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Paternal Ages and Genetic Diseases and Congenital Anomalies

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PATERNAL AGES AND GENETIC DISEASES AND CONGENITAL ANOMALIES

by

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ABSTRACT

The purpose of this thesis is to investigate the link between advanced paternal ages (APA) (i.e., APA ≥ 35 years and APA ≥ 50 years) and genetic diseases and congenital anomalies. Currently, the relationship between both advanced paternal ages and genetic diseases and congenital anomalies remains unclear. There is room for improvement, however, to investigate systematically the relationship between specific congenital anomalies in newborns and advanced paternal ages. More recently, the link between advanced paternal age (as opposed to existing studies analyzing advanced maternal age alone) and genetic diseases has been recognized by researchers, epidemiologists, and various health experts. Thus, this study serves to examine the effect of advanced paternal ages on the likelihood of birth defects using a new dataset intended to discover those relationships.

I create three different datasets and utilize 12 statistical models to analyze the relationship between advanced paternal ages (APA ≥ 35 years and APA ≥ 50 years) (while including advanced maternal age or AMA [AMA ≥ 35 years]) and genetic diseases and congenital anomalies. I focus on Down syndrome, cleft lip with or without cleft palate, and meningocele/spina bifida and explore the relationship between both advanced parental ages. I explore whether (a) the advanced paternal ages and (b) the advanced maternal age increase the likelihood of newborn reproductive defects: (a) Down syndrome, (b) cleft lip with or without cleft palate, and (c) meningocele/spina bifida. This study includes all U.S. births between 2016 and 2019 using the CDC Natality Registry[1] database (2020). I perform the analyses using logistic

[1] Collection of data for all variables used in this research are obtained with full permission from: United States Department of Health and Human Services (US DHHS), Centers for Disease Control and Prevention" "(CDC), National Center for Health Statistics (NCHS), Division of Vital Statistics, Natality public-use data 2016-2019, on CDC" WONDER Online Database, October 2020. Accessed at http://wonder.cdc.gov/natality-expanded-

regression models (to estimate odds ratios) that provide explanations of the relationship between each birth defect and advanced paternal ages. Analysis results suggest that advanced paternal ages (APA ≥ 35 years and APA ≥ 50 years) are positively associated with Down syndrome, whereas advanced paternal age (APA ≥ 35 years) is negatively associated with cleft lip with or without cleft palate. The results from the advanced paternal ages models do not suggest any causal relationship/effect on spina bifida. The results of this study are expected to offer some insight of the following reproductive defects: (a) Down syndrome, (b) cleft lip with or without cleft palate, and (c) meningocele/spina bifida.

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For the children in India

Table of Contents

LIST OF TABLES

INTRODUCTION

The Question

In some parts of the world, there are some views that women's suitable [childbearing] age is about ten years less than men's age (Allendorf et al., 2017). The "gender gap in marital timing[2]" or biological clock may be influenced by societal taboos and myths on fertility and attributing reproductive defects to the mother's age only (Ibid., 2017). Medical studies on high-risk pregnancies in aging mothers (AMA ≥ 35 years) have been linked to an aged reproductive system and an aged body (Lampinen et al., 2009). As the mother ages, the integrity of the ova deteriorates, which may be linked to both decreased fertility and higher risk of chromosomal abnormalities (Heffner, 2021). Consequently, many studies have focused on the connection between advanced maternal age (AMA ≥ 35 years) and genetic diseases to support the theory on aging female fertility and reproductive abnormalities (Ibid., 2021). However, the identified advanced maternal age only offers half the answer to reproductive anomalies (Cnattingius et al., 1992; Bianco et al., 1996; Dollberg et al., 1996; Breart et al., 1997; Jolly et al., 2000; Hollier et al., 2000; Reefhuis et al., 2004). Thus, the potential reproductive anomalies cases relate to the question I focus on in this thesis. Do the advanced paternal ages (APA ≥ 35 years and APA ≥ 50 years) and advanced maternal age (AMA ≥ 35 years) contribute to genetic diseases and congenital anomalies in newborns?

Studies suggest advanced maternal age is viewed to be linked with a higher prevalence of chronic medical anomalies (Lampinen et al., 2009; Carolan and Nelson, 2007). Essentially, the

[2] On average, the suitable age range for a woman to marry is 20 to 25, whereas the man's acceptable age range is between 23 and 30 (Allendorf et al., 2017).

literature often studies the relationship between female age and birth anomalies in newborn children, and the alternate relationship between the male's advanced age on reproductive abnormalities remains scarce and unclear (Conti and Eisenberg, 2016). There is room for developing this topic, however, to investigate both paternal ages and maternal age on specific anomalies in infants. More recently, the link between advanced paternal ages and genetic diseases has become a topic of interest among researchers, epidemiologists[3], and various health experts. Thus, this study serves to explore the effect of advanced paternal ages on the likelihood of birth defects[4] such as genetic diseases and congenital anomalies.

The Main Argument

Birth defects, which may manifest their signs at birth or later in the affected individual's life, may be genetic in nature. From the onset of human reproduction, "abnormal genes in the reproductive cells of one or both parents, [among various predictors] cause[s] mutations" (Kalumuck, 2019). In 1912, Weinberg suggested that if a study were intended to analyze more directly the link between mutations and last-born children, we could better understand the susceptibility to dwarfism (known as achondroplasia) (Crow, 2000: 40). Weinberg's (1912) achondroplasia trait observation established the initial conception of human genetics (Crow and Hardy, 1999). Weinberg's (1912) original observation was first studied by Haldane (1947) who suggested the level of male mutation is approximately ten times higher than that of the female, which despite uncertain valuations, was substantiated in incidences of Haemophilia A[5] (a genetic

[3] Epidemiologists practice the field of medicine that focuses on the "incidence, distribution, and possible control of diseases and other factors relating to health" (Lexico, 2020).
[4] "Birth defects" is also used as an alternative when referencing genetic diseases and congenital anomalies (Grewal et al., 2012).
[5] Becker et al., 1996.

deficit usually impacting males) and Hemophilia B[6] (a genetic bleeding disorder)" (Crow, 2000: 40). Advancing Weinberg's (1912) initial theory on genetic mutations can be used to further our understanding of other genetic diseases regulated by biological functions.

Based on Haldane's (1947) study, Penrose (1955) further studied the genetic mutations model and suggested that paternal age may possibly be the only cause of Weinberg's original 1912 study (Crow, 2000: 40). Since [advanced] paternal age may be linked to genetic mutation, which may heighten the risk of birth defects, Penrose's proposed 'copy error' theory (1955) was developed (Grewal et al., 2012). Penrose (1955) claimed that since male germ cells experience constant mitotic divisions, there may be a higher likelihood of spontaneous mutations in the male germ line than that of the female (Grewal et al., 2012: 387). Therefore, the higher rate of germ cell division or spermatogenesis in advanced paternal age relative to the female germ line (or oogenesis) in advanced maternal age may be associated with congenital anomalies (Materna-Kiryluk et al., 2008). In human males, the time of spermatogenesis is between 72 - 74 days, which involves division of germ cells during the meiosis and mitosis phases, increasing the vulnerability of "cytotoxic damage" or altering the "DNA[7] sequence" resulting in genetic mutations (Ibid., 2008: 30). By the time a male is 35 years, the spermatozoa (or motile sperm cells) will have undergone about "540 divisions and replications" that accumulate in the older father's sperm, thus increasing the likelihood of congenital malformations (Grewal et al., 2012: 389). Based on the literature that analyzes the link between paternal age and genetic abnormalities, I adopt APA $\geq$ 35 years as the advanced reproductive age for the father. Vogel

[6] Ketterling et al., 1999. Green et al., 1999.

[7] DNA is the abbreviation for Deoxyribonucleic acid, which is a molecule that consists of the biological directions that exclusively define the characteristics of each breed (National Human Genome Research Institute, 2020).

and Motulsky (1997) add that males undergo 840 spermatic replications by age of 50, potentially increasing the likelihood of transcription errors throughout the replication process (Penrose, 1955). Thus, considering a higher rate of spontaneous mutations in older fathers' sperm, I argue that when paternal ages are APA ≥ 35 years and APA ≥ 50 years—combined with the advanced maternal age of AMA ≥ 35 years—there may be a higher probability of birth defects in the couple's newborn (Halvaei et al., 2020).

Building on Penrose's (1955) theory, epidemiologists have identified over four thousand "gene disorders" that are inherited, and such chromosomal abnormalities may be identified as numerical[8] and/or structural[9] (Powell-Hamilton, 2018). While science has advanced in the classification and categorization of chromosomal abnormalities, there is insufficient focus on advanced paternal age and genetic mutations. Despite the existing theory used to justify the possible connections between disorders in newborns and paternal age, studies remain unsystematic in comparing the advanced paternal ages and advanced maternal age on the effect of genetic diseases (Thompson, 2019).

According to Grewal et al. (2012), some studies analyzed and suggested a positive relationship between APA and heart defects in newborns (Lian et al., 1986; Olshan et al., 1994; Yang et al., 2007; and Materna-Kiryluk et al., 2008), neural tube and upper limb defects (McIntosh et al., 1995), orofacial clefts (Materna-Kiryluk et al., 2008; McIntosh et al., 1995; Polednak, 1976; and Bille et al., 2005) and malformations in the nervous system (Kazaura et al.,

[8] Numerical abnormalities contain an extra chromosome (i.e., Trisomy) and a missing chromosome (i.e., Monosomy) (Powell-Hamilton, 2018).
[9] Structural abnormalities contain anomalies whereby an entire chromosome (or parts of chromosomes) do(es) not properly connect with other chromosomes (i.e., Translocations) and deletions and duplications of several chromosomal parts (Powell-Hamilton, 2018).

2004). Conversely, similar studies found a negative relationship suggesting there is a higher likelihood of "congenital heart defects" among young paternal age (Zhan et al., 1995), "neural tube defects" (McIntosh et al., 1995; Kazaura et al., 2004), "anencephaly and spina bifida" (Yang et al., 2007; Kazaura et al., 2004), and "gastroschisis" (Yang et al., 2007; Materna-Kiryluk et al., 2008; and Archer et al., 2007). However, these studies are inconsistent in using reliable sources to obtain data on birth defects, do not include the partner's age in their analyses (i.e., the mother's age), and obtain limited sample size of birth defects in their analyses (Grewal et al., 2012). While the said findings offer guidance concerning the advanced father's age as a potential factor affecting birth defects in newborns, there are some discrepancies that are not accounted for in the aforementioned analyses (i.e., the female partner's age, a larger sample, and observations using reliable sources). Hence, it is difficult to analyze fully the predictors of birth defects that manifest at birth when only one parent's age characteristics is considered. Thus, the inclusion of both advanced parents' ages may provide some explanation in the empirical study of advanced paternal ages and reproductive anomalies.

Since the previous studies use Penrose's (1955) copy error theory to analyze the link between APA and reproductive anomalies, I argue that when advanced paternal ages (APA $\geq$ 35 years and APA $\geq$ 50 years) are in place, the higher rate of spontaneous mutations may increase the likelihood of birth defects when including the theory on advanced maternal age (AMA $\geq$ 35 years) in the analyses.

According to CDC Natality Registry report, genetic diseases that become apparent or detected at birth are known as congenital anomalies (2020). Accordingly, this research aims to take a more nuanced approach by identifying genetic diseases that manifest into congenital

anomalies in newborns using the CDC Natality Registry[10] (2020) to analyze the link between APA and reproductive anomalies. In particular, this research will analyze Down syndrome, cleft lip with or without cleft palate, and meningocele/spina bifida[11] (2020). Since these three congenital anomalies may appear because of idiosyncratic genetic code—a complex mechanism in the physiology of reproduction—this research provides additional analyses to determine a potential link between advanced paternal ages and the three congenital anomalies. The literature considers that both parents' family history relates to some reproductive anomalies, but the literature that analyzes the relationship of APA, while accounting for AMA, on genetic abnormalities is limited at best (Powell-Hamilton, 2018). Basically, this research assumes that if there is an advanced maternal age effect, then there ought to be a similar structure to the advanced paternal age effect, such that the select congenital diseases will appear sharply for the higher parental age-groups (APA $\geq$ 35 years and APA $\geq$ 50 years and AMA $\geq$ 35 years) (Stene et al., 1977).

[10] Collection of data for all variables used in this research are obtained with full permission from: United States Department of Health and Human Services (US DHHS), Centers for Disease Control and Prevention" "(CDC), National Center for Health Statistics (NCHS), Division of Vital Statistics, Natality public-use data 2016-2019, on CDC" WONDER Online Database, October 2020. Accessed at http://wonder.cdc.gov/natality-expanded-current.html on Jun 6, 2021, 1:24:47 PM;" United States Department of Health and Human Services (US DHHS), Centers for Disease Control and Prevention" "(CDC), National Center for Health Statistics (NCHS), Division of Vital Statistics, Natality public-use data 2016-2019, on CDC" "WONDER Online Database, October 2020. Accessed at http://wonder.cdc.gov/natality-expanded-current.html on Jun 6, 2021, 1:29:36 PM;" And United States Department of Health and Human Services (US DHHS), Centers for Disease Control and Prevention""(CDC), National Center for Health Statistics (NCHS), Division of Vital Statistics, Natality public-use data 2016-2019, on CDC" "WONDER Online Database, October 2020. Accessed at http://wonder.cdc.gov/natality-expanded-current.html on Jun 6, 2021, 1:07:36 PM."

[11] Spina Bifida is Meningocele, which is characterized with "a sac fluid [that] comes through an opening in the baby's back" (CDC, 2020). In this research, I will use "spina bifida" when discussing this reproductive anomaly.

Significance of Research

The exact cause of congenital anomalies—also called birth defects—are not known, but there are certain factors (e.g., advanced parental ages, "nutritional deficiencies, certain types of infection and other illness in the mother . . . and hereditary disorders") that have been linked with a higher likelihood of such birth defects (Berkow et al., 1997: 1223). While the literature suggests that genetic and chromosomal factors are inherited by "one or both parents", most studies analyze advanced maternal age (AMA $\geq$ 35 years) on the likelihood of a chromosomal abnormality (Ibid., 1997: 1224). Hence, the premise of this study is to account for advanced paternal ages (APA $\geq$ 35 years and APA $\geq$ 50 years), alongside the mother's advanced age, on the propensity of genetic diseases that manifest into congenital anomalies in newborns. The need for this study allows for a comparable understanding using quantitative statistical methods to demonstrate the link between APA on Down syndrome, cleft lip with or without cleft palate, and spina bifida—all of which are classified under genetic diseases. It is the genetic material that mainly contribute to the development of congenital anomalies in infants (Stene et al., 1977). Thus, concentrating on the replication factor that may enhance the proclivity of mutation with advanced paternal age, it is plausible to hypothesize that advanced paternal ages (while including the advanced maternal age in the analyses) may increase the likelihood for such congenital anomalies (Penrose and Smith, 1966).

My research offers three novel datasets that analyze the link between APA and three separate congenital anomalies (i.e., Down syndrome, cleft lip with or without cleft palate, and spina bifida) using the CDC Natality Registry from 2016-2019. Thus, this research advances the

existing literature by examining the effect of two advanced paternal ages (APA ≥ 35 years and APA ≥ 50 years) and three reproductive anomalies.

THEORETICAL FRAMEWORK

Chromosomal diseases result when there is in an abnormality in one's genetic structure during the fertilization process (Lobo and Zhaurova, 2008). Such genetic birth abnormalities can be classified into three categories: "chromosomal abnormalities (e.g., Down syndrome), single-gene defect (e.g., sickle cell disease, cystic fibrosis, Huntington disease, muscular dystrophy, and Fragile X syndrome), and multifactorial influences (e.g., cleft lip and palate, spina bifida) whereby the mixture of genetic mutations and teratogens/environmental factors interact leading "to the development of multifactorial birth defects" (Ibid., 2008). In this research, I focus on genetic birth abnormalities classified under chromosomal abnormalities (Down syndrome) and multifactorial birth defects (cleft lip with or without cleft palate and spina bifida).

Theory

Chromosomal diseases have been linked to a complex interaction of one's composition and—in some cases—environmental influencers. Down syndrome results from a chromosomal abnormality alone, whereas cleft lip and spina bifida may manifest from the interaction of genetic mutations and environmental effects leading to multifactorial influences (Lobo and Zhaurova, 2008). Thus, it is reasonable to argue that congenital anomalies have been traced to genetic mutation using the copy error mechanism to explain the manifestation of genetic birth abnormalities (or genetic diseases) (Penrose, 1955: 312-313). While it is not considered the only mechanism behind congenital anomalies in newborns, the copy error theory proposes that numerous "cell divisions during spermatogenesis allow for the introduction of transcription errors" throughout the replication process, which are later transmitted as "gene mutations" (Penrose, 1955: 312-313). Since Penrose's (1955) copy error theory, researchers further

developed his body of research to analyze the possible relationships between birth defects and APA (Thompson, 2019). I utilize the copy error mechanism in the aging father to explain the potential relationship between advanced paternal ages and reproductive abnormalities.

While extensive studies suggest AMA raises the likelihood of congenital anomalies, very few consider the effect of APA on various congenital anomalies (McIntosh et al., 1995). Additionally, studies that have evaluated APA on congenital anomalies have a small number of observations and often focus on "advanced paternal age and Down syndrome", neglecting other dominant mutations at birth (McIntosh et al., 1995). Additionally, later investigations overlooked the mother's age to test properly the role of both advanced paternal ages on various birth defects (King and Bearman, 2010; Durkin et al., 2010; King et al., 2010; Reichenberg et al., 2010). More recently, though, there have been a few discussions suggesting a connection between APA and "neonatal and pediatric disorders" but there is a lack of quantitative analyses on this observation (Thompson, 2019: 2; Andersen and Urhoj, 2017; Sigman, 2017). Therefore, it is important to include both paternal ages to properly understand the implications of the advanced paternal ages during reproduction. Drawing on the effect of copy error theory, I conduct separate analyses of the three congenital anomalies: Down Syndrome, cleft lip with or without cleft palate, and spina bifida. I use the copy error theory to discover the nexus between APA and said birth defects.

Down Syndrome

Trisomy 21 (Down syndrome), trisomy 13 (Patau syndrome), and trisomy 18 (Edwards syndrome) are the highest recurring birth disorders among chromosomal abnormalities (Lobo and Zhaurova, 2008). Down syndrome is one of the most common chromosomal abnormalities, which affects 1 in 800 babies, with an average of 6,000 newborns affected annually in the U.S. (Children's Hospital of Philadelphia, 2020). This incurable disorder yields moderate to severe intellectual, physical, and neurological disabilities and concurring health issues (e.g., "heart defects [congenital heart disease], leukemia, and Alzheimer's disease") in the affected individual (Lobo and Zhaurova., 2008). According to the Global Down Syndrome Foundation (Children's Hospital of Philadelphia, 2020), the precise cause of the disease remains ambiguous, but research suggests that Down syndrome "increases with maternal age", especially after a female is 35-years or older (Lobo and Zhaurova, 2008). More generally, Down syndrome (identified as Trisomy 21) is more likely to occur from females than males but there is lack of sufficient evidence to exclude males in the causation of this disease (Hassold et al., 1996). Thus, it is reasonable to include the father's age to determine more clearly the possible relationship between advanced age and chromosomal abnormalities in newborns.

During the non-disjunction process—when the chromosomes fail to separate properly—Down syndrome develops when three copies of chromosome[12] exist rather than two (Dreamscape, 2020). In confirmed Down syndrome cases, non-disjunction occurs in "anaphase, when a pair of homologous chromosomes do not separate before being distributed into two

[12] The packaged arrangement of Deoxyribonucleic acid (DNA) is called chromosome (National Human Genome Research Institute, 2020).

daughter cells", which results in cells where "one cell has two copies of a chromosome", while the other cell does not have any copies (Sparkes and Crandall, 1972). According to Thompson (2019), it is imperative to ascertain the exposures that yield non-disjunction in oocyte development, which is overwhelmingly age-related, and non-disjunction in sperm development, whereby the paternal age effect is still ambiguous. Thus, based on Penrose's (1955) theory, which suggests that gene expression[13] may also stem from the [aging] male, incorporating the father's age in epidemiological and/or genetic studies "may provide leads to causal factors" to various types of genetic disorders (Crow, 2000: 44).

Cleft Lip

Cleft lip with or without cleft palate (NSCL/P) affects "1 in 700 - 1,000 newborns" annually in the U.S. and such a congenital craniofacial defect has become increasingly common in newborns (Beiraghi et al., 2007). Research suggests a greater emphasis on paternal age as opposed to maternal age in cases related to autosomal dominant congenital diseases (e.g., cleft lip and palate) (Conti and Eisenberg, 2016). To ascertain this phenomenon, Vogel and Rathenberg (1975) and Drost and Lee (1995) explain a difference in gametogenesis between genders (Conti and Eisenberg, 2016). Gametogenesis is known as the process whereby cells experience meiosis to form gametes in the sexual reproduction to create a zygote (ThoughtCo., 2020). In particular, every oocyte undergoes 23 chromosome replications in total (i.e., "22 germ-cell divisions and two meiotic divisions"), but there is no DNA replication in the last meiotic division (Ibid., 421). Since a female is born with all oocytes (i.e., immature egg/ovum) "she will

[13] When instructions found in DNA are changed into a "functional product, such as protein", it is known as the gene expression process (Yourgenome, 2020).

ever have" (Gurevich, 2020), "cell divisions [in females] are completed before birth, there is no increases [in DNA replication] with postnatal age" (Crow, 2000:41). This phenomenon of DNA replication in females, researchers propose, yields a lower likelihood of genetic mutations during the human reproduction stage.

However, spermatogenesis in males is continuous throughout one's age, thus "[increasing] the number of chromosome replication events" and reaching on average 840 spermatic chromosome replication events by the age of 50 (Conti and Eisenberg, 2016: 421; Vogel and Motulsky, 1997; Kong et al., 2012). The continuous DNA replication processes may produce higher proclivities for copy error mutations, which places a potential causal relationship between paternal aging and genetic diseases (Conti and Eisenberg, 2016).

Spina Bifida

The literal meaning of spina bifida is "cleft spine," and such a neural tube defect has become increasingly common in newborns affecting on average 1,500 – 2,000 out of 4 million newborns annually in the U.S. (National Institute of Neurological Disorders and Stroke, 2020). This disease is described by the partial growth of the spine, brain, and/or the layer shielding the spine and brain (Ibid., 2020). Experts believe spina bifida may be caused by multiple factors such as genetic, environmental, and nutritional agents (Ibid., 2020). Additionally, recent studies using birth data suggest that the likelihood of neural tube defects (NTD[14]s) in newborns such as spina bifida may be linked to AMA (i.e., $\geq$ 35 years) (Vieira and Castillo Taucher, 2005). Studies including paternal age on incidence of spina bifida are scarce although this condition is a genetic

[14] Deak et al., 2010.

disorder. Thus, this study fills the gap in the literature pertaining to the link between APA and genetic diseases and congenital anomalies.

Hypotheses

By utilizing the copy error theory (Penrose, 1955) to the advanced paternal ages-genetic diseases relationship, I aim to offer clarification on how advanced paternal ages may affect the likelihood of three separate genetic diseases in newborns. I derive three hypotheses that empirically test my theory:

Hypothesis 1:

(A) Advanced reproductive aging in both father (paternal age 35+) and mother (maternal age 35+) are more likely to have a newborn with Down syndrome than both parents with non-advanced reproductive aging.

(B) The interaction between the advanced reproductive aging in both father (paternal age 35+) and mother (maternal age 35+) are more likely to have a newborn with Down syndrome than both parents with non-advanced reproductive aging.

(C) Advanced reproductive aging in both father (paternal age 50+) and mother (maternal age 35+) are more likely to have a newborn with Down syndrome than both parents with non-advanced reproductive aging.

(D) The interaction between the advanced reproductive aging in both father (paternal age 50+) and mother (maternal age 35+) are more likely to have a newborn with Down syndrome than both parents with non-advanced reproductive aging.

Hypothesis 2:

(A) Advanced reproductive aging in both father (paternal age 35+) and mother (maternal age 35+) are more likely to have a newborn with cleft lip with or without cleft palate than both parents with non-advanced reproductive aging.

(B) The interaction between the advanced reproductive aging in both father (paternal age 35+) and mother (maternal age 35+) are more likely to have a newborn with cleft lip with or without cleft palate than both parents with non-advanced reproductive aging.

(C) Advanced reproductive aging in both father (paternal age 50+) and mother (maternal age 35+) are more likely to have a newborn with cleft lip with or without cleft palate than both parents with non-advanced reproductive aging.

(D) The interaction between the advanced reproductive aging in both father (paternal age 50+) and mother (maternal age 35+) are more likely to have a newborn with cleft lip with or without cleft palate than both parents with non-advanced reproductive aging.

Hypothesis 3:

(A) Advanced reproductive aging in both father (paternal age 35+) and mother (maternal age 35+) are more likely to have a newborn with spina bifida than both parents with non-advanced reproductive aging.

(B) The interaction between the advanced reproductive aging in both father (paternal age 35+) and mother (maternal age 35+) are more likely to have a newborn with spina bifida than both parents with non-advanced reproductive aging.

(C) Advanced reproductive aging in both father (paternal age 50+) and mother (maternal age 35+) are more likely to have a newborn with spina bifida than both parents with non-advanced reproductive aging.

(D) The interaction between the advanced reproductive aging in both father (paternal age 50+) and mother (maternal age 35+) are more likely to have a newborn with spina bifida than both parents with non-advanced reproductive aging.

RESEARCH DESIGN

I extend the study on the link between advanced paternal ages and genetic diseases consisting of congenital anomalies: Down Syndrome, cleft lip with or without cleft palate, and spina bifida. I drew on the CDC Natality Registry (CDC, 2020) database that systematically compiles reported live births in the U.S., which is provided by CDC Wonder (2020). This research covers the period from 2016 to 2019. I eliminate all missing or unreported data from the overall number of observations in each model. I characterize all variables in this research as dichotomous. Thus, I use a different logistic regression model for each congenital anomaly.

Dependent Variables

I use three dependent variables to test my hypotheses. Specifically, I include genetic diseases that manifest into three different congenital anomalies: Down syndrome, cleft lip with or without cleft palate, and spina bifida. All the dependent variables in this study are dichotomous (0, 1) whereby I code the non-confirmed congenital anomaly as "0" and code the confirmed congenital anomaly as "1". Therefore, the method of analysis is logistic regression using the Stata 17.0 computer program (StataCorp., 2021). I use the CDC Natality Registry database (2020) that consists of the United States Births Vital Statistics[15] from 2016-2019 to create my three unique datasets.

[15] The data available in the CDC Natality Registry are "derived from [live] birth certificates issued" from the 2016 to 2019 years (CDC, 2020).

Down Syndrome:

An observed Down syndrome case is coded as "1" if there is a confirmed case in the newborn and coded as "0" if not. In this model *N*= 3,295,210 (Table 3).

Cleft Lip with or without Cleft Palate:

An observed cleft lip with or without cleft palate case is coded as "1" if there is a confirmed case in the newborn and coded as "0" if not. In this model *N*= 3,296,177 (Table 5).

Spina Bifida:

An observed spina bifida case is coded as "1" if there is a confirmed case in the newborn and coded as "0" if not. In this model *N*= 3,296,177 (Table 7).

Main Independent Variables

The likelihood of reproductive anomalies among two parents may be linked to their advanced ages. The models in this study include the following main independent variables: (1) Advanced Paternal Age [APA ≥ 35 years], (2) Advanced Maternal Age [AMA ≥ 35 years], (3) Advanced Parental Ages (Interaction Effect of Advanced Paternal Age and Advanced Maternal Age [APA ≥ 35 years * AMA ≥ 35 years]). While the advanced paternal age begins at 35 years considering the 540 spermatic replications (Grewal et al., 2012), Vogal and Motulsky (1997) suggest that a male undergoes 840 spermatic replications by the age of 50 years—increasing the transcription errors throughout the replication process (Penrose, 1955). Thus, I include two more independent variables that include advanced paternal age of 50 years: (4) Advanced Paternal Age [APA ≥ 50 years], and (5) Advanced Parental Ages (Interaction Effect of Advanced Paternal Age and Advanced Maternal Age [APA ≥ 50 years * AMA ≥ 35 years]). To determine the link

between advanced paternal ages and the three congenital anomalies, I restrict my observations to include only the known/reported ages of both parents found within the Natality Registry database (2020) to examine the reported congenital anomaly (i.e., Down syndrome, cleft lip, and/or spina bifida) of each live birth from 2016-2019.

Using the CDC Natality Registry (2020), I use observations that include disclosed age ranges of both partners (i.e., paternal age and maternal age) to include the father's age range. Paternal age ranges are distributed into eight groups that begin from 15 years of age (with five-year intervals): 15 – 19 years; 20 – 24 years; 25 – 29 years; 30 – 34 years; 35 – 39 years; 40 – 44 years; 45 – 49 years; 50 – 54 years; and 55 – 98 (CDC, 2020:15). In the APA ≥ 35 years variable, the father's age range grouped below 35 years [P < 35 years] is coded as "0", and the father's age range equal to or greater than 35 years [APA ≥ 35 years] is coded as "1". In the 50 years and above variable, the father's age range grouped below 50 years [P < 50 years] is coded as "0", and the father's age range equal to or greater than 50 years [APA ≥ 50 years] is coded as "1". The conceptualized advanced paternal ages (i.e., APA ≥ 35 years and APA ≥ 50 years) are both binary variables (0, 1).

Using the CDC Natality Registry (2020), I use observations that include disclosed age ranges of both partners (i.e., paternal age and maternal age) to include the mother's age range. Eight maternal age groups starting from 15 years of age (with five-year intervals) are identified: 15 – 19 years; 20 – 24 years; 25 – 29 years; 30 – 34 years; 35 – 39 years; 40 – 44 years; 45 – 49 years; and 50 – 54 years (CDC, 2020:13). Like the APA coding method, once the maternal age groups are identified, they are coded accordingly: if the mother's age range is less than 35 years [M < 35 years], then it is coded as "0"; and if the mother's age range is equal to or greater than

35 years [AMA ≥ 35 years], then it is coded as "1". The advanced maternal age is a binary variable (0, 1).

Independent Variable 1: **(APA ≥ 35 years)**

1. Advanced Paternal Age 1

Independent Variable 2: **(AMA ≥ 35 years)**

2. Advanced Maternal Age

Independent Variable 3: **(APA ≥ 35 years * AMA ≥ 35 years)**

3. Interaction Effect of Advanced Paternal Age 1 and Advanced Maternal Age

Independent Variable 4: **(APA ≥ 50 years)**

4. Advanced Paternal Age 2

Independent Variable 5: **(APA ≥ 50 years * AMA ≥ 35 years)**

5. Interaction Effect of Advanced Paternal Age 2 and Advanced Maternal Age

Control Variables

The CDC Wonder (2020) data base includes various types of maternal factors such as nutritional, health, lifestyle, pregnancy complications, etc., which may influence the birth outcome. I use two multifactorial influences: Tobacco use during pregnancy and gestational diabetes (CDC, 2020). All models include these two control variables (i.e., tobacco use during pregnancy and gestational diabetes) to determine the propensity of Down syndrome, cleft lip with or without cleft palate, and spina bifida. In this study's analyses, Table 1 includes the 12 models.

Tobacco Use During Pregnancy

One known maternal risk factor during pregnancy is the consumption of tobacco (CDC, 2020). Tobacco consumption during pregnancy—considered as both nutritional and

environmental factors (or teratogenic)—increases the likelihood of reproductive anomalies in newborns (Zhang et al., 2017). It has been confirmed that tobacco smoke includes different forms of poisonous mixtures, containing cadmium, nicotine, benzo[a]pyrene, and other carbon monoxide, whereby such components may yield long-lasting teratogenic impacts on mammals (Ibid., 2012). Consequently, I predict that a pregnant mother's consumption of tobacco increases the chances of Down syndrome, cleft lip with or without cleft palate, and spina bifida. According to CDC Wonder (2020), "tobacco use during pregnancy" reflects any form of cigarette consumption during pregnancy (Ibid., 2020). The consumption of cigarettes was recorded by the mother during the 1st, 2nd, and/or 3rd trimesters of pregnancy, ranging from 1 cigarette to 98 or more cigarettes consumed during said pregnancy (Ibid., 2020). Using the CDC Natality Registry database (2020), I include observations that reported known tobacco use by the mother during the observed pregnancies (coded as "1"), and mothers who did not use tobacco during pregnancy (coded as "0").

Gestational Diabetes

The relationship between AMA on the outcome of pregnancy have been examined carefully and the adverse reproductive outcomes speak volumes (Heffner, 2004). I include gestational diabetes mellitus (GDM) as a control variable because there is new information to suggest that complications during pregnancy and reproductive outcomes may be linked to this factor (Zhao and Weiler, 2010; CDC, 2020). Usually during prenatal physician consultations, mothers are informed about the link between various levels of hyperglycemia and the severity of negative effects (Cho et al., 2016). Thus, including gestational diabetes as a possible multifactorial influence on the three congenital anomalies may improve our understanding of these relationships. Using the CDC Natality Registry database (2020), I include observations that

reported the gestational diabetes diagnosis during pregnancy (coded as "1") and those that were not diagnosed with gestational diabetes during pregnancy (coded as "0").

Table 1: Models to be Analyzed

		Down Syndrome[16]				Cleft Lip (w or w/o Palate)[17]				Spina Bifida[18]			
	Model #	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**
Independent Variables	Advanced Paternal Age 1 (35+)	✓	✓			✓	✓			✓	✓		
	Advanced Maternal Age 35+	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
	Interaction term of Advanced Parental Ages (APA ≥ 35 years * AMA ≥ 35 years)		✓				✓				✓		
	Advanced Paternal Age 2 (50+)			✓	✓			✓	✓			✓	✓
	Interaction term of Advanced Parental Ages (APA ≥ 50 years * AMA ≥ 35 years)				✓				✓				✓
Control Variables	Tobacco Use	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
	Gestational Diabetes	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓

[16] United States Department of Health and Human Services (US DHHS), Centers for Disease Control and Prevention" "(CDC), National Center for Health Statistics (NCHS), Division of Vital Statistics, Natality public-use data 2016-2019, on CDC" WONDER Online Database, October 2020. Accessed at http://wonder.cdc.gov/natality-expanded-current.html on Jun 6, 2021, 1:24:47 PM."

[17] United States Department of Health and Human Services (US DHHS), Centers for Disease Control and Prevention" "(CDC), National Center for Health Statistics (NCHS), Division of Vital Statistics, Natality public-use data 2016-2019, on CDC" "WONDER Online Database, October 2020. Accessed at http://wonder.cdc.gov/natality-expanded-current.html on Jun 6, 2021, 1:29:36 PM."

[18] United States Department of Health and Human Services (US DHHS), Centers for Disease Control and Prevention""(CDC), National Center for Health Statistics (NCHS), Division of Vital Statistics, Natality public-use data 2016-2019, on CDC" "WONDER Online Database, October 2020. Accessed at http://wonder.cdc.gov/natality-expanded-current.html on Jun 6, 2021, 1:07:36 PM."

Methods

According to this study, the three dependent variables Down syndrome, cleft lip with or without cleft palate, and spina bifida are all binary (0, 1), therefore, I use logistic regression to perform three separate analyses.

The functional form of Models 1 – 4 with the dependent variable **Down Syndrome** is as follows: (Model 1) Down syndrome = *f* (APA 35+, AMA 35+, tobacco use, gestational diabetes). Model 2 includes a third term by interacting the advanced paternal age 35+ * the advanced maternal age 35+. (Model 2) Down syndrome = *f* (APA 35+, AMA 35+, [APA 35+ * AMA 35+], tobacco use, gestational diabetes). Model 3 tests whether the advanced paternal age 50+ increases the likelihood of Down syndrome: (Model 3) Down syndrome = *f* (APA 50+, AMA 35+, tobacco use, gestational diabetes). And finally, Model 4 tests whether the interaction effect between the advanced paternal age 50+ * the advanced maternal age 35+ affects the likelihood of Down syndrome: (Model 4) Down syndrome = *f* (APA 50+, AMA 35+, [APA 50+ * AMA 35+], tobacco use, gestational diabetes).

The functional form of Models 5 – 8 with **Cleft Lip with or without Cleft Palate** as the dependent variable is as follows: (Model 5) Cleft Lip = *f* (APA 35+, AMA 35+, tobacco use, gestational diabetes); (Model 6): Cleft Lip= *f* (APA 35+, AMA 35+, [APA 35+ * AMA 35+], tobacco use, gestational diabetes); (Model 7) Cleft Lip = *f* (APA 50+, AMA 35+, tobacco use, gestational diabetes); and (Model 8) Cleft Lip = *f* (APA 50+, AMA 35+, [APA 50+ * AMA 35+], tobacco use, gestational diabetes).

The functional form of Models 9 – 12 with **Spina Bifida** as the dependent variable is as follows: (Model 9) Spina Bifida = *f* (APA 35+, AMA 35+, tobacco use, gestational diabetes); (Model 10): Spina Bifida = *f* (APA 35+, AMA 35+, [APA 35+ * AMA 35+], tobacco use,

gestational diabetes); (Model 11) Spina Bifida = *f* (APA 50+, AMA 35+, tobacco use, gestational diabetes); and (Model 12) Spina Bifida = *f* (APA 50+, AMA 35+, [APA 50+ * AMA 35+], tobacco use, gestational diabetes). In the next section, I describe the results of the analyses.

Summary Statistics

The Summary Statistics (test statistic reports) found in Appendix A show the comparison of two groups (e.g., Down syndrome and APA 35+) to measure the causal relationship. For example, I utilize the 2 x 2 matrix that displays the distribution of confirmed Down syndrome cases resulting from fathers who are 35 years of age or above. The *Pr value* is interpreted as the probability (p-value) of each matrix in Appendix A. For each dependent variable, Appendix A provides the frequency distribution of the independent variables (i.e., Advanced Paternal Age ***1*** (35+), Advanced Paternal Age ***2*** (50+), Advanced Maternal Age 35+, Interaction term of Advanced Parental Ages [APA ≥ 35 years * AMA ≥ 35 years], and Interaction term of Advanced Parental Ages [APA ≥ 50 years * AMA ≥ 35 years]). Appendix A displays the joint distribution of the dependent variables and independent variables by providing the frequency data and corresponding percentage in each matrix. The frequency data that reflect statistical significance suggest a causal relationship between the respective dependent and independent variables.

RESULTS

This section describes the results of the three dependent variables: (1) Down syndrome, (2) cleft lip with or without cleft palate, and (3) spina bifida.

Results—Down Syndrome and Paternal Ages

I test the link between advanced paternal ages and Down syndrome using varying combinations of advanced paternal ages and advanced maternal age. Table 3 reports the results of my analyses for each relationship. In Table 3, Models 1 – 4 report the odds ratios derived from each logistic regression analysis. I hypothesized that advanced paternal ages increase the likelihood of Down syndrome. Accordingly, I summarize my hypotheses on the link between Down syndrome and advanced paternal ages and provide the analyses of my results:

Hypothesis 1 and Analysis:

Model I: Advanced reproductive aging in both father (paternal age 35+) and mother (maternal age 35+) are more likely to have a newborn with Down Syndrome than both parents with non-advanced reproductive aging: The effects of both advanced parental ages are consistent with my expectations and are statistically significant. Hassold et al. (1996) and Thompson (2019) suggested that scientific studies need to include the father's advanced age in the potential causation of Down syndrome since the lack of evidence on this relationship is unclear. My findings suggest that there is a 38% more likelihood of a father age 35+ (APA $\geq$ 35 years) to have a confirmed Down syndrome case in his newborn. While this is a modest research, it does offer some promising information to further investigate the link between APA and Down syndrome (Hassold et al, 1996; Thompson, 2019).

Model II: The interaction between the advanced reproductive aging in both father (paternal age 35+) and mother (maternal age 35+) are more likely to have a newborn with Down Syndrome than both parents with non-advanced reproductive aging: The coefficient of the interaction variable yielded an opposite effect than predicted and is not statistically significant. However, the advanced paternal age (APA ≥ 35 years) as a separate variable in Model II is significant and suggests a 43% more likelihood of having a newborn with Down syndrome among fathers ages 35+.

Model III: Advanced reproductive aging in both father (paternal age 50+) and mother (maternal age 35+) are more likely to have a newborn with Down Syndrome than both parents with non-advanced reproductive aging: The effects of that father age 50+ and mother age 35+ are consistent with my hypothesis and are statistically significant. The advanced paternal age (APA ≥ 50 years) is significant and suggests a 60% more likelihood of having a newborn with Down syndrome among fathers ages 50+.

Model IV: The interaction between the advanced reproductive aging in both father (paternal age 50+) and mother (maternal age 35+) are more likely to have a newborn with Down Syndrome than both parents with non-advanced reproductive aging: The interaction effect of both advanced parental ages here are not consistent with my expectations and are not statistically significant. But in this model, only the maternal age 35+ was consistent with my expectations and statistically significant. Table 2 displays the predicted probabilities[19] of the models that produced statistically significant results:

[19] Predicted probabilities are the probability or likelihood of an event (i.e., Down syndrome, cleft lip with or without cleft palate, and spina bifida) that is calculated from the available data (StataCorp., 2021)

Table 2: Predicted Probabilities of Down Syndrome

Main Independent Variables	***Model***			
	I	**II**	**III**	**IV**
Paternal Age ≥ 35 years = 0	0.0005***	0.0050***		
Paternal Age ≥ 35 years = 1	0.0004***	0.0004***		
Paternal Age ≥ 50 years = 0			0.0005***	0.0005***
Paternal Age ≥ 50 years = 1			0.0006***	0.0005***

*Confidence Level Notes: *p<0.1; **p<0.05; ***p<0.01*

Results—Down Syndrome and Control Variables

In all four models in Table 3, tobacco use during pregnancy is not statistically significant and thus has no effect on Down syndrome[20] in newborns. However, gestational diabetes is consistent with my expectations and is statistically significant in all four models.

[20] United States Department of Health and Human Services (US DHHS), Centers for Disease Control and Prevention" "(CDC), National Center for Health Statistics (NCHS), Division of Vital Statistics, Natality public-use data 2016-2019, on CDC" WONDER Online Database, October 2020. Accessed at http://wonder.cdc.gov/natality-expanded-current.html on Jun 6, 2021, 1:24:47 PM."

Table 3: Relationship between Down Syndrome and Advanced Paternal Ages

Table 3: Relationship between Down Syndrome and Advanced Paternal Ages

	Dependent variable: Down Syndrome			
	All Advanced Parental Ages			
	(I)	(II)	(III)	(IV)
Advanced Paternal Age 35+	1.382***	1.433***		
	(0.126)	(0.176)		
Advanced Maternal Age 35+	4.594***	4.818***	5.487***	5.499***
	(0.416)	(0.672)	(0.397)	(0.401)
Advanced Parental Ages 35+		0.923		
		(0.167)		
Advanced Paternal Age 50+			1.599***	1.791
			(0.283)	(0.901)
Advanced Parental Ages Father 50+, Mother 35+				0.880
				(0.473)
Tobacco Use	1.258	1.256	1.236	2.714
	(0.207)	(0.207)	0.203	(1.388)
Gestational Diabetes	1.307**	1.306**	1.312**	0.470**
	(0.144)	(0.144)	(0.145)	(0.237)
Constant	0.000***	0.000***	0.000***	0.000***
	(7.010)	(7.390)	(6.920)	(6.950)
Observations	3,295,210	3,295,210	3,295,210	3,295,210
Likelihood Ratio	595.85***	596.04***	589.39***	589.44***
Pseudo-R2	0.040	0.040	0.039	0.039

*Notes: Values in parentheses are Standard Error (SE); *p<0.1; **p<0.05; ***p<0.01*

Results—Cleft Lip with or without Cleft Palate and Paternal Ages

I test the link between advanced paternal ages and Cleft Lip with or without Cleft Palate[21] using varying combinations of advanced paternal ages and advanced maternal age. Table 5 displays the findings of my analyses for each relationship. In Table 5, Models 5 – 8 report the odds ratios derived from each logistic regression analysis. I hypothesized that APA increases the likelihood of cleft lip with or without cleft palate in newborns. Accordingly, I summarize my hypotheses on the link between cleft lip with or without cleft palate and advanced paternal ages and provide the analyses of my results:

Hypothesis 2 and Analysis:

Model V: Advanced reproductive aging in both father (paternal age 35+) and mother (maternal age 35+) are more likely to have a newborn with cleft lip with or without cleft palate than both parents with non-advanced reproductive aging: Surprisingly, I found a negative effect of paternal age 35+, which was statistically significant, on cleft lip with or without cleft palate. Consistent with the results found in the studies conducted by McIntosh et al. (1995) and Kazaura et al. (2004), they suggest that younger fathers have a higher likelihood of having newborns with neural tube defects like cleft lip. My findings suggests that fathers over 35 are about 15% less likely to have a child with cleft lip than fathers who are under 35 years of age. Since cleft lip has multifactorial influences, it is reasonable to argue that younger fathers (below 35 years) are more likely to be exposed to environmental and nutritional agents, for example, that may negatively

[21] United States Department of Health and Human Services (US DHHS), Centers for Disease Control and Prevention" "(CDC), National Center for Health Statistics (NCHS), Division of Vital Statistics, Natality public-use data 2016-2019, on CDC" "WONDER Online Database, October 2020. Accessed at http://wonder.cdc.gov/natality-expanded-current.html on Jun 6, 2021, 1:29:36 PM."

interact with inherent genetic mutations leading to birth defects (Lobo and Zhaurova, 2008). I found no support that maternal age 35+ increases the likelihood of cleft lip.

Model VI: The interaction between the advanced reproductive aging in both father (paternal age 35+) and mother (maternal age 35+) are more likely to have a newborn with cleft lip with or without cleft palate than both parents with non-advanced reproductive aging: I found no support that the interaction of both advance paternal ages (APA ≥ 35 years * AMA ≥ 35 years) increases the likelihood of cleft lip. But like Model V, I found statistical significance and a negative relationship between cleft lip and APA ≥ 35 years, suggesting a 15% less likelihood of cleft lip in newborns whose father's age is 35+ years. Also, I found no support that maternal age 35+ increases the likelihood of cleft lip.

Model VII: Advanced reproductive aging in both father (paternal age 50+) and mother (maternal age 35+) are more likely to have a newborn with cleft lip with or without cleft palate than both parents with non-advanced reproductive aging: While I did not find a relationship between cleft lip and paternal age 50+, I found a negative relationship between cleft lip with or without cleft palate and maternal age 35+ with statistically significant results. Essentially, the findings suggest that mothers 35+ years and older have a 14% less likelihood to have a child with cleft lip than mothers who are younger than 35 years of age.

Model VIII: The interaction between the advanced reproductive aging in both father (paternal age 50+) and mother (maternal age 35+) are more likely to have a newborn with cleft lip with or without cleft palate than both parents with non-advanced reproductive aging: The interaction effect of both advanced parental ages in Model VIII are not consistent with my expectations and are not statistically significant. But I found a negative relationship between cleft lip with or without cleft palate and maternal age 35+ with statistically significant results. This suggests that

mothers 35+ years and older have a 14% less likelihood to have a child with cleft lip than mothers who are below the age of 35 years.

Table 4 displays the predicted probabilities of the models that produced statistically significant results:

Table 4: Predicted Probabilities of Cleft Lip with or without Cleft Palate

Main Independent Variables	***Model***			
	V	**VI**	**VII**	**VIII**
Paternal Age ≥ 35 years = 0	0.0002***	0.0002***		
Paternal Age ≥ 35 years = 1	0.0028***	0.0003***		
Paternal Age ≥ 50 years = 0			0.0002***	0.0002***
Paternal Age ≥ 50 years = 1			0.0004***	0.0004***

*Confidence Level Notes: *p<0.1; **p<0.05; ***p<0.01*

Results—Cleft Lip with or without Cleft Palate and Control Variables

In all four models in Table 5, tobacco use during pregnancy and gestational diabetes are consistent with my expectations and are statistically significant. This suggests that tobacco use during pregnancy and gestational diabetes increase the likelihood of cleft lip with or without cleft palate in newborns.

Table 5: Relationship between Cleft Lip and Advanced Paternal Ages

Table 5: Relationship between Cleft Lip with or without Cleft Palate and Advanced Paternal Ages

	Dependent variable: Cleft Lip with or without Cleft Palate			
	All Advanced Parental Ages			
	(V)	(VI)	(VII)	(VIII)
Advanced Paternal Age 35+	0.847**	0.851**		
	(0.055)	(0.062)		
Advanced Maternal Age 35+	0.970	0.987	0.869**	0.868**
	(0.075)	(0.134)	(0.058)	(0.059)
Advanced Parental Ages 35+		0.974		
		(0.161)		
Advanced Paternal Age 50+			1.190	1.152
			(0.247)	(0.366)
Advanced Parental Ages Father 50+, Mother 35+				1.058
				(0.444)
Tobacco Use	1.504***	1.503***	1.503***	1.503***
	(0.149)	(0.149)	(0.149)	(0.149)
Gestational Diabetes	1.218**	1.218**	1.209**	1.209**
	(0.111)	(0.111)	(0.110)	(0.110)
Constant	0.000***	0.000***	0.000***	0.000***
	(0.000)	(0.000)	(0.000)	(0.000)
Observations	3,296,177	3,296,177	3,296,177	3,296,177
Likelihood Ratio	30.16***	30.19***	24.20***	24.22***
Pseudo-R2	0.001	0.001	0.001	0.001

*Notes: Values in parentheses are Standard Error (SE); *p<0.1; **p<0.05; ***p<0.01*

Results—Spina Bifida and Advanced Paternal Ages

I test the relationship between advanced paternal ages and Spina Bifida[22] while employing different dimensions of the father's advanced age. Table 7 displays the findings of my analyses for the relationship between spina bifida and (a) APA ≥ 35 years and AMA ≥ 35 years; (b) APA ≥ 35 years and AMA ≥ 35 years, and the interaction between APA ≥ 35 years * AMA ≥ 35 years; (c) APA ≥ 50 years and AMA ≥ 35 years; and (d) APA ≥ 50 years and AMA ≥ 35 years, and the interaction between APA ≥ 50 years * AMA ≥ 35 years. Accordingly, I summarize my hypotheses on the link between spina bifida and advanced paternal ages and provide the analyses of my results:

Hypothesis 3 and Analysis:

Model IX: Advanced reproductive aging in both father (paternal age 35+) and mother (maternal age 35+) are more likely to have a newborn with spina bifida than both parents with non-advanced reproductive aging: The advanced paternal age (APA ≥ 35 years) has a negative effect but is not statistically significant. Whereas the advanced maternal age (AMA ≥ 35 years) has a positive effect but is not statistically significant. Although spina bifida is classified as a genetic birth abnormality, this birth defect manifests from the interaction of genetic mutations and environmental (or multifactorial) influences (Lobo and Zhaurova, 2008). There are numerous multifactorial influences (e.g., the combination of each parent's environment, daily diet, nutritional deficiencies, maternal infections, consumption of alcohol, drugs, and/or tobacco use, etc.) that become confounding variables in this type of genetic disease (Berkow et al., 1997).

[22] United States Department of Health and Human Services (US DHHS), Centers for Disease Control and Prevention""(CDC), National Center for Health Statistics (NCHS), Division of Vital Statistics, Natality public-use data 2016-2019, on CDC" "WONDER Online Database, October 2020. Accessed at http://wonder.cdc.gov/natality-expanded-current.html on Jun 6, 2021, 1:07:36 PM."

Thus, it is difficult to produce definitive results when genetic diseases that have multifactorial influences.

Model X: The interaction between the advanced reproductive aging in both father (paternal age 35+) and mother (maternal age 35+) are more likely to have a newborn with spina bifida than both parents with non-advanced reproductive aging: Similar to Model IX, Model X yielded similar findings that do not support my predictions. Thus, the inclusion of the interaction variable is not significant for the relationship between spina bifida and the explanatory variable (i.e., advanced paternal age [APA $\geq$ 35 years]).

Model XI: Advanced reproductive aging in both father (paternal age 50+) and mother (maternal age 35+) are more likely to have a newborn with spina bifida than both parents with non-advanced reproductive aging: These explanatory variables do not affect the probability of spina bifida and do not reflect statistical significance.

Model XII: The interaction between the advanced reproductive aging in both father (paternal age 50+) and mother (maternal age 35+) are more likely to have a newborn with spina bifida than both parents with non-advanced reproductive aging: The interaction variable of paternal age 50+ and maternal age 35+ does not affect the probability of spina bifida and does not reflect statistical significance.

Table 6: Predicted Probabilities of Spina Bifida

Main Independent Variables	***Model***			
	IX	**X**	**XI**	**XII**
Paternal Age ≥ 35 years = 0	0.0001***	0.0001***		
Paternal Age ≥ 35 years = 1	0.0001***	0.0001***		
Paternal Age ≥ 50 years = 0			0.0001***	0.0001***
Paternal Age ≥ 50 years = 1			0.0001**	0.0001

*Confidence Level Notes: *p<0.1; **p<0.05; ***p<0.01*

Results—Spina Bifida and Control Variables

In all four models in Table 7, tobacco use during pregnancy and gestational diabetes are consistent with my expectations and are statistically significant. This suggests that tobacco use during pregnancy and gestational diabetes increase the likelihood of spina bifida in newborns. The combination of genetic and environmental agents suggests that spina bifida has multifactorial influences as supported by this study (Conti and Eisenberg, 2016). Tobacco use during pregnancy and gestational diabetes are classified as confounding variables (i.e., environmental and nutritional agents) that may influence the reproduction stage, resulting in the birth defect.

Table 7: Relationship between Spina Bifida and Advanced Paternal Ages

Table 7: Relationship between Spina Bifida and Advanced Paternal Ages

	Dependent variable: Spina Bifida			
	All Advanced Parental Ages			
	(IX)	(X)	(XI)	(XII)
Advanced Paternal Age 35+	0.882	0.819		
	(0.106)	(0.114)		
Advanced Maternal Age 35+	1.165	0.912	1.086	1.074
	(0.159)	(0.242)	(0.126)	(0.126)
Advanced Parental Ages 35+		1.424		
		(0.446)		
Advanced Paternal Age 50+			0.766	0.410
			(0.346)	(0.411)
Advanced Parental Ages Father 50+, Mother 35+				2.400
				(2.698)
Tobacco Use	1.423*	1.429*	1.427*	1.429*
	(0.269)	(0.270)	0.270	(0.270)
Gestational Diabetes	1.515***	1.518***	1.508***	1.508***
	(0.232)	(0.233)	0.231	(0.231)
Constant	0.000***	0.000***	0.000***	0.000***
	(7.930)	(8.180)	(7.280)	(7.300)
Observations	3,296,177	3,296,177	3,296,177	3,296,177
Likelihood Ratio	11.37**	12.71**	10.630**	11.340**
Pseudo-R2	0.001	0.001	0.001	0.001

*Notes: Values in parentheses are Standard Error (SE); *p<0.1; **p<0.05; ***p<0.01*

CONCLUSION

Does advanced paternal ages affect the likelihood of genetic diseases and congenital anomalies in newborns? This thesis seeks to fully appreciate whether advanced paternal ages may, too, contribute to birth defects in infants. Limited studies have investigated the link between advanced paternal age and genetic disease and congenital anomalies systematically (Thompson, 2019). The extent of the study on the relationship between advanced age and reproductive anomalies rely mainly on AMA, while the possible link between APA and reproductive anomalies has been recently reviewed (Halvaei et al., 2020). Thus, this thesis conducts a study to analyze the link between advanced paternal ages and genetic diseases and congenital anomalies to shed light on the topic. This study's findings confirm a positive relationship between advanced paternal ages (APA $\geq$ 35 years and APA $\geq$ 50 years) and Down syndrome. Fathers 35+ and 50+ are associated with a higher likelihood of having a newborn with Down syndrome. Essentially, these findings suggest an elevation in the risk for advanced paternal ages and the manifestation of Down syndrome. Considering these outcomes, future studies may be able to analyze these predictions by including more recent observations (e.g., 2020 – 2023) to reinforce the scientific relationship between APA and Down syndrome.

Interestingly, findings confirm a negative relationship between advanced paternal age (APA $\geq$ 35 years) and cleft lip with or without cleft palate, which suggests that a father who is younger than age 35 years has a higher likelihood of having a newborn with cleft lip with or without cleft palate. These results are consistent with McIntosh et al. (1995) and Kazaura et al. (2004) who suggest a negative effect of APA and NTDs such as cleft lip.

Is paternal age just a number when it comes to reproductive anomalies? This study provides some explanation to the literature by exploring the effect of advanced paternal ages on genetic diseases and congenital anomalies. My substantive results suggest a causal relationship between advanced paternal ages and Down syndrome, but the study finds no support for Hypotheses 2 and 3 (i.e., the relationship between advanced paternal ages and cleft lip and the relationship between advanced paternal ages and spina bifida). The literature provides important guidance in advancing the research on advanced paternal ages and genetic diseases and congenital anomalies. This study furthers Penrose's (1955) copy error theory by distinctively examining the father's advanced age in relation to birth defects by explicitly stating that when a father's age is 35 and older, he may have an increased likelihood of having a newborn with genetic diseases and congenital anomalies. Considering the positive relationship found between Down syndrome and advanced paternal ages and the negative relationship between cleft lip and advanced paternal age (APA $\geq$ 35 years), this study may be helpful to potential studies in the areas of epidemiology and genetics. The implications in the results may motivate scholars to develop models by incorporating added confounding variables (e.g., socio-economic factors, father's diet containing consumption of alcohol and/or drugs, etc.). Including more confounding variables is important in furthering our understanding on how the father's age may affect the likelihood of reproductive anomalies. Based on the growing interest in analyzing the father's age in studies on reproductive health, it may be useful for future studies to build on the empirical findings of this study.

APPENDIX A: SUMMARY STATISTICS[23]

[23] Collection of data for all variables used in this research are obtained with full permission from: United States Department of Health and Human Services (US DHHS), Centers for Disease Control and Prevention" "(CDC), National Center for Health Statistics (NCHS), Division of Vital Statistics, Natality public-use data 2016-2019, on CDC" WONDER Online Database, October 2020. Accessed at http://wonder.cdc.gov/natality-expanded-current.html on Jun 6, 2021, 1:24:47 PM;" United States Department of Health and Human Services (US DHHS), Centers for Disease Control and Prevention" "(CDC), National Center for Health Statistics (NCHS), Division of Vital Statistics, Natality public-use data 2016-2019, on CDC" "WONDER Online Database, October 2020. Accessed at http://wonder.cdc.gov/natality-expanded-current.html on Jun 6, 2021, 1:29:36 PM;" And United States Department of Health and Human Services (US DHHS), Centers for Disease Control and Prevention""(CDC), National Center for Health Statistics (NCHS), Division of Vital Statistics, Natality public-use data 2016-2019, on CDC" "WONDER Online Database, October 2020. Accessed at http://wonder.cdc.gov/natality-expanded-current.html on Jun 6, 2021, 1:07:36 PM."

Summary Statistics – Down Syndrome

Down Syndrome	Father ≥ 35 years					Father ≥ 50 years			
	No		Yes			No		Yes	
No	2,223,877	99.99%	1,070,525	99.95%		3,250,496	99.98%	43,906	99.92%
Yes	313	0.01%	495	0.05%		774	0.02%	34	0.08%
Total	**2,224,190**	**100%**	**1,071,020**	**100%**		**3,251,270**	**100%**	**43,940**	**100%**
N=3,295,210	Pr= 0.000					Pr= 0.000			

Down Syndrome	Mother ≥ 35 years					Both Parental Ages ≥ 35 years			
	No		Yes			No		Yes	
No	2,647,487	99.99%	646,915	99.93%		2,760,605	99.99%	533,797	99.92%
Yes	339	0.01%	694	0.07%		404	0.01%	404	0.08%
Total	**2,647,826**	**100%**	**2,647,826**	**100%**		**2,761,009**	**100%**	**534,201**	**100%**
N=3,295,210	Pr= 0.000					Pr= 0.000			

Down Syndrome	Father ≥ 50 years * Mother≥ 35 years			
	No		Yes	
No	3,267,729	99.98%	26,673	99.89%
Yes	778	0.02%	30	0.11%
Total	**3,268,507**	**100%**	**26,703**	**100%**
N=3,295,210	Pr= 0.000			

Summary Statistics – Cleft Lip

Cleft Lip	Father ≥ 35 years				Father ≥ 50 years			
	No		Yes		No		Yes	
No	2,223,524	99.95%	1,071,077	99.96%	3,250,642	99.95%	43,959	99.95%
Yes	1,123	0.05%	453	0.04%	1,552	0.05%	24	0.05%
Total	**2,224,647**	**100%**	**1,071,530**	**100%**	**3,252,194**	**100%**	**43,983**	**100%**
N=3,296,177	Pr= 0.001				Pr= 0.514			

Cleft Lip	Mother ≥ 35 years				Both Parental Ages ≥ 35 years			
	No		Yes		No		Yes	
No	2,647,013	99.95%	647,588	99.96%	2,760,218	99.95%	534,383	99.06%
Yes	1,298	0.05%	278	0.04%	1,355	0.05%	221	0.04%
Total	**2,648,311**	**100%**	**647,866**	**100%**	**2,761,573**	**100%**	**534,604**	**100%**
N=3,296,177	Pr= 0.044				Pr= 0.018			

Cleft Lip	Father ≥ 50 years * Mother≥ 35 years			
	No		Yes	
No	3,267,870	99.95%	26,731	99.95%
Yes	1,562	0.05%	14	0.05%
Total	**3,269,432**	**100%**	**26,745**	**100%**
N=3,296,177	Pr= 0.733			

Summary Statistics – Spina Bifida

Spina Bifida	Father ≥ 35 years					Father ≥ 50 years			
	No		Yes			No		Yes	
No	2,224,333	99.99%	1,071,385	99.99%		3,251,740	99.99%	43,978	99.99%
Yes	314	0.01%	145	0.01%		454	0.01%	5	0.01%
Total	**2,224,647**	**100%**	**1,071,530**	**100%**		**3,252,194**	**100%**	**43,983**	**100%**
N=3,296,177	Pr= 0.675					Pr= 0.647			

Spina Bifida	Mother ≥ 35 years					Both Parental Ages ≥ 35 years			
	No		Yes			No		Yes	
No	2,647,949	99.99%	647,769	99.99%		2,761,196	99.99%	534,522	99.98%
Yes	362	0.01%	97	0.01%		377	0.01%	82	0.02%
Total	**2,648,311**	**100%**	**647,866**	**100%**		**2,761,573**	**100%**	**534,604**	**100%**
N=3,296,177	Pr= 0.426					Pr= 0.339			

Spina Bifida	Father ≥ 50 years * Mother≥ 35 years			
	No		Yes	
No	3,268,977	99.99%	26,741	99.99%
Yes	455	0.01%	4	0.01%
Total	**3,269,432**	**100%**	**26,745**	**100%**
N=3,296,177	Pr= 0.886			

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