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The Role of Placental Genes on Intellectual Disability and Developmental Delay

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THE ROLE OF PLACENTAL GENES ON INTELLECTUAL DISABILITY AND DEVELOPMENTAL DELAY

by

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This manuscript has been read and accepted for the Graduate Faculty in Cognitive Neuroscience in satisfaction of the thesis requirement for the degree of Master of Science.

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ABSTRACT

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The complex interaction between gene expressions and environmental factors plays a key role in the pathogenesis of various diseases, including neurodevelopmental disorders (Lenroot & Giedd, 2008). This study aimed to evaluate first, the magnitude of association between placental gene Methyl-CpG Binding Protein 2 (*MeCP2*) and intellectual disability (ID) in the offspring and second, the synergy between placental gene Forkhead box protein 1(*FOXP1)* and developmental delay (DD) in the offspring. We focused on assessing two specific paradigms, i) placental gene expressions of *MeCP2* among children that have ID vs. children without ID; and ii) placental gene expression of *FOXP1* among children that have DD vs. children without DD. We measured the presence and severity of ID using Full Scale Intelligence Quotient (FSIQ) derived from the Wechsler Preschool & Primary Scale of Intelligence IV (WPPSI-IV) as well as the presence and severity of DD using a composite score from Bayley Scales of Infant Development (Bayley-III) and examined gene expressions of *MeCP2* and *FOXP1* in the placenta. Previous studies found concrete evidence that both *MeCP2* and *FOXP1* are implicated with neurodevelopmental disorders such as ID and DD (Chahrour et al., 2008; Meerschaut et al., 2017). We proposed to test whether hyper expressions of those two genes (*MeCP2 and FOXP1)* in placenta will be associated with children that have higher intellectual and developmental scores. We further examined the influence of prenatal stress, as measured by exposure to Hurricane Sandy, on the relationship between the

placental expression of the two genes, and neurodevelopmental disorders ID and DD. A linear regression model demonstrated that there is a significant moderately positive correlation between *MeCP2* gene expression and FSIQ score for children with ID. In contrast, we found that hyper-expression of the *FOXP1* gene was associated with lower scores on the three domains of DD: motor development, language development and general adaptive development areas. In addition, the magnitude of the association between *FOXP1* and DD in areas of language and general adaptive development was different in relation to exposure to Hurricane Sandy among mothers during pregnancy. Children whose mothers were exposed to Hurricane Sandy on average had a hyper-*FOXP1* expression along with lower DD score. This suggests that prenatal stress further aggravates the magnitude of the association between *FOXP1* and DD. These results demonstrate the intricate roles of genes (*MECP2 and FOXP1*) and environment (Hurricane Sandy) on neurodevelopmental disorders (ID and DD). To further advance our understanding of *MECP2, FOXP1*, ID and DD, more studies should be conducted to examine the impact of specific mutations in placental genes such as *MECP2 & FOXP1* on ID and DD respectively, in offspring.

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Table of Contents

Introduction *1*

THE PLACENTA 2

INTELLECTUAL DISABILITY 3

DEVELOPMENTAL DELAY 5

METHYL CPG BINDING PROTEIN 2 9

FORKHEAD BOX P1 10

Present Study Hypotheses 13

Method *14*

PARTICIPANTS 14

PLACENTA ACCRETION AND GENE EXPRESSION ANALYZATION 15

WPPSI-IV 16

BAYLEY-III 18

STATISTICAL ANALYSIS 20

Results *21*

Discussion and Conclusion *27*

Bibliography *33*

Tables and Figures

Figure 1 Four Domains of FSIQ and their prospective subsets.....17
Figure 2 Bayley-III and WPPSI-IV scoring guidelines.....19
Table 1 Demographic Characteristics of Participants.....24
Figure A MeCP2 Gene Expression Among Children with ID and without ID.....25
Figure B MeCP2 Gene Expression Among Children with ID and without ID.....25
Figure C The Influence of FOXP1 Gene on Motor Development.....25
Figure D The Influence of FOXP1 Gene on Motor Development.....25
Figure E The Association between FOXP1 and General Adaptive Development.....26
Figure F The Association between FOXP1 and General Adaptive Development.....26
Figure G The Role of FOXP1 Gene on Language Development.....26
Figure H The Role of FOXP1 Gene on Language Development.....26

Introduction

Several epidemiology models have described the interaction between genes and environmental factors using models that account for the way genetic outcomes can be modified by different types and levels of environmental exposure (Hunter, 2005). In the past century, a growing number of researchers have studied the impact of genes and environmental factors, both singly and jointly, on the functionality of the brain. From neurodevelopmental disorders in early years in life to cognitive decline in later years, there is a clear interrelation between genes, environmental disposition and diseases that attenuate the functionality of the brain (Lenroot & Giedd, 2008). It is well established that there is a strong linkage between the pathogenesis of neurodevelopmental disorders and epigenetics that is not a result of single mutation but alters gene expression patterns. Maternal factors during gestation have been shown to trigger epigenetic mechanisms that alter the expression of various placental genes. Those genes play a significant role in fetal development without causing mutation (Salilew-Wondim et al., 2014). In this study, we focused on assessing two specific paradigms, i) gene expressions of *MeCP2* among children that have ID vs. children without ID; and ii) pattern of *FOXP1* gene expressions among children that have DD vs. children without DD.

Previous studies have shown evidence that both *MeCP2* and *FOXP1* are implicated in neurodevelopmental disorder such as ID and DD (Chahrour et al., 2008; Meerschaut et al., 2017). As such, we examined expressions of those two genes collected and analyzed from the quadrant midway of mothers' placenta, postpartum. We examined the influence of exposure to Hurricane Sandy on the relationship between the placental genes, *MeCP2*, *FOXP1* and neurodevelopmental disorders, ID and DD. The group of mothers exposed to Hurricane Sandy included all mothers who were pregnant when Hurricane Sandy struck New York in 2012 (N=95).

Neurodevelopmental disorder is a collective term that denotes diagnostic outcomes resulting in abnormal brain development at the neonatal stage and cognitive impairment. This includes both structural defects such as neural tube defects and neuropsychological deficits such as impairments in motor and sensory organization, delayed speech and language, difficulties in learning, and other social interactions. These impairments can lead to disabilities that negatively affect the children's quality of life (van Loo & Martens, 2007).

The Placenta

The placenta is an endocrine organ that connects maternal and fetal functions through a variety of biological pathways (Zhang et al., 2020a). It develops inside the uterus during pregnancy and is discharged shortly after birth (Garnica,et al., 1996). The health and functionality of the placenta is highly impacted by gene regulations, prenatal stress, maternal age, a break in water before labor, birth delivery method, high blood pressure, blood clotting disorder, previous uterine surgery, substance use, alcohol use, and abdominal trauma (Garnica & Chan, 1996; Key et al., 2007a; Knopik et al., 2012; Martinelli et al., 2018; Zhang et al., 2020a). Of these, one of the most pertinent factors is prenatal stress, which can impact the function of the placenta. Specifically, prenatal stress can modify the placenta's development during early pregnancy, alter structures that might interfere blood flow to the placenta arteries or change gene expressions that might interrupt the functionality of vital proteins (Zhang et al., 2020a). Further, because of the dynamics of placental function and its key contribution to energy expenditure, gene expressions in the placenta are tightly regulated.

The placenta has been shown to be affected by several maternal factors that lead to abnormal epigenetic regulation of various developmental genes such as *MeCP2* and *FOXP1*. This

abnormal epigenetic regulation may result in neurodevelopmental disorders in the offspring (Wilhelm-Benartzi et al., 2012). Epigenetic regulation of placental gene expression is achieved via one of the three mechanisms: i) DNA methylation; ii) circulation of microRNA; noncoding short RNAs that regulates mRNA expression; or iii) histone modification (Tsochandaridis et al., 2015; Vaiman, 2017). In this study, we will assess the contribution of prenatal stress (in terms of exposure to Hurricane Sandy) on the relationship between placental gene expressions of *MECP2*, *FOXP1*, and neurodevelopmental disorders, ID and DD, respectively.

Intellectual Disability (ID)

ID is the most common developmental disability affecting over 6.5 million Americans, in which approximately 545,000 are children between the ages of 6 and 21 years (Lee et al., 2021). ID is characterized by a significant limitation both in intellectual functioning and adaptive behavior in an individual. These limitations often lead to a range of disadvantage to the individual's daily life in personal and social functioning (Bach, 2007; Nagi, 1991; Oliver, 1996). Intellectual functioning describes the mental capability of an individual in the areas of reasoning, problem solving, planning, abstract thinking, judgment, comprehending complex ideas, learning rate, and learning from personal and societal experience, whereas adaptive behavior comprises conceptual, social, and practical skills that have been learned and performed by an individual with ID from people around his/her environment (Wechsler, 2012). The operational definition of ID and diagnostic criterial measures intellectual functioning and adaptive behavior (Schalock, 2015).

In recent years, the term ID is widely used internationally and has replaced the previous term mental retardation, which has taken on a derogatory connotation. The new term, ID, better represents the multidimensionality of the disability. ID is operationally defined as having

significantly subaverage general intellectual functioning along with deficits in adaptive behavior that is manifested during the developmental period, that adversely affects a child's quality of life. The diagnostic criteria of ID are deficit in intellectual functioning that results in an intellectual quotient (IQ) of 70 or below and deficit in adaptive behavior measured using standardized, culturally appropriate tests. The current definition emphasizes the importance of the interaction between the person and their environment and the role that an individualized support system can play in enhancing individual functioning. ID also looks at the individual's overall participation in a society: it analyzes the individual's performances in actual activities, in social life domains and comfortability of the individual in a society. Furthermore, it assesses roles and interactions of an individual in the areas of home, work, education, leisure, spiritual, and cultural activities (Wehmeyer et al., 2008).

Similarly, the etiology of ID is a multifactorial construct, that can change across time. The updated etiology of ID replaces the old approach to diagnosis. In recent years, researchers have developed a more comprehensive criteria for etiology of ID by dividing the etiology of ID into four main risk factors categories – biomedical, social, behavioral and educational factors – that interact across time (Emerson et al., 2007). Biomedical factors are related to biologic processes, such as genetic disorders, nutrition, maternal illness, or parental age. Social factors look at social and family influences, such as poverty, maternal malnutrition, and adult responsiveness. Behavioral factors relate to potentially causal behaviors, such as treacherous activities or maternal substance abuse. And lastly, educational factors analyze the availability of educational supports promoting mental development and the development of adaptive skills (Walker et al., 2007). The etiology of ID provided a framework for screening variables in our study: independent variable

(gene expressions), moderating effector (prenatal stress – exposure to Hurricane Sandy) and confounding variable (maternal age, smoking history, and education).

Generally, contributing factors to ID include both environmental and personal factors. Environmental factors include physical, social and attitudinal ambience in which people live and conduct their daily lives. Personal factors are phenotypes and traits of the person such as genetic disposition, gender, race, age, coping styles, education, past and current experiences and struggles, resiliency, and psychological assets. Analyzing the interrelated environmental and personal factors in the individual's everyday life produces a more detailed background of an individual's life. This detailed information can help us understand the source of limitations and how it causes disadvantages to an individual (WHO, 2001). Limitations in all or any combination of these factors could play a key role in the manifestation of ID (Luckasson & Reeve, 2001; Schalock, 2015; Schalock et al., 2010).

Assessment tools such as the Wechsler Preschool and Primary Scale of Intelligence (WPPSI) are designed to account for this multidimensionality and precisely evaluate the level and severity of ID. The Full-Scale Intelligence Quotient (FSIQ) section of the WPPSI probes ID via subtests in areas of verbal comprehension, perceptual reasoning, working memory, and processing speed (Syeda & Climie, 2014; Wechsler, 2012). We used the WPPSI assessment to evaluate the presence and severity of ID in this study.

Developmental Delay (DD)

DD manifests when a child fails to reach developmental milestones due to impairments as compared to children in his/her age group. An array of impairments in motor, speech, language, cognitive performance, social, psychological, and general daily activity often are associated with

DD. Delay is often caused by biological factors, such as alteration in genes; delay can also be caused by environmental factors, such as maternal depression, maternal stress and/or maternal drug abuse (Choo et al., 2019; Ross et al., 2015).

A standardized method to screen for DD is by looking at impairments in one of the four main domains. The first domain is gross and fine motor development which includes physical developments such as rolling over, sitting, standing, hopping, and unclenching fist voluntarily before the age of three months. Most clinicians and researchers assess fine motor function at early ages with a set of blocks to evaluate proper hand-mouth coordination. As the child gets older, areas of impairment are easier to assess more directly. By the age of 1.5 years, a baby should be able to play with blocks; by the time the baby reaches the age of 2 years, the baby should be able to assemble a short tower. A three-year-old should be able to make a tower of 6 to 8 blocks, a four-year-old a tower of 10 blocks, and a five-year-old a complex building or staircase with the blocks (Chen, 1999). As the baby gets older, the height and the complexity of the tower should progress, as well.

The second domain looks at language development. Language development encompasses the extent of the child's language performance, expressive as well as receptive, and the characteristics of the environment in which the child is learning a language. The developmental period from age 3 to 5 years old is a critical language learning time (Scarborough Hollis S. & Dobrich Wanda, 1990). From birth to 5 months, a child should vocalize pleasure and displeasure sounds differently (laughs, giggles, cries, or fusses) and make noise when talked to. In the age range between 6 to 12 months, a child should understand the word “no”, bubbles random words without understanding the meaning such as “ba-ba-ba”, “ma-ma”, “da-da”, and attempts to repeat short words. From the age of 12 to 23 months, child should reach certain milestones such as answer

simple questions, expand vocabulary collection, ask for common foods by name and make animal sounds. In the age range between 2 to 4 years old, a child should comprehend spatial concepts, pronouns and descriptive words, answer to simple questions, form short sentences and repeat sentences. Between the age of 4 to 5 years, a child should understand complex questions and time sequences, deliver understandable speech with some pronunciation mistakes, express ideas and feelings, and use imagination to create stories (Luinge et al., 2006; Nelson et al., 2006).

The third domain describes cognitive development. In the age range of newborn to 4 years old, motor and language milestones are often the best proxy to assess cognitive functionality. For example, from eight to nine months a child should comprehend object permanence. If a child cannot recognize that a hidden object is still present, the child might fail to make the appropriate mental connections. A child in the age range of 1 to 1.5 years old should begin to demonstrate an understanding of cause and effect: parents should be asked whether the child loves to throw a toy down just so the parent can pick it up. Once the parent picks up the object, the child should have a positive reaction such as laughing, understanding that the cause of throwing the toy has an effect of the parent picking it up (Chen, 1999; Martin et al., 2012). As a child grows, increased attention to size and shape relations, symbolic thoughts and play, as well as the development of more formal language should indicate comprehension of both concrete and expanding abstract thinking (Mackrides & Ryherd, 2011a).

The last domain is psychosocial development which covers behavioral abnormalities which could be a possible indicator of difficulties in emotional development. Although it is normal to have behavioral obstacles as a child, clinicians and parents must assess quantity, severity, nature, and duration of these episodes. Infants who refuse to eat, ruminate (chew excessively), or have an abnormal desire to eat substances not normally eaten, severe sleep disturbances, overexcitability,

or apathy, and toddlers and preschoolers with signs of extreme aggressiveness, fearfulness, or substantial defecation problems should be referred for psychological or behavioral testing (Chen, 1999).

In the United States alone, 12 to 16 percent of children have at least one type of developmental delay. However, 6 to 8 percent of the children with DD aren't identified up until they enter kindergarten. Failure to identify DD during early infancy results in missed opportunities for effective intervention. The sooner DD is identified, the earlier an effective intervention can be implemented, and hence the higher the chance to reduce long-term disability due to DD (Mackrides & Ryherd, 2011b).

The etiology of DD consists of three main risk factor categories: 1) biological risk factors which include prenatal or perinatal insult, 2) environmental factors, and 3) established risks, such as clearly diagnosed disorder in infancy (Rihar et al., 2018). In addition, factors, such as low birth weight as a result of intraventricular hemorrhage, sepsis or meningitis, metabolic disturbances, and nutritional deficits can have a subsequent impact on brain growth (Tseng, 2017). Children at increased environmental risk include those whose mothers are young and inexperienced and those with limited financial and familial resources. Children living in families troubled by drugs, alcohol, and violence are particularly vulnerable to poor developmental outcome as well (Tseng et al., 2016; Wilder, 2015).

In order to assess developmental delay in our study, we used data of children that had available developmental assessments from the Bayley-III. The Bayley-III is an individually administered instrument designed to assess the developmental functioning of infants, toddlers, and young children aged between 1 and 42 months (Bayley & Aylward, 2019).

Gene 1: Methyl CpG Binding Protein 2 (*MeCP2*)

MeCP2, first identified in 1992, is a protein that specifically binds symmetrically to only methylated DNA. The *MeCP2* gene is highly conserved across mammals; samples from humans and mice (which diverged from common ancestors) showed a 95% homogeneity at the amino acid level (Guy et al., 2011). The high homogeneity allows researchers to manipulate the gene and generate mutation of *MeCP2* in mouse models and draw a conclusion that can be closely translated to human body functions.

In healthy cells, MeCP2 protein plays a transcriptional regulatory role through a mechanism that involves global binding to DNA and regulation of tertiary structures (Hite et al., 2009). MeCP2 defines both structural and functional properties of neurons during the stages of neurodevelopment and adulthood. *MeCP2* gene begins being expressed for the first time in mid-gestation with a persistent high level of expression in mature neurons. The MeCP2 protein is highly abundant in the brain (Gulmez Karaca et al., 2019). In general, MeCP2 regulates brain development and maintains the function of mature neurons throughout adulthood. During early embryonic development, neuronal maturation, and circuit formation, MeCP2 monitors neuronal differentiation. When cells are undergoing the process of differentiation and maturation, MeCP2 facilitates chromocenter clustering, therefore contributes adequately to the foundation of the typical chromatin structure of mature neurons (Gulmez Karaca et al., 2019). In adulthood, MeCP2 is a critical factor in the maintenance of the neuronal function: it maintains the chromatin structure and regulates the neuronal transcriptomic profile (Adkins & Georgel, 2011). Moreover, it maintains permissive state for stimulus-dependent gene transcription and regulates cognitive function (Gulmez Karaca et al., 2019).

In a mouse model, via Cre recombinase-mediated excision of exon of *MeCP2*, scientists demonstrated the essential role of *MeCP2* for embryo viability and placenta development. Multiple reports have illustrated that *MeCP2* are abundantly expressed in the placenta (Itoh et al., 2012). Collectively, these studies demonstrate the important role of *MeCP2* gene in brain functions.

Mutation in *MeCP2* can cause other neurobehavioral abnormalities such as learning disability, autism, x-linked ID and infantile encephalopathy. In the classic case, a nonsense and missense mutation in a X-linked *MeCP2* gene causes Rett syndrome, a severe and progressive neurodevelopmental disorder that is highly characterized by mild to profound ID (Gulmez Karaca et al., 2019). The x-linked mutation in mammals leads to different outcomes depending on gender. In general, x-linked *MeCP2* mutations affect males much more severely than females due to hemizygosity (Guy et al., 2011). Surprisingly, a duplication and overexpression of *MeCP2* also has been linked with equally detrimental damage to the brain functionality (Chahrour et al., 2008).

Gene 2: Forkhead Box P1 (*FOXP1*)

The *FOXP1* gene belongs to the forkhead family of winged helix transcription factor genes. *FOXP1*, a transcriptional suppressor plays a vital role in the regulation of tissue and cell specific gene transcription during developmental period and adulthood (Ferland et al., 2003). Via transcriptional repression mechanism, FOXP1 protein regulates embryogenesis and preserves differentiated tissue in the early stage of life. FOXP1 protein is expressed as early as gestational week 14 and persist into adulthood. FOXP1 is highly expressed in the developing and mature basal ganglia. A series of experiments found moderate gene expression of *FOXP1* in the cerebral cortex (layers 3–5), hippocampus (CA1), and thalamus (Bacon et al., 2015). FOXP1 protein is expressed

in distinct brain regions of developing bird, mice and human brains; these brain regions are associated with production and processing of vocalization and language (Hamdan et al., 2010).

FOXP2, the closest forkhead family member to *FOXP1* and with high homogeneity at amino acid level to *FOXP1*, was the first gene associated with pathogenesis of speech development and language disorder (Takahashi et al., 2013). The functional relationship between *FOXP1* and *FOXP2* and their role in pathogenesis of developmental language disorders has been described in many studies using mouse models and human subjects (Meerschaut et al., 2017).

Mutation in the *FOXP1* gene has been linked with neurodevelopmental disorders such as ID, autism spectrum disorder and developmental language disorder. These findings suggest that *FOXP1* might have a key role in cognitive and social processes (Bacon et al., 2015). Some reports have shown that individuals with DD, ID, developmental speech and language disorders, autism spectrum disorder, and motor development delay demonstrated some type of *FOXP1* mutation including specific deletions, nonsense mutations and chromosomal breakpoints that essentially led to interruption of protein functionality (Bacon et al., 2015; Le Fevre et al., 2013). Another study showed that mutation in *FOXP1* causes intellectual disability and specific language disorder, along with or without autistic traits. Patient with *FOXP1* mutation also displayed neuromotor delay (Gheorghe et al., 2009).

Transcription factor, *FOXP1* has been associated with many recognizable cognitive phenotypes. In a study with humans, Meerschaut and her colleagues analyzed the correlation between 48 clinical patients with defected *FOXP1* and their cognitive phenotype. The sample comprised molecular data of 25 novel and 23 previously reported patients with *FOXP1* defects. The research evaluated *FOXP1* activity using in vitro luciferase model and FOXP1 protein stability in vitro by western blot. All patients with defected *FOXP1* showed ID, neuromotor delay, language

impairment along with behavioral problems and autistic traits. In a further analysis, severity of ID, neuromotor delay and language impairment varied depending on location of deletion of *FOXP1*: patients with interstitial 3p deletions (14 patients) had a more severe cognitive phenotype compared to patients with monogenic *FOXP1* defects (34 patients).

It is worth mentioning that both monogenic *FOXP1* mutations and more extensive 3p chromosomal deletions surrounding *FOXP1* were implicated with language disorder. Most of these mutations led to premature truncation and nonsense-mediated mRNA decay: only truncation resulted in nonfunctional protein. The most common disorder observed amongst the 48 subjects were developmental language delay, neuromotor delay and ID (Meerschaut et al., 2017).

In a mouse model, *FOXP1* mutant mice generated via Cre-lox system had significantly reduced striatum volume compared to wildtype mice. Mice with a mutation to FOXP1 show a reduction in the striatum and less densely packed neurons in CA1 of the hippocampus. These finding suggests that FOXP1 modulates striatum and CA1 functions. The striatum plays a key role in facilitating voluntary movement, such as motor and action planning, decision making, and speech movement and CA1 of the hippocampus is important for representing space in the environment: individual cells in CA1 are responsible for encoding for space and therefore long-term memory for space and attentional modulation (Wolfgang, 2015, Kandel et al., 2014). *FOXP1* mutant mice showed strikingly reduced exploratory behavior in all categories of the experiment: nest-building ability of *FOXP1* knockout mice was drastically impaired, with no attempt made to construct a nest after nesting material was provided (Bacon et al., 2015). This finding suggests that *FOXP1* has an essential role in spatial memory formation related to motor functions.

Present Study Hypotheses

Hypothesis 1: Studies have shown that the maternal factors during gestation trigger epigenetic mechanisms that may alter the expression of various placental genes that play a significant role in fetal development. There is a clear linkage between pathogenesis of neurodevelopmental disorders and epigenesis that do not result in a mutation, but rather alter the gene expression pattern (Salilew-Wondim et al., 2014). Based on the literatures discussed above, we hypothesized that there is a positive linear relationship between *MeCP2* placental gene expression and FSIQ score in children with ID. Although, a lot of the past studies on *MeCP2* and ID focused on the causal relationship between a specific type of mutation in *MeCP2* and its detrimental impact on ID, this study focused on the correlative relationship between gene expression of *MeCP2* and ID across generation.

Hypothesis 2: A mutation in *FOXP1* has been implicated with pathogenesis of language, motor, social and cognitive delay. Although, a lot of the past studies on *FOXP1* and DD focused on the causal relationship between a specific type of mutation in *FOXP1* and its detrimental impact on DD, this study focused on the correlative relationship between gene expression of *FOXP1* and DD across generation. Based on the literatures discussed in the DD and *FOXP1* gene sections, we propose that there is a positive linear relationship between *FOXP1* gene expression and developmental scores in areas of language development, cognitive development, motor development, and general-adaptive development.

Method

Participant:

The Stress in Pregnancy (SIP) Study is an ongoing longitudinal study that investigates the impact of prenatal stress on child neurodevelopment. The participants are pregnant women recruited from the obstetrics clinics at Mount Sinai Hospital and New York Presbyterian/Queens in New York City. All recruited participants were at least in their second semester. All mothers were equipped with detailed follow-ups prospectively. For this study, a total of 275 participants were included in which placental biopsies were collected along with relevant delivery information (mode of delivery, use of assisted delivery devices, etc.). We restricted our analysis from the general SIP study participants to those that had available placental gene analysis and their offspring's cognitive functioning scores from the WPPSI-IV and the Bayley-III assessments. After cross referencing the molecular data of mother's placental gene analysis and offspring that have completed the WPPSI and the Bayley assessments, we had a total of 266 subjects (163 that have completed the WPPSI and 103 that had the Bayley assessments). Written consent was obtained from all participants and the study was approved by the Institutional Review Boards (IRB).

Exclusion criteria: mothers were excluded from the study based on HIV infection, maternal psychosis, maternal age < 15 years, life-threatening maternal medical complications, and congenital or chromosomal abnormalities in the fetus.

The mean age of mothers was 27 years old with standard deviation of 5.74 years. Among the offspring that completed the WPPSI or the BAYLEY, 48.4 % were female. The mothers were Hispanic/Latino (53%), Black (24%), White (9%), Asian (8%) and other (6%). Though 58% of mothers attended college, only 18% had completed a bachelor or graduate degree. A small

majority of mothers were single (57%), while 40% were married or in a common law marriage. 34.4% (N = 95) of mothers were exposed to Hurricane Sandy: Of the 95 exposed mothers, 66 participants experienced the storm during their first trimester and 29 mothers during the 2nd or 3rd trimesters.

The Institutional Review Boards at the City University of New York, Icahn School of Medicine at Mount Sinai, and New York Presbyterian/Queens approved the study.

Placenta accretion and gene expression analyzation:

At delivery, researchers gathered medical birth records and collected placentas. Placenta biopsies, free of maternal decidua, were collected from each quadrant midway between the cord insertion and the placenta rim within one hour of delivery in order to prevent RNA degradation. The placentas were snap-frozen in liquid nitrogen and stored at -80℃. RNA was extracted with the Maxwell 16 automated DNA/RNA extraction equipment, using the proprietary extraction kits following the manufacturer's protocol. RNA was quantified with Nanodrop spectrophotometer at Thermo Electron North America in Madison, WI. Placental RNA was profiled using nCounter by NanoString Technologies in Seattle, WA. Nanostring data were normalized using the NanoString Norm package. First, raw code counts were normalized against the geometric mean of spike-in controls to account for differences in hybridization and recovery. Differences in sample content were accounted for by normalizing the data against the geometric mean of housekeeping genes (GAPDH, RPL19, and RPLP0). The background threshold was set to the limit of detection divided by the square root of two to maintain sample variability (Zhang et al., 2020b).

Wechsler Preschool and Primary Scale of Intelligence-IV (WPPSI-IV)

The WPPSI-IV is a reliable and valid tool to measure the presence and severity of ID (Syeda & Climie, 2014)(Wechsler, 2012). The WPPSI measures overall intellectual disability on Full Scale Intelligence Quotient (FSIQ). The FSIQ measures an individual's overall level of general cognitive and intellectual functioning. In this study, we used FSIQ composite score derived from administration of subtests from the WPPSI to assess the presence and severity level of ID. Of the participants for whom placental genetic data was collected and analyzed, we only included placental gene expression analysis data for mothers who had corresponding assessments for their children - completed the test before seven years of age.

The WPPSI is composed of multiple subtests to assess the intellectual ability and cognitive functioning of children as young as 2 years,6 months old to 7 years, 3 months old. The score summary is divided into five main domains which include FSIQ, Verbal IQ (VIQ), Performance IQ (PIQ), Processing Speed (PIQ), and Global Language (GLC). For the purpose of this specific study, we focused primarily on the FSIQ score, which is the most representative indicator of ID. The FSIQ provides us with a composite score of four different areas which include verbal comprehension, perceptual reasoning, working memory, and processing speed (Wechsler, 2012). Each four domains are further broken down to subtests as demonstrated as in Figure 1.

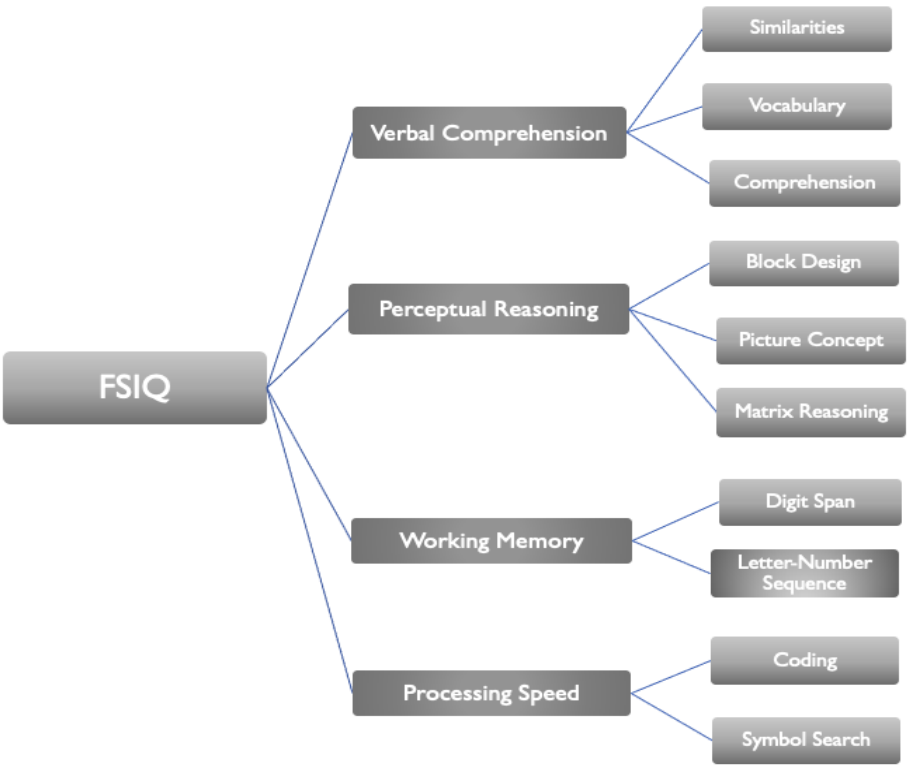

Figure 1: Four Domains of FSIQ, and their prospective subsets.

The WPPSI-IV is administered to two age groups: 2 ;6(indicates 2 years, 6 months) to 3;7; vs. 4to 7;7; . Subtest scores which were used to form the FSIQ score differed between the two-age group. For children in the age range of 2;6 to 3;7, the FSIQ was based on five core subtests: Information, Receptive Vocabulary, Block Design, Object Assembly and Picture Memory. The subtests for this age range provides a more extensive measure of general intellectual functioning with the addition of Picture Memory which measures working memory. For children in the 4;0 to 7;7 age range, the FSIQ score is based on six core subtests: Information, Similarities, Block Design, Matrix Reasoning, Picture Memory, and Bug Search. The subtests for this age range provides a more comprehensive measure of processing speed with the addition of Bug Search (Wechsler, 2012). Refer to *Figure 2* for the breakdown of FSIQ score in terms of measuring ID.

Bayley Scales of Infant and Toddler Development Screening Test (Bayley-III)

In order to assess developmental delay in our study, our analysis included children that had available developmental score from the Bayley-III. The Bayley-III is an individually-administered instrument designed to assess the developmental functioning of infants, toddlers, and young children aged between 1 and 42 months (Bayley & Aylward, 2019). The five main developmental domains assessed include cognitive, language, motor, adaptive, and socio-emotional development.

Cognitive scale: encompasses a process by which knowledge is gained from perceptions or ideas. The cognitive scale assesses the child's abilities in visualization, memory, and attention skills (Madaschi et al., 2016). Toddlers are examined on how they explore new toys, how they solve problems, and their learning process (Bayley & Aylward, 2019).

Language scale: encompasses receptive communication (RC) and expressive communication (EC) to assess the child's understanding of descriptive words, prepositions, and paralinguistic skills. RC is assessed through tasks that measure the child's ability to identify pictures, follow directions, and understand sizes/colors (Harman, 2010). EC is assessed through the child's ability to use gestures, put words together in their native language, and their use of nonverbal expressions (Bayley & Aylward, 2019).

Motor scale: the motor scale assesses both fine and gross motor abilities. Fine motor (FM) assesses the child's ability to use their appendages to make things happen such as grabbing an object, stacking blocks, and drawing shapes. Gross motor (GM) assesses how well the child can move their body through sitting, walking, jump, and maintaining coordination (Bayley & Aylward, 2019).

Adaptive scale: the scale highlights how the child communicates their needs, crawls, plays, and how he/she is in their personal relationships and in socialization. The adaptive scale is primarily assessed through a questionnaire that the parent completes (Harman, 2010).

Social-emotional development scale: The social-emotional scale is primarily assessed through a questionnaire that the parent completes. The scale consists of various items exploring the way in which the child reacts to his/her name, when interrupted in play, and their understanding of inhibitory words (Harman, 2010).

The Bayley-III uses both raw and scaled scores as well as composite scores and percentile ranks for each domain. The standard score allows the examiner to measure the child's development compared to other children his/her age and categorizes this into one of the seven levels. The seven levels include extremely low, borderline, low average, average, high average, superior and very superior (Madaschi et al., 2016). FSIQ score from the WPPSI has an identical break down of score to measure and assess the presence and severity of ID. In this study, we used the composite scores that is dissected as follow:

Composite Score	Classification
130 and above	Very Superior
120-129	Superior
110-119	High Average
90-109	Average
80-89	Low Average
70-79	Borderline
69 and below	Extremely Low

Figure 2: Bayley-III and WPPSI-IV scoring guidelines

Statistical Analysis

The magnitude of association between *MeCP2* and ID was evaluated using a simple linear regression. A predictive model was then generated using the lm function, $FSIQ_{score} = \alpha + \beta(MeCP2) + error$. The α represents y-intercept and β represents the slope of the model. In addition, we used an independent sample *t*-test to assess if there is a mean difference of *MeCP2* gene expression among children with ID vs. those without ID. Similarly, a linear regression model was also used to appraise the correlation between *FOXP1* and DD. The predictive model estimated the presence and level of DD via the model, $DD_{score} = \alpha + \beta(FOXP1) + error$. We also conducted an independent sample *t*-test to determine the significance of mean difference of *FOXP1* gene expression between children with DD vs. children without DD. All regression models were then visualized using ggplot package in R-studio. A Secondary Analysis evaluated the moderating effect of exposure to Hurricane Sandy on the magnitude of association between placental genes *MeCP2* and *FOXP1,* and neurodevelopmental disorders ID and DD, respectively via multiple linear regression model. We also evaluated the impact of certain confounding variables such as maternal age, smoking history, education and race via a multiple linear regression model. All confounding variables have been described in past studies as having an impact and/or an association with neurodevelopmental health of the offspring (Key et al., 2007b; Janecka et al., 2019; Morgan et al., 2017; Harper, 2017). The significance level for all models was set at $p < 0.05$.

Result

The Magnitude of Association between *MeCP2* and ID

Figure A shows a strong significant positive association between *MeCP2* gene expression and FSIQ score among children with ID (N=36). Children with a FSIQ score of 70 or below were categorized as children with ID *(figure 2).* The graph demonstrates the variation of *MeCP2* gene expression filtered by children with ID (turquoise shade) and without ID (light pink shade). For children with ID, the predictive model is $FSIQ_{score} = -339 + 47(MeCP2) + error$ with a *p*-value of 0.028 and an adjusted R-squared value of 0.123. The slope is defined by 47 units: for every increase by 47 units in *MeCP2*, there is a one unit increase in FSIQ score. However, for a one-unit change in gene expression of *MeCP2*, a -292 FSIQ score is obtained which suggests that this is a non-linear relationship. For children without ID (N=125), a weak non-significant positive correlation is observed between M*eCP2* and FSIQ score among children without ID. The Moderating effect of Hurricane Sandy on the relationship between *MeCP2* and ID was insignificant (p =0.069).

Figure B demonstrates that children with ID have a higher average of *MeCP2* gene expression compared to children without ID. An independent sample *t*-test was conducted to examine children with ID ($\bar{x}$ = 8.16, σ= 0.267, N=37) and children without ID ($\bar{x}$ = of 8.112, σ = 0.325, N=126) *MeCP2* gene expression average. No significant difference was found p = 0.446).

The impact of Covariates on the relationship between *MeCP2* and ID

Maternal smoking had a significant effect on the magnitude of the association between *MeCP2* and FSIQ score; mothers that were smoking during pregnancy had a hypo-*MeCP2* expression along with lower FSIQ score (p = 0.047, adjusted R^2 = 0.163). Lastly, we adjusted the regression for maternal age and found a significant effect of maternal age on the relationship between *MeCP*2 and FSIQ score. Mothers that were older than the age of 35, on average, had a lower *MeCP2*

expression and lower FSIQ score (p = 0.046, adjusted R^2 = 0.165). There was no significant effect of race or education on the relationship between *MeCP2* and FSIQ score.

The Correlation between *FOXP1* and Motor Developmental Score (MDS)

For children with DD (N=35), those who had developmental score of 70 or less, there is a negative correlation between gene expression of *FOXP1* and MDS with an adjusted R-squared of 0.345 and a p-value of 0.034. The predictive model generated for this relationship is, MDS= 126-8.50(*FOXP1*) + error. A slope of -8.50 indicates that as *FOXP1* expression decreases by 8.50 units, there is an increase in motor development score by one unit (*Figure C*). Exposure to Hurricane Sandy didn't have a significant moderating effect on the relationship between *FOXP1* and MDS. However, when adjusted for smoking history, there was a significant interaction between mothers that smoked during pregnancy, *FOXP1* expression and MDS with (p-value = 0.030, adjusted R^2 = 0.569). This suggests that mothers that were smoking during pregnancy had a stronger negative impact on the correlation between *FOXP1* and motor development score: a higher *FOXP1* expression was associated with a lower MDS, on average. *Figure D* displays that the average *FOXP1* gene expression for children with motor DD is higher than children without motor DD. An independent sample t-test was conducted to examine children with DD in motor area ($\bar{x}$= 6.54, σ = 0.52) and children without DD in motor area ($\bar{x}$= 6.45, σ= 0.49) *FOXP1* gene expression. No significant difference was observed (pe = 0.55).

The Magnitude of Correlation between *FOXP1* and General Adaptive Score (GAS)

Looking at general adaptive development, there was a strong magnitude of association between GAS and *FOXP1* gene expression (p = 0.026, adjusted R^2 = 0.380). A slope of -9.54 in *Figure E* demonstrates a negative correlation between *FOXP1* and GAS for children with DD. *Figure F* displays that children with DD in general adaptive domain ($\bar{x}$ =6.604, σ= 0.454) had hyper- *FOXP1*

gene expression compared to children without DD ($\bar{x}$ =6.441303, σ= 0.495). The mean difference was not significant (p = 0.301).

The Magnitude of Association between *FOXP1* and Language Developmental Score (LDS)

We also observed a significant negative correlation between language development score and *FOXP1* gene expression (p = 0.033; adjusted R-squared = 0.132). *Figure G* shows a slope of -5.649, displaying an inverse relationship between *FOXP1* and language development score among children with DD. For mothers exposed to Superstorm Sandy, there was a significant moderating effect of environmental factor on the relationship between *FOXP1* and LDS with a slope of -27 (p = 1.382e-05). In *Figure H*, a hyper-*FOXP1* mean was observed for children with LDS ($\bar{x}$ =6.509, σ =0.402) compared to children without LDS ($\bar{x}$ =6.451, σ =0.523). However, an independent sample *t*-test demonstrated that the mean difference wasn't significant (p = 0.548).

Table 1. Demographic characteristics of participants

		Superstorm Sandy exposure status		
	Total Sample (N=303)	Exposed (N=208)	Unexposed (N=95)	statistics
Maternal race, N (%)				
White	27 (9%)	15 ((7%)	12 (13%)	
Black	74 (25%)	58 (28%)	16 (17%)	
Hispanic/Latino	159 (53%)	111 (53%)	48 (51%)	
Asian	23 (7.7%)	10 (5%)	13 (14%)	
Others	14(4.68%)	13 (6%)	5(5%)	
Missing	2 (0.69%)	1 (~0%)	1 (1%)	X^2 *(5)=0.976* *p-value=0.323*
Maternal education, N (%)				
Primary school	6 (2.3%)	5 (2%)	1 (1%)	
Some high school	53(17%)	47 (23%)	6 (6%)	
High school graduate	68(22%)	48 (23%)	20 (21%)	
Some college	91(30%)	66 (32%)	25 (26%)	
Associate degree	30 (9.9%)	16 (8%)	14 (15%)	
Bachelor's degree	30 (9.9%)	14 (7%)	16 (17%)	
Graduate degree	25(8.5%)	12 (6%)	13 (14%)	X^2 *(6)=19.9* *p-value= 0.000*
Marital status, N (%)				
Married	101 (33%)	50 (24%)	51 (54%)	
Common law	21 (7%)	14 (7%)	7 (7%)	
Single	173(57%)	140(67%)	34 (36%)	
Widowed	2 (1%)	2(1%)	0 (0%)	
Divorced/separated	3 (1%)	1 (.5%)	2 (2%)	
Missing	2 (1%)	1 (.5%)	1 (1%)	X^2*(5)=27.0* *p-value= 5.862*
Child sex, male, N (%)	158(52%)	94(59%)	64(41%)	F= 2.249
Maternal age, Mean (SD)	27(5.74)	27(6.1)	27(5.13)	F=0.7304
Birthweight (grams), Mean (SD)	3268(594)	3308(643)	3211(536)	F=14.83
Gestational age at birth (weeks), M (SD)	39.2(2.07)	39.2(2.22)	39.04(2.02)	F= 2.879
Maternal prenatal smoking, N (%)	24(12.8%)	6(0.25%)	18(0.75%)	X^2(1)= 1.112 p-value = 0.292
Intellectual Disability, N (%)	36(22.3%)	5(13.8)	31(86.1)	X^2(1)=6.51 p-value= 0.011

MeCP2 Gene Expression Among Children with ID and without ID

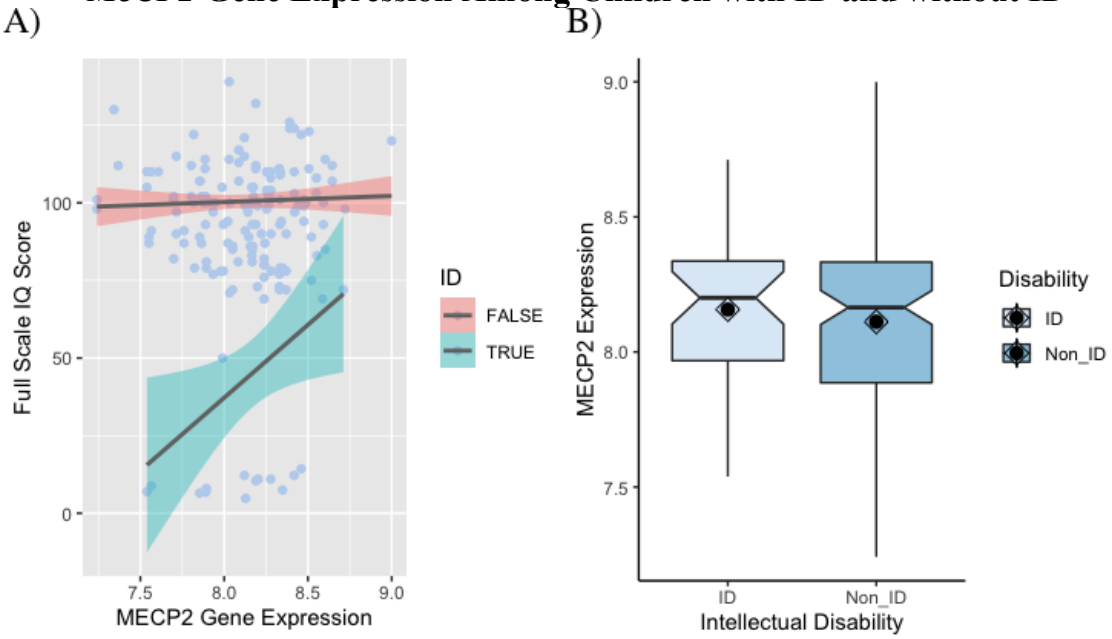

Figure A*: The magnitude of association between MeCP2 gene expression and FSIQ score among children with ID (turquoise, TRUE) and children without (light pink, FALSE).* ***Figure B*** *compares the mean MeCP2 expression for children with ID vs. children without ID.*

The Influence of *FOXP1 Gene* on Motor Development

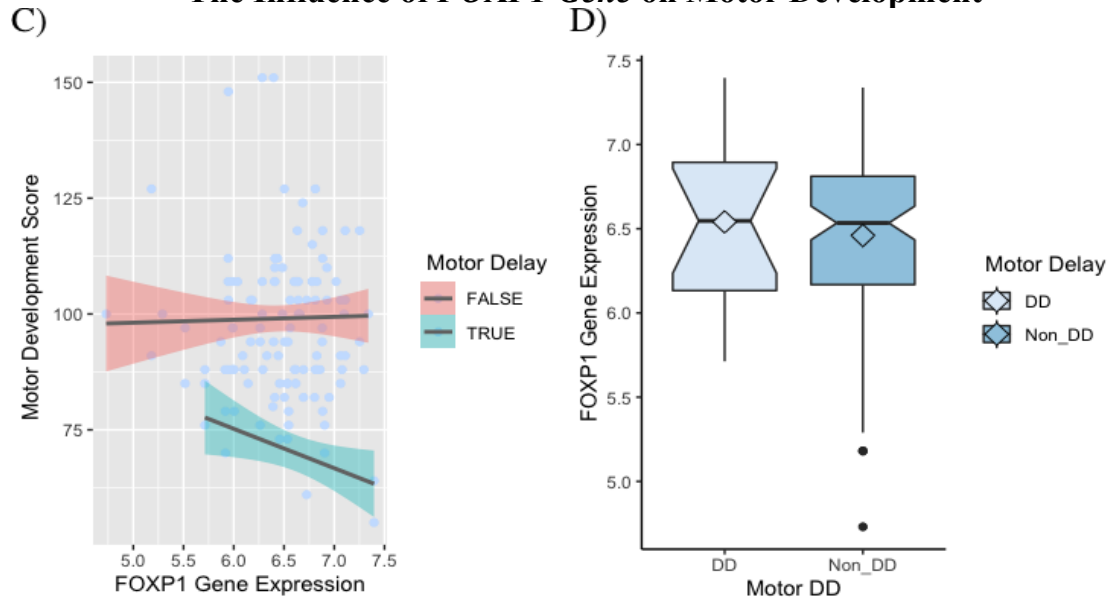

Figure C *shows the association between FOXP1 gene expression and motor development composite score for children with DD (turquoise, TRUE) and without DD (light pink, FALSE).* ***Figure D*** *compares the mean value of FOXP1 gene expression among children with DD and children without DD in motor developmental area.*

The Association between *FOXP1* and General Adaptive Development

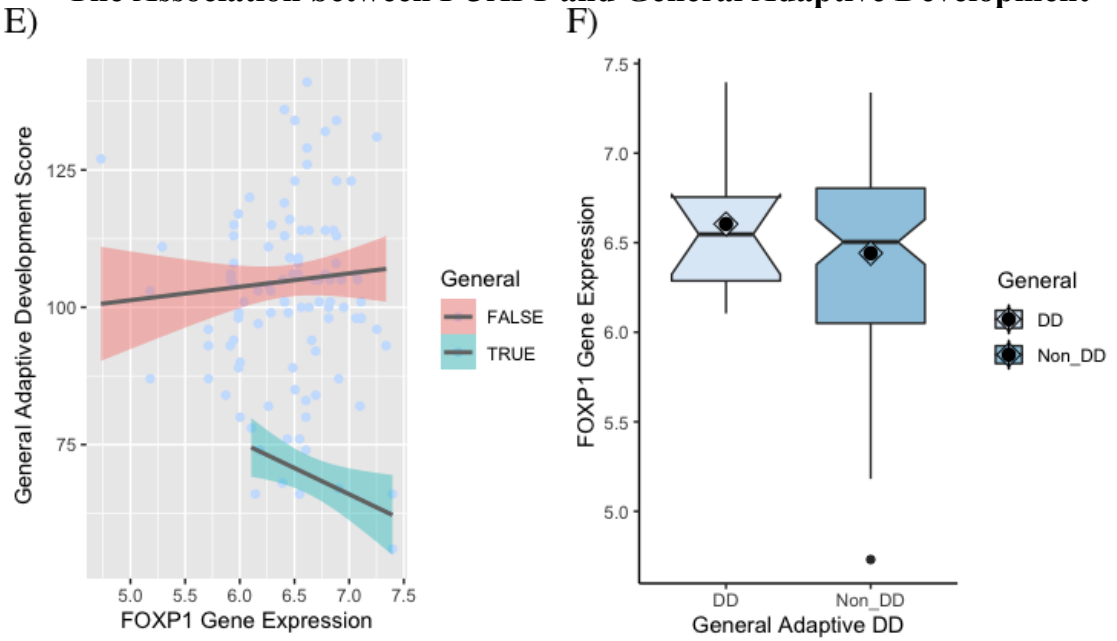

Figure E*: a linear regression demonstrating the association between FOXP1 gene expression and General Adaptive neurodevelopment score among children with DD (turquoise, TRUE) vs. children without DD(light pink, FALSE).* ***Figure F****: comparison of mean of FOXP1 gene expression among children with general adaptive DD and children without general adaptive.*

The Role of *FOXP1* Gene on Language Development

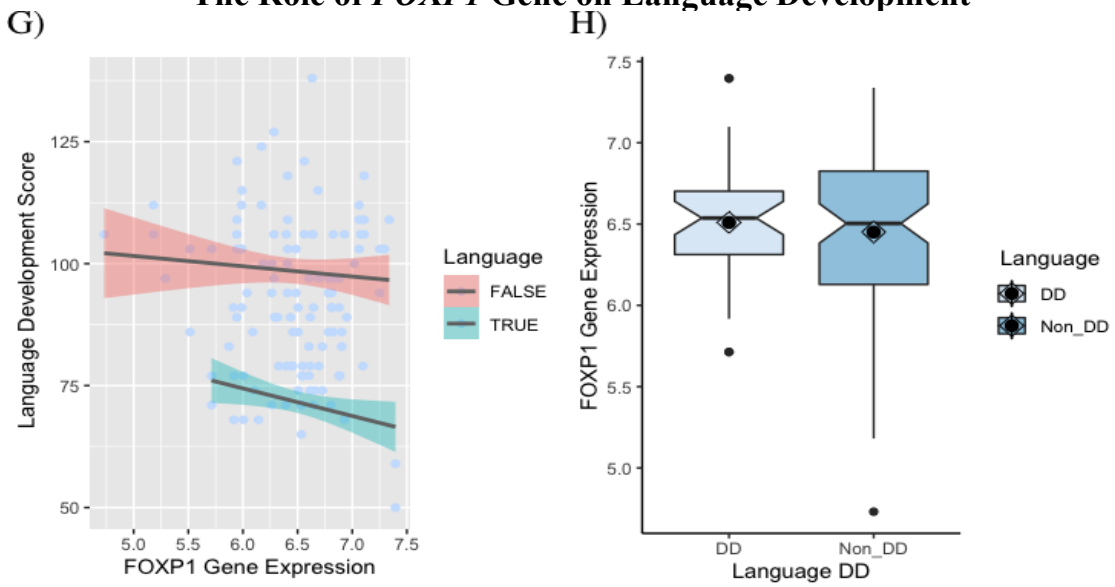

Figure G *a linear regression demonstrating the association between FOXP1 gene expression among children with DD in Language development score (turquoise, TRUE) vs. children without DD(light pink, FALSE).* ***Figure H****: comparison of mean of FOXP1 gene expression among children with language DD and children without language DD.*

Discussion and Conclusion

Our results showed a significant association between *MeCP2* gene expression and FSIQ score of children with ID. We observed a positive strong correlation between *MeCP2* gene expression and FSIQ score of children with ID, and a non-significant association between *MeCP2* gene expression and children without ID (Figure A). *MeCP2* is a critical factor in the maintenance of the neuronal function; it maintains the chromatin structure and regulates the neuronal transcriptomic profile, playing a vital role in pathogenesis of many neurodevelopmental disorders including ID. The observed result from this study supports our hypothesis and aligns with previous studies. Hyper-*MeCP2* gene expression was associated with higher developmental score among children with ID.

This study focused on the correlative relationship between placental gene expression of *MeCP2, FOXP1* and neurodevelopmental disorder of ID and DD, respectively. We had no molecular data on mutation of these genes, we simply assessed the magnitude of association between gene expressions of *MeCP2, FOXP1* and neurodevelopmental disorders, ID and DD. However, this study is unique in that it compared maternal placental gene expressions with diagnosis of the offspring as ID or DD. Past studies discussed in the introduction section focused on *MeCP2* and *FOXP1* mutations in patients and presence and severity of ID and DD in the same patients. This study assessed the role of placental gene expression on neurodevelopmental disorder across generation: placental genes from the mother and ID and DD diagnoses from the offspring. The main rationale for this approach is that the candidate gene expressions were from a specific organ that connects and shapes multiple biological mechanisms between mother and offspring, the placenta.

The maternal factors during gestation trigger epigenetic mechanisms that may alter the expression of various placental genes that play a significant role in fetal development (Salilew-Wondim et al., 2014). The placenta has been shown to be affected by environmental factors, leading to abnormal epigenetic regulation of various developmental genes; this abnormal regulation may result in neurodevelopmental disorders in the offspring (Wilhelm-Benartzi et al., 2012).

There are several epigenetics mechanisms that alter gene expression pattern without altering the DNA sequence via mechanisms such as DNA methylation, post-translational modifications of histone proteins, and transcriptional regulation by non-coding RNAs including miRNA, siRNA, piRNA (Cedar & Bergman, 2011). In addition, several reports have shown that the process of early embryonic development in the fetus is highly susceptible to epigenetic modulation ((Reik et al., 1993; Resendiz et al., 2014). It is well established that there is a clear link between pathogenesis of neurodevelopmental disorders and epigenesis that does not result in a single mutation but, rather, alters gene expression pattern. Therefore, in this study, we focused on assessing two specific paradigms, i) the placental gene expression pattern of *MeCP2* among children that have ID vs. children without ID, ii) pattern of *FOXP1* gene expression among children that have DD vs. children without DD.

The main limitation in the manipulation of this study is that we assessed maternal gene expression patterns among offspring with DD and ID subjects instead of a specific mutation. A future direction of this study will evaluate the relationship between maternal placental gene expression of *MeCP2* and *FOXP1.* This evaluation will include specific mutations of *MeCP2* and *FOXP1*genes and their offspring's ID and DD scores. An expansion of this study would involve recruiting mothers that have a nonsense or missense mutation in *MeCP2* gene of the placenta and

evaluating whether their offspring are at higher risk for ID. In a parallel study, mothers with a placental 3p deletions or monogenic *FOXP1* mutation would have their offspring evaluated for any signs of developmental delay. Such studies will allow us to evaluate whether *MeCP2* and *FOXP1* mutations in maternal placenta can cause neurodevelopmental disorders (ID, DD) in children; it will allow us to assess impact of epigenesis across generation, from mother to offspring.

The *FOXP1* gene expression pattern was significant among children that have DD in three specific developmental domains: motor development, language development and general adaptive development areas. All three developmental areas had a moderate to strong negative correlation with *FOXP1* gene expression among children that have DD. No significant association was observed between *FOXP1* gene expression and developmental areas in children without DD. We did not expect to find a negative correlation between *FOXP1* gene expression and DD children. On average children with lower score in motor, language and general adaptive developmental areas had a hyper-*FOXP1* expression. These findings neither support our hypothesis nor align with findings from past literature on function of *FOXP1*. We expected that higher gene expression of *FOXP1* would be correlated with higher developmental scores.

This unexpected inverse linear relationship between *FOXP1* gene expression and DD scores could be accounted for by the small sample size we had for children with DD. Even though we had adequate data from mothers regarding placental gene expression, too few of the offspring in the sample had a DD assessment. To confirm our findings, we suggest replicating a similar study with a much larger sample size of children with DD.

For *FOXP1*, we observed higher mean gene expression average for DD group than non-DD group. Again, considering that mutation of *FOXP1* causes delays in language, motor and intellectual functions, we were surprised that children with DD had higher gene expression of

FOXP1 on average. We expected to find that, on average, children without DD will have a higher expression of *FOXP1* that children with DD. However, an independent sample *t*-test showed that this mean difference was not significant.

A future study should assess whether there is a specific range of *FOXP1* gene expression that is correlated with DD vs. non-DD. Similarly, expression of *MeCP2* was higher among children with ID vs non-ID. We found, on average, hyper- *MeCP2* among children with ID. There are some studies that reported on the relationship between overexpression of *MeCP2* with ID: more analysis needs to be done but our result does coincide with these findings (Chahrour et al., 2008). More study needs to be done to evaluate, i) what qualifies as overexpression of *MeCP2*, ii) what impact does placental overexpression of *MeCP2* has on offspring neurodevelopment.

The impact of Hurricane Sandy on the relationship between placental genes and neurodevelopmental disorder was significant in almost all models. The correlative relationship between placental genes and neurodevelopmental disorders was more pronounced (in the direction of the findings from the primary analysis) in mothers that were exposed to Hurricane Sandy. These finding are parallel with previous findings on the influence of environmental factors on genes and pathogenesis of diseases. This study design was not able to address whether these environmental factors had a causal role in on the relationship between placental genes and neurodevelopmental disorders. Hurricane Sandy had the most moderating effect on the magnitude of association between *FOXP1* gene expression and language DD; it's worth noting that the sample size for the children identified with DD (N= 29) that also had mothers exposed to Hurricane Sandy was the greatest sample size compared to other groups. This observation suggests that a bigger sample size of mothers that were exposed to Hurricane Sandy is needed to accurately evaluate the moderating effect of prenatal stress on the magnitude association between gene expressions and

neurodevelopmental disorders. In groups where we didn't see a significant impact of prenatal stress (exposure to Hurricane Sandy), the sample size was very small. There appeared to be a moderating effect of Hurricane Sandy on the association between hypo-*MeCP2* expression and lower FSIQ scores, but the statistical modeling indicated that this effect was not significant. However, it is not clear if this is due to a small sample size or actual lack of association. Notably, there was only 5 cases of ID among children whose mothers were exposed to Hurricane Sandy.

The biggest limitation of this study is a small sample size; our sample size was restricted only to SIP study patients. A correlative study such as this one would be much stronger with data across the nation instead of a data that is restricted only to participants from Mount Sinai Hospital and New York Presbyterian/Queens Hospital in New York City. Although, we started out with 275 participants that had placental gene analysis, when we cross referenced and merged the molecular data on placental genes with the children who had also completed the WPPSI or the Bayley-III assessment, our sample size was significantly reduced. A great expansion of this study would be a meta-analysis study that investigates the association between placental gene expressions and neurodevelopmental disorder across the nation or even the world.

ID and DD are amongst the most common neurodevelopmental disorders in American. Both ID and DD generate limitations that interrupts many aspects of individual daily life. These limitations often lead to a range of disadvantage for the individual, posing obstacles and challenges in the individual's daily life in personal functioning and performance of roles and tasks that are expected of an individual within a social environment.

Over 6.5 million Americans have mild to severe ID and about 6 to 8 percent of the entire population have some form of DD. These numbers are alarming. A number of reports have shown that there is a clear link between epigenetic regulation and neurodevelopmental disorders such as

ID and DD. Dissecting the relationship between environmental factors, genes and neurodevelopmental phenotypical outcome is an important step in understanding this complex association. Advancing our understanding of this intricate relationship can help us identify risk factors for ID and DD. Subsequently, identifying risk factors allows parents and clinicians to implement certain interventions and measures that could potentially reduce disabilities and delays in a child's life. Collectively, as a society we should began focusing on the impact epigenetics as in the health of our children.

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