Epigenetic Pathogenesis of Neurological Disorders in utero and Considerations for Genetic Counseling

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Abstract

Epigenetic modifications are a major focus of study in the pathogenesis of many disorders regarding metabolism, aging, neurodevelopment, and neurodegeneration. Epigenetic mechanisms are present throughout life but are especially vital to guiding fetal development. The precise timing of gene activation and deactivation guides stem cell differentiation through each embryonic stage. After exposure to environmental stimuli, gene expression can be altered by transcription factors, resulting in observable phenotypes and even pathology. Here, the epigenetic mechanisms responsible for the pathogenesis of neurodevelopmental and neuropsychiatric disorders are explored in response to environmental perturbations in utero. The present goal is to identify correlations between biochemical mechanisms of neurodevelopmental etiologies and those of psychiatric disorders. Based on these identified commonalities, future research of targeted epigenetic therapies may be suggested in addition to novel genetic counseling strategies or therapies with epigenetic foundations.

Epigenetic Pathogenesis of Neuropsychiatric Disorders in utero

The genome is the blueprint to life, comprised of information to execute all activities required for a cell to survive and thrive. Epigenetics (literally "above genetics") is the study of how gene function is altered apart from direct changes in gene sequence (Kubota et al., 2015; Millan, 2013). These alterations may be inherited mitotically or meiotically and can be regulated based on the needs of the organism as well as in response to environmental signals. Both the genome and epigenome affect aspects of physiology, biochemistry, and pathophysiology. Sometimes, even in the absence of a genetic code mutation, an epigenetic change may ultimately lead to observable deviations from normal functioning. The underlying biochemical mechanisms of disease development and progression are the subject of much research in genetics, physiology, and psychology and lead to greater understanding of normal and abnormal development. Specifically, fetal neurological development is vulnerable to the effects of epigenetic alterations, leading to permanently altered biochemistry that may later result in neurodevelopmental disorders or even psychosis (Millan, 2013).

The epigenome may be explored for sources of neurological disease (ND) due to peculiarities observed in the manifestations of these disorders that cannot be explained by genetics alone. Parent-of-origin effects, sexual dimorphisms, and pathological inconsistencies observed in monozygotic twins are only a few examples of observable epigenetic ramifications. Environmental hazards such as maternal nutrition, exposure to toxins, and occurrences of stress or trauma can also negatively influence the fetal epigenome, resulting in abnormal neurodevelopment and potentially disease later in life. Environmental influences reverberate through the epigenome for generations, and the study of how they are integrated in ND etiology

will lead to a better understanding of specific risk factors, enhancing the breadth and depth of knowledge regarding fetal development. Because the epigenome is dynamic, changes to it may be reversible; this allows for future research in the development of epigenetic therapies, further contributing to medical knowledge, therapeutic strategies, and even shaping the future of genetic counseling.

Prevalence of Neurodevelopmental Disorders

Neurodevelopmental disorders (NDDs) have been a focus of psychological, biological, and genetics research for years, first officially introduced as a class of disorders in the DSM-5 in 2013 (Morris-Rosendahl & Crocq, 2020). The psychological definition specifies this group of disorders to have onset in the developmental period and involve deficits that lead to decreased functioning; included are learning and intellectual disorders, communication disorders, autism spectrum disorders (ASD), attention deficit and hyperactivity disorders (ADHD), and those that involve abnormal motor functions (i.e. tics) (American Psychiatric Association, 2013). Similar neuropathology, symptoms, and shared genetic mutations have been discovered throughout the group so that there is evidence to view it as a single spectrum rather than compartmented disorders (Hansen et al., 2018). Classified by atypical cognitive development, a possible low intelligence quotient, and certain functional or behavioral abnormalities that are unique to every case, there are obvious clinical challenges to classifying these disorders (Márquez-Caraveo et al., 2021).

NDDs are emerging as the leading cause of childhood morbidity, decreasing quality of life for the children affected as well as their families, and comorbidities further hinder treatment options and prognosis (Hansen et al., 2018). The worldwide distribution and prevalence of

NDDs constitutes a public health focus and explains why they are a primary focus of genetics and biochemical research. With advancements in these areas of study, NDDs can be better understood on a biological level, furthering medical knowledge in fields of psychiatry, medical genetics, pediatrics, and genetic counseling. There may be epigenetic bases for the origination of NDDs in utero after exposure to environmental risk factors, and these etiologies may be shared with those of neuropsychiatric disorders (NPDs) – with this understanding, future outlooks of genetics research and genetic counseling for neurological disorders rooted in the epigenome may be suggested.

The Search for Biological Roots of NDDs

Environmental and genetic associations to ND development are constantly being discovered. Modern science and technology have allowed for rapid advancements to be made in studying the genetics and biochemistry of NDDs and NPDs, there is hope to draw more connections between genotypes and phenotypes. However, most cases are not observed to have Mendelian patterns of inheritance; rather, they are the outcome of a combination of many pathogenic variances and possibly environmental influences (Morris-Rosendahl & Crocq, 2020). This suggests that although gene mutations may prompt the development of some neurological disorders, modifications of DNA activity by chemical alterations to the base sequence is more likely in some cases (Zahir & Brown, 2011). Many still have no genetic links, suggesting that the biological root of these cases may instead be found outside (or on 'top of') the genetic code.

Mutational Origins of NDDs

Over time, searching within the genome has exposed certain pathological variants that contribute to the development to physiological and psychological disorders. Point mutations,

chromosomal rearrangements, and copy number variations have all been associated with clinical cases of neurological dysfunction, among other genetic mechanisms (Parenti et al., 2020). These molecular diagnoses are helpful in drawing connections between genotype and phenotype, but challenges are still present due to the heterogeneity of clinical manifestations even in cases of similar genetic etiology (Parenti et al., 2020). It is therefore postulated that NDDs may arise from environmental influences or the interaction of multiple mutations (Zahir & Brown, 2011). For this reason, familial studies are helpful to study genetic and environmental influences on the development of NDDs, and twin studies are particularly insightful for this analysis (Parenti et al., 2020). Tracing the genetic roots of NDDs helps to unveil disordered molecular functioning. Whether the dysfunction is due to a change in gene sequence or change in gene regulation sheds light on the genetic or epigenetic nature of NDD development.

Many disease genes, especially within the context of NDDs, have been discovered during a "reverse genetics" research process, whereby phenotypes are observed and their correlating common genetic markers are subsequently identified among patients with that phenotype versus a set of controls (Morris-Rosendahl & Crocq, 2020). This process is akin to finding a needle in a haystack, as it is difficult to pinpoint a gene with little to no knowledge of its protein or function. However, this technique is less daunting with the advent of genome-wide analysis techniques that integrate new information to adjust definitions of phenotypes based on discoveries of common alleles. The goal of this process is to correlate phenotypic data with allele frequencies to define molecular and genetic subtypes of a disease without prior knowledge of the affected biochemical pathway or protein product (Orkin, 1986).

Reverse phenotyping has aided in the discovery of many NDD-causing genes (as well as many other physiological disorders which are beyond the scope of this paper). However, this is not a linear process; extensive heterogeneity is observed in this class of disorders. More than one NDD may be linked to a pathogenetic mutation, and variable expressivity of single gene variances may also be observed (Cardoso et al., 2019). However, many NDDs display molecular convergence, reflecting different epigenetic landscapes, binding patterns, and gene expression that give rise to one disorder over another (Chen et al., 2014). While the same biochemical pathways (or genes) may be etiologically involved in separate disorders, it is the regulation of these genes or pathways that determine the fate of NDD and psychosis progression. Moreover, by studying the molecular manifestations of NDDs, it can be observed that not only does the genetic code play a role in guiding normal and pathological functioning, but the epigenome may as well.

Biochemical Mechanisms of NDD and NPD Progression

There is mounting evidence not only for biological roots of NDDs and NPDs (together referred to as neurological disorders, NDs), but that the presence of certain alleles in conjunction with environmental factors may determine the trajectory of disease development due to pleiotropic effects (Rees et al., 2021). Over the past decade, researchers have sought to elucidate the biochemical mechanisms by which NDs arise, such as the Psychiatric Cell Map Initiative, aiming to determine the molecular pathophysiology of NDs and define key biological pathways affected in this class of disorders (Parenti et al., 2020). Many of the same genes have been associated with multiple NDs with varying prevalence and are mostly involved in a few main biological pathways, including protein synthesis, regulation of transcription, and synaptic

signaling (Belger et al., 2011). Identified mutations may accumulate and further predispose a person to developing a ND, or may support a two-hit model of ND etiology whereby two mutated alleles must be present to fully incapacitate normal neurodevelopment (Parenti et al., 2020).

Many ND pathologies can be traced to the molecular level: ultimately it is altered biochemistry that leads to observed neurological manifestations, suggesting that genetic or epigenetic roots are possible. If altered base pair sequences can pathologically influence the biological pathways described above, so may an alteration in the regulation of those genes. Some of these molecular pathologies particularly influence fetal neurodevelopment, such as disturbances of DNA regulation, dysregulation of protein transcription, and alterations to synaptic signaling (Maston et al., 2006; Monk et al., 2019; Mossink et al., 2021).

Disturbances of DNA Methylation Cause NDDs

DNA methyltransferases (DNMTs) are proteins that act on chromatin to methylate certain gene sequences, inhibiting their transcription. These enzymes are essential to determine a methylation signature during prenatal development via a global demethylation process at conception and subsequent, specific remethylation of the genome (Mossink et al., 2021). DNA methylation is indispensable for proper fetal development by guiding tissue-specific gene expression, silencing repetitive DNA elements, and maintaining genomic stability (Morris-Rosendahl & Crocq, 2020). The crucial role of DNMTs during neurodevelopment is further confirmed by the observation that the highest levels of methylation are found within the brain (Moore et al., 2013).

Mutations in DNMTs underlie NDDs due to their imperative role in cell differentiation and maturation (Cristancho & Marsh, 2020; Moore et al., 2013). Downregulation of DNMTs in response to fetal stress due to prenatal malnutrition or toxin exposure may ultimately lead to impaired development, causing reduced neuronal size and synaptic plasticity, correlating to decreased functioning in learning and memory when the hippocampus is affected (Feng et al., 2007; Kubota et al., 2015). DNA methylation and demethylation are important for activation of long-term potentiation (LTP) genes during neurological development by altering the transcription factor binding sites (Muñoz et al., 2016). LTP genes are associated with the strengthening of neurological synapses; diminished expression causes decreased hippocampal-dependent memory formation (Jung et al., 2013). Decreased LTP has also been attributed to defects in the MeCP2 protein, a transcription factor responsible for regulating methylated gene expression and proper neural functioning, with deficits ultimately leading to decreased synaptic connectivity and subsequent abnormal motor function and learning (Jung et al., 2013; Muñoz et al., 2016). These neural abnormalities have been associated with ASD and other NDDs, suggesting a potential etiological role of this type of epigenetic disturbance (Jung et al., 2013; Maag et al., 2017).

Dysregulation of Transcription Causes NDDs

According to the central dogma of molecular biology, DNA codes for mRNA codes for proteins. Therefore, the transcriptional process is integral to the development and maintenance of healthy cells and organisms. However, only about 1% of the entire genome codes for final protein products, and the majority largely contributes to the regulation of those protein products via promoters, enhancers, silencers, and insulators (Gloss & Dinger, 2018; Maston et al., 2006).

Other RNA products also contribute to transcriptional regulation of DNA, such as microRNA (O'Brien et al., 2018). The roots of many disorders have been identified in the dysregulation of DNA transcription either locally or globally (Izumi, 2016). For example, mutations in genes coding for histone modification enzymes, chromatin remodelers, and transcription factors can cause facial malformations, cognitive deficits, and other physiological conditions. Both loss of function and gain of function mutations can contribute to dysregulated DNA transcription, resulting in congenital physiological and cognitive abnormalities (Izumi, 2016).

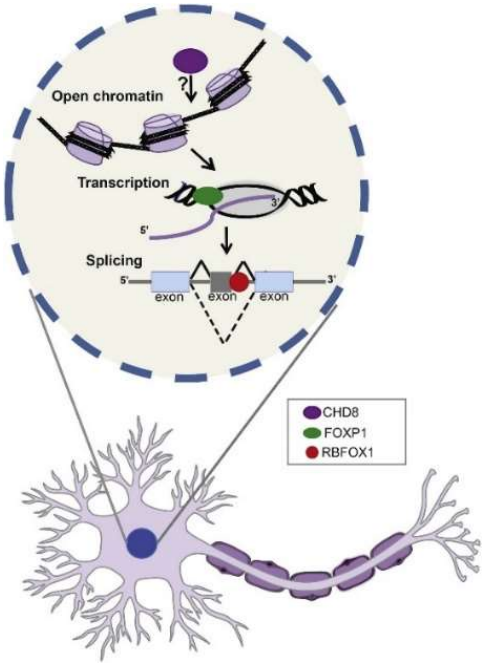
Neocortex development is an integral part of fetal development, highly susceptible to perturbations in utero that may ultimately result in cognitive dysfunction. It is primarily the role of transcription factors to guide this development, mediating neuronal specification, cell migration, and circuit wiring (Kwan, 2013). Many of these transcription factors have been disrupted in cases of ASD, characterized by defects in higher functions mediated by the neocortex (Kwan, 2013). Some of these variants have also been reported in schizophrenia (SCZ), reflecting the pleotropic nature of epigenetic modifications and their clinical manifestations (Ayhan & Konopka, 2019). Dysregulation of neuronal circuitry often leads to the cognitive dysfunction characteristic of ASD via de novo mutations in transcription factor genes (Ayhan & Konopka, 2019).

Some transcription factors crucial for neurodevelopment include TRB1 (T-box brain factor 1), responsible for mediating axonal differentiation and cortical connection; SOX5 (sex-determining region Y-box 5), important in stem cell differentiation and cell fate specification; FEZF2 (FEZ family zinc-finger 2), also important for corticofugal connectivity; and FOXP1 (forkhead box transcription factor 1), which regulates numerous transcriptional processes in the

brain (illustrated in Figure 1). Loss of any of these transcription factors has negative impact on fetal neurodevelopment, contributing to cognitive and social deficits (Ayhan & Konopka, 2019; Kwan, 2013). Ultimately, disrupted transcription may arise from multiple avenues, be genetically or environmentally influenced, and result in larger-scale deficits in brain function, altogether contributing to the clinical manifestations of NDDs and psychiatric disorders.

Figure 1

Disturbances in Regulatory Genes Associated with NDDs



Note. Common transcription factors with NDD-associated disruptions. CHD8 is a chromatin remodeler with numerous downstream effects on ASD-risk associated genes. FOXP1 is necessary for proper neurodevelopment. RBFOX1 binds RNA and mediates RNA metabolism. Reproduced with permission from "Regulatory genes and pathways disrupted in autism spectrum disorders" by F. Ayhan and G. Konopka, 2019, *Progress in Neuro-Psychopharmacology & Biological Psychiatry, 89,* p. 58 (https://10.1016/j.pnpbp.2018.08.017).

Altered Synaptic Signaling Causes NDDs

The formation of synapses is a necessary element of neurodevelopment. Many classes of proteins are involved in the processes of synaptic formation and signaling, and through the use of techniques such as whole exome sequencing, mutations in genes coding these proteins have been associated with ASD, ADHD, and SCZ (Hsueh, 2019). Cell adhesion proteins are localized to the cell surface and are highly expressed at synapses, crucial to creating and maintaining cellular and synaptic integrity (Batool et al., 2019). Early in neurodevelopment, these proteins are involved in cell migration and axon growth, preparing for their late-stage role as structural stabilizers of the synapse (Batool et al., 2019).

Neuroligins and neurexins are responsible for the connection of newly developed synapses; epigenetic perturbations in any of the genes coding for these proteins result in synapse dysfunction and intellectual deficits. Synaptic stability defines the overall functional quality of neural circuits; deficits are associated with the development of ASD, and such as has been observed with the amygdalar and cortico-striatum circuits (Hsueh, 2019). Without the structural integrity of these synapses, the connectivity of brain circuitry is compromised and neurodevelopment is impaired. Despite the heterogeneity of underlying genetic perturbations, the resulting functional disturbances observed in NDDs tend to converge on neurogenesis and synapse formation, consistent with the polygenic nature of neurodevelopment (Belger et al., 2011).

The Neurodevelopmental-Neuropsychiatric Disorder Continuum

There is becoming a more widely accepted consensus that disturbances during fetal neurodevelopment can contribute to the pathogenesis of neuropsychiatric disorders as well as

neurodevelopmental disorders (Owen & O'Donovan, 2017). This belief is so widely held that there is a push to consider these disorders as less distinct etiologically and more on a continuum, especially with emerging evidence of common genetic roots. On a larger scale, it is known that the presence of a NDD is associated with a higher risk of development of a mental health disorder later in life, possibly because of similar pathologies (So et al., 2020). Many genetic variations associated with NDDs are also found when investigating underlying genetic causes of NPDs; hence, it follows that common molecular pathways and other functional domains are observed between the two classes of disorders, such as protein synthesis, synaptic transmission, and cell growth and differentiation (Cristino et al., 2014). These processes may be affected by genetic mutations as well as epigenetic perturbations, revealing the vulnerability of fetal neurodevelopment to changes within the base sequence and above it.

The observance of pathological commonalities is not to say that significant variances between the disorders do not exist; the aim is to establish a basis for defining a continuum of NDDs and NPDs, especially in the context of fetal epigenetics. The discovery of common genetic etiologies between the disorders may assist in future research on environmental or epigenetic factors that contribute to the development of more severe symptoms and reveal why some genetic variations ultimately manifest in psychosis. One aim is to identify environmental or epigenetic risks specific to the timing of fetal development that determine the pathology and progression of the NDD-NPD continuum. Epigenetics may explain some of the non-Mendelian aspects of these NDs, such as nonidentical cases between monozygotic twins, sexual differences, varying disease course, symptoms that fluctuate with endocrine variations, and parent-of-origin effects (Gavin & Sharma, 2010).

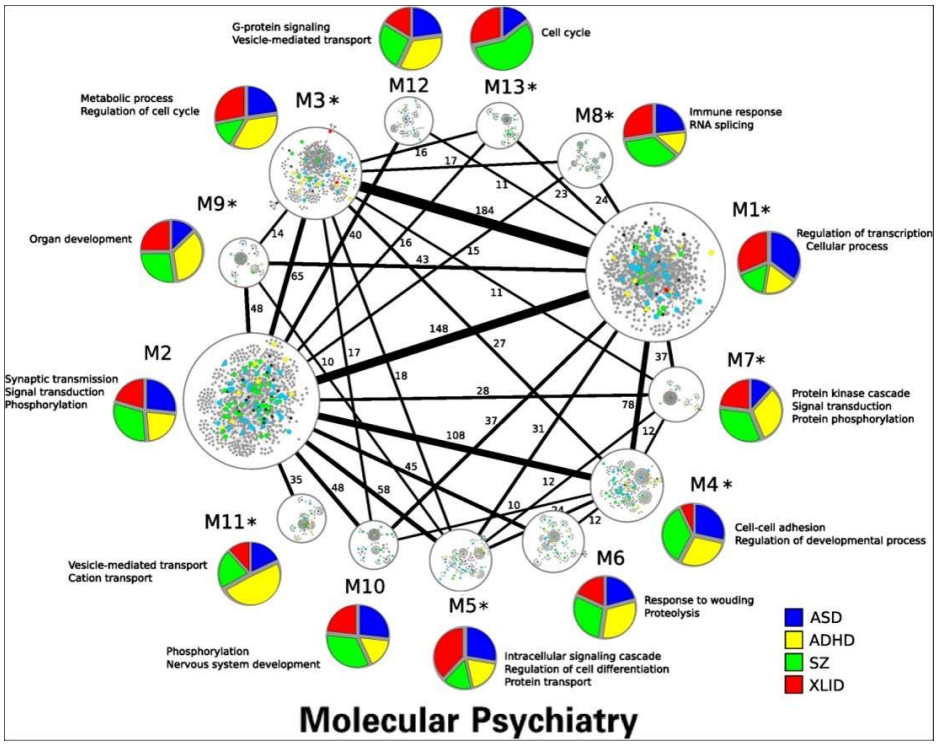
Biochemical Origins of NDDs and Schizophrenia

Schizophrenia has been observed to share several underlying genetic abnormalities with NDDs such as ASD, ADHD, and even bipolar disorder (Cristancho & Marsh, 2020). Some environmental risk factors are common between the classes of disorders, which will be further discussed later on. SCZ and other NPDs (such as mood disorders, which together constitute the major focus of this review) are often first observed around adulthood or adolescence, with fewer occurrences of childhood onset. This contrasts with NDDs, which have onset either congenitally or during early childhood (Owen & O'Donovan, 2017).

Many of the same molecular processes are controlled by the same genes where mutations may result in both NDD and NPDs: Cristino et al. recognized over 4,000 genetic mutations responsible for the development of these disorders and were able to organize the variants into 13 functional domains (Figure 2). Many of these identified genes encode proteins involved in similar higher order biological functions such as transcription regulation, intercellular communication, signal transduction pathways, cell cycle regulation, and nervous system development (Cristino et al., 2014). However, these domains were not all equally represented in the disorders studied: some functions, such as signal transmission, proteolysis, phosphorylation, and G-protein signaling were common across NDDs and NPDs, but regulation of transcription, vesicular transport, and protein kinase signal transduction were less evenly distributed (Cristino et al., 2014).

Figure 2

Functional Protein Domains that Contribute to Neurological Disorders



Note. The size of each functional domain (M1-M11) is proportional to the number of proteins associated with it. The pie charts reflect the extent to which the NDs are related (ASD, ADHD, SZ (schizophrenia), XLID (X-linked intellectual disability)). The intersecting lines and numbers represent the protein interactions between domains, and the asterisk (*) represents significantly different gene frequencies between disorders. Reproduced with permission from "Neurodevelopmental and neuropsychiatric disorders represent an interconnected molecular system" by A. S. Cristino, S. M. Williams, Z. Hawi, J. Y. An, M. A. Bellgrove, C. E. Schwartz, L. d. F. Costa, and C. Claudianos, 2014, *Molecular Psychiatry, 19*(3), p. 298 (https://10.1038/mp.2013.16).

Different epigenetic mechanisms may contribute to the development of SCZ including DNA methylation and histone modifications. For example, many genome studies have identified SCZ-associated variants as predominantly involved in the enhancement or silencing of gene expression (Hannon et al., 2021). This provides part of the basis to search the epigenome for biological roots of schizophrenia. DNA methylation is a primary epigenetic modification for gene repression, and certain variances in the methylome have been associated with an increased risk of developing SCZ; whether they are the etiological root or simply a side effect is not entirely clear and may be situational (Hannon et al., 2021).

Many SCZ-associated differentially methylated positions are associated with cellular pathways involved in the maintenance of extracellular structure and cell-cell adhesion (Hannon et al., 2021). Increased methylation at certain genes involved in synaptic plasticity (e.g. *RELN*) and formation of neurotransmitter GABA (e.g. *GAD67*) have also been associated with SCZ due to high concentrations of CpG islands (Gavin & Sharma, 2010). Moreover, SCZ can be caused by epigenetic modifications (not limited to those listed above) that are shared by NDD etiologies, supporting the idea that the development of these disorders is not limited to merely genetics or environment, but perhaps a combination of both.

Similar Pathogenesis of Neurodevelopmental and Neuropsychiatric Disorders

The emphasis on the relationship between schizophrenia and NDDs is due to the evidence of underlying biological similarities between the classes of disorders. ADHD (predominantly observed during childhood) may presage mood, anxiety, and substance abuse disorders in adulthood, indicating a potential biological link that could be traced to disturbances in circadian rhythms or dopaminergic dysfunction (Bădescu et al., 2016). Dopamine regulation

has a significant role in the development of ADHD and mood disorders via the reward processing system (Bădescu et al., 2016). ADHD is associated with irregular circadian rhythms and altered sleep schedules, which are also commonly observed in mood disorders. Certain genes that contribute to the maintenance of these biological rhythms may contribute to the genetic pathology of ADHD, and some medications even target these genes directly (Bădescu et al., 2016). The environmental maintenance of circadian rhythms may be a potential therapeutic strategy for psychiatric mood disorders but may not address the true genetic cause of the disorder.

Dopaminergic dysregulation is also a key feature of ASD and SCZ, further confirming the idea that these classes of disorders are interconnected (Campbell et al., 2019; Howes et al., 2016). It is believed that at least one form of ASD can be caused by a mutation in the dopamine transporter (DAT), causing the two gates of the transporter to become uncoupled and act independently. This causes decreased uptake of dopamine in the brain, which translates into abnormal behavioral patterns such as those observed in ASD (Campbell et al., 2019). One mutation studied in drosophila (ΔN336, a deletion in the DAT gene) resulted in an overall decrease in dopamine uptake in the brain due to increased efflux and subsequent increased concentrations of extracellular dopamine. The researchers postulated that this difference caused the ΔN336 flies to exhibit hyperactivity and repetitive movements, as well as a prolonged fear response and other atypical social functioning (Campbell et al., 2019).

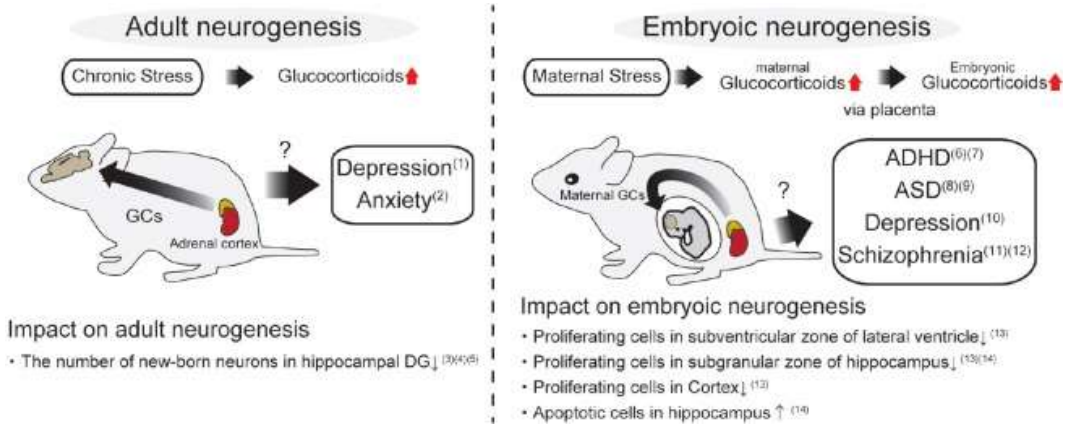
In contrast, SCZ tends to display increased synthesis and release of dopamine (Howes et al., 2016; Sonnenschein et al., 2020). The consistently observed dopamine dysfunction in SCZ is thought to be involved with the hallucination and delusional symptoms of the disorder in

addition to cognitive deficits. It is also known that increased functioning of the dopamine system over time leads to more full expression of the disorder (i.e. psychosis) (Sonnenschein et al., 2020). Most of the SCZ-associated genes are involved in dopaminergic signaling or neurotransmission in general with downstream effects on dopaminergic signaling (Howes et al., 2016). Moreover, there is extensive evidence pointing to similar biochemical and neurological manifestations of these classes of disorders, which may indicate a deep etiological relationship.

Changes to gene expression in response to environmental factors have also been associated with development of NPDs. The risk of developing depression later in life increases substantially in response to chronic stress, early life stress, and even stress experiences of preceding generations through inherited epigenetic alterations (Nestler, 2014). Chronic stress has been correlated to global chromatin acetylation and methylation levels, reflecting a relatively open or repressive state of the genome, respectively. These modifications may work together to change the expression of genes involved in neurological signaling. For example, glucocorticoid receptor (GR) is commonly downregulated in major depressive disorder (MDD) in response to early-life stress (Fass et al., 2014; Nestler, 2014). Fass et al. (2014) observed that hypermethylation caused decrease of GR in rat hippocampi and found reductions even into adulthood. Resulting elevations in glucocorticoid levels caused a hyperactive hypothalamic-pituitary-adrenal (HPA) axis, a hallmark of depression and anxiety-related disorders (Fass et al., 2014). These same phenomena during fetal neurodevelopment have different effects, resulting in NDDs or NPDs (Odaka et al., 2017). This timing difference may reflect epigenetic roles in the development and progression of certain disorders, supported by observations of increased stress susceptibility in generations after occurrences of early-life stress (Nestler, 2014).

Figure 3

Effects of Stress on Postnatal vs. Prenatal Neurodevelopment



Note. Hypothesis of ND development after exposure to stress as an adult vs. exposure to maternal stress as a developing fetus. Proposed glucocorticoid effects are demonstrated. Reproduced with permission from "Impact of glucocorticoid on neurogenesis," by H. Odaka, N. Adachi, and T. Numakawa, 2017, *Neural Regeneration Research, 12*(7), p. 1031, (https://10.4103/1673-5374.211174). Licensed CC BY-NC-SA 3.0.

Genetic Counseling for NDDs

Because of known genetic variants associated with NDs, genetic testing is utilized to diagnose some NDDs and is becoming a standard of pediatric primary care (Savatt & Myers, 2021). After receiving a genetic diagnosis, families may be referred to genetic counselors to help them understand the diagnosis, assess family history, evaluate recurrence risk, as well as assist with psychosocial adjustment to receiving the information. Genetic counseling is becoming a more integral part of health management and aids immensely in these scenarios. Genetic counselors train in both human genetics and interpersonal counseling to help families understand

genetic etiologies, and may even assist with diagnostic evaluation or lifestyle management with the knowledge of a genetic condition (Blesson & Cohen, 2020). Many NDDs are diagnosed by a pediatric clinician and may then be referred to a pediatric genetic counselor for testing and family counseling sessions. The clinical suspicion for diagnosing an NDD is usually based upon missing behavioral or functional milestones during development, which may lead to neurodevelopmental deficits in areas such as cognition, language and communication, motor skills, or occupational functioning (Savatt & Myers, 2021).

Understanding the genetic etiology for an NDD gives a fuller understanding of the condition and may provide insight into a more accurate prognosis or more characterizable disease progression. This knowledge may even lead to the development or acquisition of specific treatments or therapies. It is especially helpful for this class of disorders to have a reference library of known genetic or molecular causes because the penetrance is so variable (Tărlungeanu & Novarino, 2018). Individuals affected by NDDs display different symptoms and severity of symptoms, so the discovery of a common genetic cause would be a helpful basis for biological therapies and symptom management. This knowledge also contributes to predicting risk of recurrence for family planning: de novo mutations speak to a much different risk for future children than autosomal dominant or recessive mutations present within a family, which can be communicated to extended relatives and make early intervention possible. Genetic counselors can direct families with an NDD diagnosis to specific resources, strategies for personalized caretaking, or avoidance of unnecessary diagnostic testing or irrelevant etiologically based therapies (Savatt & Myers, 2021).

Genetic Testing Techniques for NDDs

Genetic counselors utilize many genetic testing techniques to confirm NDDs for pediatric cases including gene-specific genetic testing, chromosomal microarray, or whole exome sequencing (Savatt & Myers, 2021). Genetic testing may be pursued after the onset of symptoms to confirm a diagnosis, allowing families and healthcare providers access to more information regarding the condition and etiology (Savatt & Myers, 2021). Whole exome sequencing has a high yield for diagnosing NDDs (between 20 and 40%), followed by chromosomal microarrays (about 10%) (Blesson & Cohen, 2020; Savatt & Myers, 2021). The latter can be utilized after observation of delayed or regressed neurodevelopment, multisystem involvement, and/or the presence of seizures or specific dietary triggers, which all indicate some sort of abnormal metabolism of a particular chemical (Blesson & Cohen, 2020). There are conflicting viewpoints on the use of testing for inborn errors of metabolism to diagnose nonspecific NDDs; currently, these tests are typically performed during childhood or adolescence by the order of a physician or genetic counselor (Savatt & Myers, 2021).

This information is helpful for the sake of monitoring a condition and early intervention. Studies have shown that a majority of probands with a genetic NDD have used such information to guide clinical management and make informed decisions about treatment or therapy (Tărlungeanu & Novarino, 2018). Genetic counselors are immensely helpful in this regard to explain genetic testing processing, results, and how best to use the information to guide further health management or communicate recurrence risks.

Unfortunately, even with such widely available testing, many of these tests are limited to genetic etiologies and have trouble discerning epigenetic abnormalities. For example,

chromosomal microarrays are useful to detect deletions or duplications, but does not are not sensitive to epigenetic alterations such as methylation status (Savatt & Myers, 2021). Exome sequencing also does not perceive this type of modification despite the extensiveness of the technique (Savatt & Myers, 2021). Advances in epigenetic testing techniques focusing on the methylome or RNA sequencing may provide insight into pathologies that evade detection using current techniques.

Current Limitations in Genetic Counseling

Even before undergoing genetic testing, a genetic counselor must advise a family of advantages and disadvantages of pursuing testing for an NDD to enable them to make an informed and independent decision. There is the possibility that a known genetic etiology will aid in medical management, social resources, or prognosis, but there are drawbacks as well. There is always a possibility that genetic testing may come out negative, even if there is simply no present test for the variant at hand. Further, a positive test result may not shed light on how to adapt health management, and there may be no treatments available. Often, families desire the information and pursue testing to learn about recurrence risk or seek medical and social resources. In the event that a positive test result for a NDD is received, a genetic counselor will gently share the information with the family, sensitive to their mental state. Data has shown that communication of hope, emotional support, resources, and current information is helpful to make the overall experience more positive (Savatt & Myers, 2021). It is important to engage in this conversation with emphasis on the parents' emotions and concerns regarding the diagnosis.

Many questions may be raised by the proband or proband's family during a genetic counseling session that reside outside of the genetic counselor's scope of practice. Often there

may persist an underlying feeling of parental guilt, and questions regarding obstetric complications may arise. In this case, a genetic counselor may refer to a physician, or opt to discuss the concerns and conclude that unless those complications have clear implications of brain injury, they are not likely responsible for the development of the NDD (Simonoff, 1998). If other environmental risks that occurred during pregnancy are discussed, the genetic counselor will act in a similar gentle manner due to the knowledge that certain exposures increase the chance for developing a NDD. The environment may affect the epigenome, especially at such a vulnerable time as fetal development. Genetic counselors may consider parent-of-origin effects, sex differences of symptomology or inheritance, or the penetrance of a disorder that could reflect an epigenetic etiology of the ND rather than a purely genetic root (Simonoff, 1998).

Epigenetic Causes of NDs

It is increasingly clear that there is not always a genetic basis for phenotypic disorders: while there are many potentially pathogenic variances observed in an individual's genetic code, changes in the regulation of the genome can also cause physiological or neurological disorders via epigenetic mechanisms. Epigenetic alterations of gene expression arise during development, contributing to the differentiation of cells and mediating environmental adaption (Jaenisch & Bird, 2003). These changes are heritable for several generations and contribute to a modified phenotype in offspring without changing the genetic code (Lind & Spagopoulou, 2018). Many environmental factors can cause such changes, especially during fetal development, such as maternal nutrition, exposure to toxins, and fetal stress or trauma that ultimately manifest as NDDs or NPDs.

Maternal Malnutrition

It is no surprise that maternal nutrition and thus the supply of nutrients to a fetus is a primary influence on the process of fetal development. It is imperative that the fetus receives a steady and balanced supply of lipids, carbohydrates, and amino acids in order to grow and proliferate by way of differential gene expression (Maloney & Rees, 2005). Nutrient sensors in eukaryotic cells can modulate gene expression in response to changes in nutrient levels, maintaining fetal homeostasis in response to prolonged excess or deficiency of nutrients (Maloney & Rees, 2005). These changes can have long-lasting effects on the organism with physiological manifestations such as altered metabolism, dysregulated endocrine signaling, abnormal cell development, or atypical organ structure (Barker, 1997).

Many chemicals derived from food products play a prominent role in regulating gene expression in utero, including vitamins B6 and B12, methionine, and folic acid (Thornburg et al., 2010). These compounds act as methyl donors, so deficiencies in any of them ultimately reduce levels of DNA methylation during fetal development (Gawlińska et al., 2021). The genome is essentially cleared of methylation at conception via active and passive mechanisms, but these markers are reintroduced as tissue differentiation occurs throughout the first and second gestational trimesters (Slieker et al., 2015). Studies on the methylation dynamics of fetal development have reported that regions of the genome with increasing methylation over time were associated with general growth and development, whereas as time progressed, regions that had decreased methylation were associated with tissue-specific functions (Slieker et al., 2015). This logical progression contributes to the development of specific cell types, which organize to form the tissues and organs of an organism at full gestational age.

Maternal diets deficient in these methyl-donating nutrients lead to global and gene-specific DNA hypomethylation, which may disrupt the normal progression of tissue-specific differentiation. Prenatal diets deficient in folic acid may lead to miscarriage, neural tube defects, or behavior, communication, and learning deficiencies consistent with a diagnosis of ASD (Desai et al., 2017; Greenberg et al., 2011). This relationship, however, is not simple; the observed folic acid deficiency during gestation may be due to a dietary deficiency or a reduced metabolism for the vitamin. For example, the presence of folate-receptor alpha-specific autoantibodies blocks the transport of folate to the developing fetus and is very prevalent in ASD cases (about 70%) (Desai et al., 2017).

Beyond alterations in vitamin levels, other dietary factors can contribute to epigenetic mechanisms and ultimately NDD pathology. Synaptogenesis is highly active during the intrauterine development and postnatal periods and is particularly vulnerable to disruptions (Gawlińska et al., 2021). For example, a high-fat maternal diet can alter methylation patterns of the developing fetus (specifically in males) in the regions of the brain associated with ASD dysfunctions, the frontal cortex and hippocampus (Gawlińska et al., 2021). Altered expression in 48 genes was observed in response to a maternal high-fat diet, mostly associated with synaptic functions, transcription regulation, and chromatin remodeling. Increased and decreased expression were observed for different genes, indicating a great amount of pleiotropy responsible for the heterogeneity of the ASD phenotype (Gawlińska et al., 2021). Environmental factors influence gene expression at various time points throughout development and thus take different, even opposing routes to lead to decreased neural function consistent with a diagnosis of ASD (Gawlińska et al., 2021). A general trend observed in this study was decreased levels of DNA

methylation in the frontal cortex and hippocampus, leading to increased expression of ASD-related genes (Gawlińska et al., 2021). This study contributes to the growing field of knowledge surrounding in utero environmental hazards that interact with the fetal genome and lead to the accumulation of epimutations, altering development patterns and foreshadowing ND pathologies.

Maternal Exposure to Toxins

Infections during pregnancy can disrupt fetal development and have been linked to abnormal neurological traits consistent with ASD, bipolar disorder, or SCZ (Lee et al., 2015; Weber-Stadlbauer, 2017). Bacterial infections, viruses, and other infectious agents increase the risk for development of ASD when the mother was infected at any trimester (Lee et al., 2015). Though different infections may act in unique ways, they may ultimately have similar effects on the developing fetus, leading to disrupted neurodevelopment. Epimutations may contribute to the pathology of these neurological disorders: DNA methylation, microRNAs, and histone acetylation alterations following maternal immune responses to infection all cause changes in DNA transcription for genes involved in neuronal development, synaptic transmission, and immune signaling (Weber-Stadlbauer, 2017).

Maternal smoking causes DNA hypomethylation during fetal development in genes related to neuropeptide signaling and physiological development (Tran & Miyake, 2017). Prenatal exposure to smoking has been linked to increased risk for development of ASD, ADHD, SCZ, behavioral abnormalities, dysfunctional neurotransmission, and dysfunction in the hypothalamic-pituitary-adrenal axis, a major regulator of circadian rhythms and behavioral and physiological stress responses (Buck et al., 2021; Kinlein & Karatsoreos, 2020). These disordered phenotypes are likely originated by observed changes in the DNA methylome – this

idea is supported by the heritability of these traits trans- and intergenerationally (Buck et al., 2021). DNA methylation studies in offspring exposed to maternal smoking showed local and global abnormal methylation patterns, specifically in genes associated with neural development, synaptogenesis, and differentiation of cells within the central nervous system (Buck et al., 2021). These methylation changes persist into adolescence and adulthood across tissue types, supporting the notion that these epigenetic characteristics may be useful as biomarkers and predictors of future health issues (Rauschert et al., 2019).

Prenatal alcohol exposure (PAE) is another gestational toxin linked to developmental delays, reduced growth, physical abnormalities, and behavioral deficits (Alberry et al., 2021). Fetal alcohol spectrum disorders arise from abnormal neurodevelopment, which can be traced to altered epigenetic mechanisms of cell differentiation and development. Many changes in gene expression associated with PAE affect proteins with cognitive or gene regulatory functions (Alberry et al., 2021). Noncoding RNAs are imperative for successful neurogenesis and neuronal migration, and altered microRNA levels have been associated with PAE, resulting in decreased hippocampal cell development and neurogenesis. Increased DNA methylation has also been observed in many cases of PAE, ultimately lowering expression of genes involved in cell adhesion, neurodevelopment, and hypothalamic serotonin transport (Cobben et al., 2019). Altogether, these neurodevelopmental delays may be traced to changes in brain morphology, signaling, and cell differentiation, all guided by epigenetic modifications throughout prenatal development.

Maternal Stress

Beyond physical factors such as diet or toxin exposure, psychological factors also play a part in epigenetic alterations during fetal development. It is understood that poor mental health has physiological and neurological manifestations, and these effects are not limited to one generation. Maternal stress and anxiety during gestation have been understood to influence fetal neurodevelopment and future emotional well-being (O'Donnell & Meaney, 2017; St-Pierre et al., 2016). Mice offspring of prenatally stressed mothers consistently show significantly increased fearfulness and intensity of stress response via the HPA axis, and decreased cognitive functions even after being fostered by non-stressed mothers postnatally, and there is evidence for paralleled symptoms in humans (O'Donnell & Meaney, 2017). Social and emotional functions as well as learning and memory are negatively impacted by maternal stress during the gestational period, perhaps due to changes in brain morphology or signaling, and may even contribute to the development of later mental health disorders (O'Donnell & Meaney, 2017; St-Pierre et al., 2016).

Maternal anxiety has been associated with decreased fetal methylation at the *IGF2* (insulin-like growth factor) and *H19* genes, which are highly active during fetal development (Mansell et al., 2016). Methylation of these genes tend to be specific to their parental origin and are influenced by in utero environmental factors (Mansell et al., 2016). Decreased methylation in these regions allows the binding of CTCF, a repressive transcription factor that blocks the downstream activation of the IGF2 promoter. When CTCF is bound, H19 transcribes microRNA 675, which hinders fetal growth and development (Mansell et al., 2016). This phenomenon has

been associated with low birth weight, disrupted neural cord development, and even downstream health and mental health problems (Mansell et al., 2016).

Potential mechanisms of the psychological reverberations may include the disruption of the fetal HPA axis due to increased levels of glucocorticoids crossing the placental barrier. This leads to decreased negative feedback of the pathway and changes the overall neurocircuitry (Monk et al., 2019). Fetal corticotropin-releasing hormone (CRH) in response to maternal stress is released from the placenta and reaches both the maternal and fetal pituitary, initiating a positive feedback cycle. This leads to reduced HPA axis sensitivity even into adulthood, leading to more anxious behaviors and heightened stress responses (Chan et al., 2018). A hyperactive HPA axis has also been associated with decreased expression of glucocorticoid receptor (GR) following prenatal stress and related to symptoms of psychosis (Griffiths & Hunter, 2014).

Changes in microRNA levels during critical periods of fetal development have been associated with prenatal stress and subsequent development of NDDs and NPDs (Griffiths & Hunter, 2014). microRNAs are responsive and susceptible to environmental influence as gene regulatory elements (Beversdorf et al., 2021). Maternal stress has been associated with changes in microRNA expression involved in dopaminergic synaptic signaling in the brain, a hallmark of ASD (Beversdorf et al., 2021). microRNAs have a variety of epigenetic roles and can silence or activate transcription of genes and even have post-transcriptional functions inside and out of the nucleus. This flexibility is crucial to respond to environmental conditions and shape the trajectory of gene expression and physiological development (O'Brien et al., 2018; Xu et al., 2010). Most microRNAs function primarily or exclusively in the brain and act predominantly in neuronal development (Xu et al., 2010). Changes in certain microRNA expressions have been

linked to reduced brain function as well as development of SCZ, ASD, and other disorders with neurological manifestations due to altered neuronal gene expression (Xu et al., 2010).

Varying but consistent sex differences are also noted in studies on maternal stress and fetal neurodevelopment. Some have found higher risk for development of ADHD in adolescent males when the mother had undergone loss of a child or spouse during pregnancy; others have observed a higher risk for depression in females at eighteen years of age but not males (Monk et al., 2019). There are a few potential explanations for these observations that may all contribute at least in part: the slower developmental rate of the male fetal brain may make it more vulnerable to the influence of maternal stress; or, testosterone exposure may contribute to the vulnerable state of fetal neurodevelopment (Monk et al., 2019). Another theory is related to the placenta, the shared endocrine organ that, due to the larger contribution of embryonic tissue than maternal, also shares the fetal XX or XY genotype. The placenta may mediate genotype-specific responses to maternal stress or other factors that cause sex-specific epigenetic modifications, resulting in the different susceptibilities for NDs in males vs. females (Bronson & Bale, 2016).

The Future of Epigenetic Research of Neurological Disorders

Of course, with the growing knowledge of underlying epigenetic causes of NDDs and neuropsychiatric disorders, there is an aim to develop therapeutics targeting the biological root of these disorders rather than simply symptom management (Millan, 2013). An initial challenge is present with the question of whether the goal is to administer therapy before the presentation of molecular or cellular alterations or to focus on the reversal of those alterations. Regarding this, the prevention of these disorders via their epigenetic roots may rely on the prevention of maternal environmental risk factors as described previously, but the prevention of the epigenetic

modifications themselves prove slightly more challenging due to the direct integration of environmental hazards to the developmental trajectory of the fetus. It also may be difficult to implement any sort of preventative therapeutic agent due to the early determination and onset of these disorders. Ergo, reversal of epigenetic modifications would be more realistic to implement as early as infancy to prevent later onset of neurodevelopmental and neuropsychiatric symptoms. The realistic aim of this development would be to derail, delay, or neutralize the genesis of NDs either altogether or for specific symptoms by targeting abnormal biochemical pathways affected by epimutations. Because of the variety of epigenetic roots of these disorders, much of the current research to develop epigenetic therapies is aimed at alleviating cognitive defects due to their commonality across the disorders, the vast understanding of their mechanisms, and simple experimental validation (Millan, 2013). The epigenetic mechanisms contributing to these are also quite simple to identify through literature and experimentation and include DNA methylation changes, histone alterations, and microRNAs (Millan, 2013).

DMNT and histone deacetylase (HDAC) inhibitors are clinically effective in treating a variety of NDs and provide a basis for the development of more targeted therapies (Qureshi & Mehler, 2013). DMNT inhibitors (such as clozapine, prescribed for schizophrenia) can improve learning, memory, and reward circuitry and inhibit neurodegeneration. The possibility for these mechanisms to become more efficient and specific drives the motivation to continue research in this area, aiming to mitigate the symptoms and pathogenesis of a variety of NDs (Qureshi & Mehler, 2013). HDAC inhibitors have been the predominant focus of epigenetic therapies due to the various context-dependent roles of histone acetylation and presence in many neurological disorders (Urdinguio et al., 2009). Some studies have observed a high level of specificity of

HDAC inhibitors, finding them to positively affect genes specifically related to cognition and synaptic plasticity – two clinically relevant symptoms of NDDs and NPDs (Millan, 2013; Urdinguio et al., 2009). HDAC inhibitors also have antidepressant effects which may reflect the NDD-NPD continuum as well as the specificity of targeting epigenetic roots of mood disorders (Millan, 2013).

The possibility of developing epigenetic therapies for disease does not pose the same ethical dilemma as purely genetic therapies, because in this context the genome is not being directly altered. While regulation and expression of the genome may be changed for the aim of correcting the fetal neurodevelopmental trajectory, the integrity of the unique genetic sequence is maintained. The therapeutic aims reside in the biochemical and physiological etiologies of NDDs and NPDs, and may be applicable to their continuum, preventing eventual degeneration into psychosis and ultimately improving quality of life. The pursuit of mental health therapy and/or pharmaceutical intervention for NDDs or NPDs does not pose any moral questioning, so any ethical opposition to the pursuit of biologically based pharmaceutical therapies is therefore avoided. It is common for people to rely on antidepressants, antipsychotics, stimulants, or cognition-enhancing medications that may already work in these fashions to alter brain chemistry and improve the quality of brain functioning, learning, and mental health to motivate them in other areas of life. Because of the biological and epigenetic evidence for a NDD-NPD continuum, epigenetic therapies have the possibility to prevent development of later-stage psychiatric conditions, or even fetal NDDs of offspring of those with psychiatric conditions. Epigenetic signatures may be inherited through generations; intervention therefore reduces the risk that environmental hazards will persist within a family to affect offspring years later.

Ultimately, this improves the neurological and mental health of families and communities and reduces guilt that the in utero environment shaped by maternal experiences may continue to negatively influence members of a family generations later.

Epigenetic Considerations for Neurological Genetic Counseling

Of course, the possibility to develop targeted epigenetic therapies for NDDs and NPDs provides hope to those afflicted by these disorders. This type of intervention can be ultra-precise, directly altering the biochemical etiology of neurological disorders and could even be person-centered, unique to their epigenome. By identifying the specific pathways affected (out of many possible genes, pathways, and functions, as described throughout this review), specific therapies may be administered based on the specific epigenetic pathology. Patient-specific drugs are at the advent of medical genetics research, and with this growing body of knowledge regarding epigenetic etiologies, seem to be within reach.

Along with this growing body of knowledge comes a need to communicate this information at least when medically relevant, and ideally for the improvement of public health and global health literacy. Genetic counselors seem to fit this job role, professionals trained in communicating the clinical effects of genetic and epigenetic mutations resulting in disease phenotypes. Specifically, epigenetic susceptibility to NDDs and NPDs could be communicated as early as a prenatal or preconception genetic counseling appointment, where a genetic counselor may meet with expecting parent(s) and discuss risk for development of NDDs or NPDs for the fetus. Due to the vast number of potential epigenetic etiologies, the logistical reality of testing for all possible variants seems impractical. However, knowledge of familial

predisposition for NDDs or NPDs may guide genetic counselors to look for specific indications of epigenetic modulations known to contribute to the pathogenesis of specific disorders.

This type of information may also provide a more logical basis for a NDD diagnosis based on clinical symptom severity and the underlying epigenetic cause. With this diagnosis and knowledge of the NDD-NPD continuum, a genetic counselor may be able to predict longitudinal risk for development of a NPD based on the epigenetic landscape and contributing environmental risk factors. They may then apply this projected risk to an individual's health management, family planning for carriers, or implementation of targeted therapies under the guidance of a physician. The genetic counselor is also attuned to caring for the psychosocial aspect of receiving a genetic or epigenetic diagnosis and can navigate conversations surrounding such topics with grace and empathy.

Genetic counseling sessions regarding NDDs or NPDs may be necessary for one class of disorders or the other depending on the familial context and unique situation of the proband. For example, a young couple may see a genetic counselor with knowledge of multiple NDDs or NPDs in one or both of their families and receive information that they display abnormal regulation of a gene involved in neurodevelopment, increasing the likelihood that any child of theirs will develop an NDD. Another situation may consider a proband who was gestationally exposed to toxins, maternal infection, or nutrient excess/deficiency and has recently been diagnosed with a NPD such as depression, bipolar disorder, or schizophrenia. A genetic counselor may work with this information regarding the proband's prenatal background and current situation to identify an epigenetic etiology of the psychiatric disorder with aim to mitigate its progression at the biochemical level.

These sessions require utmost attention to the psychosocial condition of the patient and how they receive this news. It is needless to say that undergoing a diagnosis of any sort, whether for oneself or for a family member, is difficult, and the acceptance of such a diagnosis is not aided much by outside input. Genetic counselors are uniquely helpful in this area due to their didactic training in counseling, psychosocial assessment, and communication of medical and genetic information based on the needs of the patient. This new world of targeted epigenetic therapies provides hope for people affected by NDDs and NPDs due to the dynamic nature of the epigenome and reversibility of resulting etiology. This hope is imperative for a genetic counselor to communicate to patients with these diagnoses to emphasize the opportunity to pursue treatment that targets the biological cause of what may be understood to be an incurable neurological disease. Apprehensions may arise at this point when dealing with treatment of one's genetic profile, but there comes the reminder that the genome is not being targeted here; the epigenome is, resulting in changes of expression of biochemical pathways, and not the genes themselves. It may even be of benefit in these situations to remind the proband of the physiological component of many of these disorders – that the pathology relies on altered protein levels, over- or underexpression of chromatin, or even larger-scale symptoms such as decreased synaptic connectivity. Epigenetic treatments are ideally able to target these symptoms rather than the genome itself, resulting in corrected expression of the unchanged genetic code.

Of course, it is not the role of the genetic counselor to persuade a proband to pursue treatment – the primary responsibility is to educate them about the genetic (or epigenetic) background of their diagnosis and allow for independent decision-making about their own health management. However the proband decides to proceed, whether making decisions about

undergoing genetic testing, treatments following a genetic/epigenetic diagnosis, or communicating the risk to family members is up to their discretion, but may be influenced by the information they receive from a genetic counselor. Education of epigenetic predisposition for disease (especially in the realms of NDDs and NPDs) is crucial to improving public knowledge of environmental risk factors that may contribute to the pathogenesis of NDDs and NPDs, particularly in utero. With the knowledge of common biological origins for these disorders and surmounting evidence of the NDD-NPD continuum, genetic counselors may grow to have extended responsibilities in epigenetic counseling for NDDs and NPDs for prenatal, pediatric, and adult patients. There is hope to be found in epigenetic etiologies for NDDs and NPDs: precise patient-centered treatments that are more accurate to unique conditions, reversibility of the effects of a hazardous in utero environment, and risk assessment for the future with knowledge of a familial background. These perspectives on the future of epigenetic medicine oblige genetic counselors to take a front-row seat due to their prime position at the intersection of psychosocial and (epi)genetic expertise.

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