable to access a physician with frequency (Loveland Cook, Selig, Wedge, & Gohn-Baube, 1999), or pay for many therapies (Pathak, Singh, & Subramanian, 2010). Thus, these conditions become serious public health concerns. While groups such as the World Health Organization and the American Diabetes Association frequently release literature that determines the guidelines of screening and

therapy, the reality is that healthcare access is still a large concern for many women. Thus, service organizations throughout the world should continue to identify these issues and find innovative ways to provide healthcare to women of all socioeconomic statuses all over the world.

Future Directions

Current research in the fields of fetal alcohol exposure and maternal GDM-induced hyperglycemia exposure has mostly been in understanding the mechanism of pathogenesis. The biochemical processes that lead to cell apoptosis, and thus congenital defects, have been elicited in great detail as a combined effort by research groups around the world. By understanding the mechanisms, scientists are able to identify the exact risks that mothers and their fetuses encounter in high-risk pregnancies.

Research has also begun to identify an assortment of treatment options for these conditions. Armed with a thorough understanding of how NCC processes go awry, researchers have been able to assess which therapies might improve congenital outcomes. However, these therapies are still not always viable from a patient standpoint. Those that have proven to be most effective in the lab, such as the addition of SOD, are not possible treatments in the clinic. Supplementation with vitamins and other such compounds is possible, but is not always effective in completely preventing any symptoms in the fetus.

Thus, future directions in this field of study should be to identify treatment options that are viable in the field. When considering communities of lower socioeconomic backgrounds, or international communities that have few resources, it is

important to design therapies that are cost-effective and easily accessible. Future research should come from a public health and pharmaceutical standpoint, focusing on creating affordable and effective therapeutics.

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