2018

Family Planning Decisions After A Child's Diagnosis Of Rett Syndrome: A Pilot Study

Erin E. Huggins
University of South Carolina

FAMILY PLANNING DECISIONS AFTER A CHILD'S DIAGNOSIS OF RETT SYNDROME: A PILOT STUDY

by

Erin E. Huggins

Bachelor of Science
Coastal Carolina University, 2016

Submitted in Partial Fulfillment of the Requirements

For the Degree of Master of Science in

Genetic Counseling

School of Medicine

University of South Carolina

2018

Accepted by:

Richard Ferrante, Director of Thesis

Lauren Baggett, Reader

Steven A. Skinner, Reader

Cheryl L. Addy, Vice Provost and Dean of the Graduate School

DEDICATION

To my beautiful grandparents-Grandma and Pop-for always believing in me, always loving me, and always guiding me. Your presence has been felt every step of the way.

ACKNOWLEDGEMENTS

This present study would not have been possible without the dedication of the entire Rett syndrome community. Special thanks to Paige and Janice of RettSyndrome.org for their commitment to this project, to Greenwood Genetic Center for their award-winning care of those with Rett syndrome, and finally, to the dedicated parents of children with Rett syndrome. Their strength and tireless advocacy was a constant inspiration for the completion of this project.

ABSTRACT

Rett syndrome (RTT) is a rare neurodevelopmental disorder that primarily affects females. In 99% of cases, RTT is believed to occur sporadically, or *de novo.* However, in rare cases, RTT can be passed down from parent to child through gonadal mosaicism or asymptomatic carrier mothers. It is known that having a child with an inherited genetic condition can lead to changes in family planning; however, little research has investigated this phenomenon in sporadic genetic conditions, such as RTT. This present study used a questionnaire to assess family planning decisions of parents of children with RTT. Forty-three percent of respondents reported that their family planning changed. The primary reason for reproductive stoppage was due to caregiver strain, and of those that chose reproductive continuation, the primary change was in the age gaps between their children. Parents were also asked to explain what they were told by healthcare providers about the recurrence of RTT and if they received genetic counseling. Seventy-eight percent reported they were told there was a 1% or less chance of recurrence of RTT and 34% received genetic counseling. There was no significant association between those who received genetic counseling and those who altered their family planning decisions.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

MECP2.....Methyl-CpG Binding Protein 2

RTT.....Rett syndrome

CHAPTER 1
BACKGROUND

A Brief History of Rett Syndrome

Rett syndrome (RTT) is an X-linked dominant neurodevelopmental disorder that occurs in about 1 in 10,000 females and very rarely affects males (Laurvick et al., 2006). Individuals with RTT develop normally for a period between 6-18 months, followed by a period of regression. Affected individuals lose purposeful speech, hand use, and ambulation; they may also develop microcephaly and/or seizures. RTT was originally described by Dr. Andreas Rett, an Austrian neurodevelopmental pediatrician, in the 1960s (Rett, 1966). Rett's papers, written in German, were not well-known among European physicians at the time. Swedish pediatric neurologist Dr. Bengt Hagberg noted girls with similar features around the same time yet was unaware of the findings of Andreas Rett. It was not until 1983 that Rett syndrome was featured in an English publication by Hagberg and colleagues in *Annals of Neurology* (Hagberg et al., 1983). This paper described 35 female patients across France, Portugal, and Sweden all with similar features, including developmental regression, severe dementia with acquired microcephaly, loss of purposeful hand movements, autistic behaviors, and truncal and gait ataxia. Hagberg et al. (1983) concluded that these features were so striking that there was likely an underlying etiology that had not been previously described. Laboratory tests were inconclusive, and an exclusively female patient population raised suspicion of an X-linked dominant form of inheritance with lethality in males, but with little evidence

to support this claim. In addition, only one familial case out of the patients described in this paper was reported; two half-sisters born to the same unaffected mother with an unaffected half-brother from a third father (Hagberg et al., 1983). Thus, the inheritance pattern of Rett syndrome became a challenge to pinpoint.

Genetics

In 1999, Amir et al. were the first to report that mutations in the *MECP2* (methyl-CpG-binding protein 2, OMIM # 300005) gene, located at Xq28, caused RTT. Mutations were identified in the coding regions of *MECP2* in 5 of 21 sporadic cases, as well as one familial case-a set of half-sisters that share the same mother (Amir et al., 1999).

A larger study in 2008 investigated genotype-phenotype correlations in a cross-sectional study of 245 females with RTT. Of note, 97% of affected girls and women in this study had a *MECP2* mutation (Neul et al., 2008). By this time, over 200 mutations had been identified; however, 8 mutations accounted for 60% of cases according to data from RettBASE (Christodoulou et al., 2003). While genotype-phenotype correlations have yet to be fully described, Neul et al. (2008) determined that specific mutations impact clinical severity. Importantly, it was observed that certain mutations are associated with increased phenotypic severity in specific categories: ambulation, hand use, and language (Neul et al., 2008).

Natural History

Individuals with RTT develop typically between the ages of 6-18 months, followed by a period of regression (Amir et al., 1999). RTT is divided into typical (classic) and atypical (variant) forms. In 2010, a revision of the 2002 diagnostic criteria (Hagberg et al., 2002) was published to streamline clinical diagnosis, add required criteria, and distinguish variant forms of RTT. These criteria are continually being evaluated and will be updated

appropriately as more data becomes available to clinicians and researchers. Currently, the main criteria for RTT are: partial or complete loss of acquired purposeful hand skills, partial or complete loss of acquired spoken language, gait abnormalities, and stereotypic hand movements. All main criteria are required for a diagnosis of typical RTT, and two of these are required for a diagnosis of atypical RTT. In addition, supportive criteria include breathing disturbances when awake, bruxism when awake, impaired sleep pattern, abnormal muscle tone, peripheral vasomotor disturbances, scoliosis/kyphosis, growth retardation, small cold hands and feet, inappropriate laughter/screaming spells, diminished response to pain, and intense eye communication (Neul et al., 2010).

Due to the wide phenotypic spectrum, variant forms of Rett syndrome have been described and named for their phenotype; for example, the "preserved speech variant," also known as the Zapella variant, is considered the mildest RTT phenotype and is the most common atypical variant. A 2009 study identified 28 out of 29 females with Zapella variant RTT as having a missense or late-truncating *MECP2* mutation. Even still, the patients with this variant exhibited variable phenotypes (Reniere et al., 2009). A second variant called the "early seizure variant," or the Hanefield variant, is characterized by early-onset seizures (typically before developmental regression). *MECP2* mutations are less common in this variant, and mutations in the *CDKL5* gene (also located on the X chromosome) are more likely (Archer et al., 2006). The third variant is called the "congenital variant," or the Rolando variant, and affected individuals present with abnormal development from birth. It has been shown that mutations in the *FOXG1* gene (located on chromosome 14) may be more likely to cause the congenital form of RTT (Ariani et al., 2008). However, *CDKL5* and *FOXG1*-associated conditions are now

recognized as distinct diagnoses from RTT and are no longer included in current studies of RTT.

MECP2 mutations are also known to be responsible for a phenotypic spectrum outside of what is typically expected in RTT. In 1999, Wan et al. described a familial case in which a woman was found to have a mild learning disability and some motor problems. This woman had a sister and daughter both with classic RTT, as well as a son who was hemizygous for the mutation and passed away at one year of age due to neonatal encephalopathy (Wan et al., 1999).

While phenotypically variable, RTT typically presents in a severe form and leads to lifelong disability. The quality of life of individuals with RTT has dramatically changed in recent years due to advanced therapies and interventions. Survival for classic and atypical RTT is greater than 70% at 45 years, and survival into the 5th decade has become typical (Tarquinio et al., 2015). According to Tarquinio et al. (2015), "presumed cardiorespiratory complications" are the leading cause of death in females with classic RTT according to data from the US RTT Natural History Study. With strong physician monitoring of nutrition, gastrointestinal issues, scoliosis, aspiration risk, and epilepsy, the lifespan of individuals with RTT may continue to increase as these risk factors are managed.

Inheritance

RTT is considered *de novo*, or "random," in approximately 99% of cases. Few familial cases have been reported and are likely due to gonadal mosaicism in either the paternal or maternal germline, or to highly skewed X- chromosome inactivation (XCI) patterns in carrier mothers (Zhang et al., 2012). Zhang et al. (2012) found mutation type varied between paternally and maternally derived mutations in a cohort of Chinese patients. It

was found that point mutations are more likely to be of paternal origin, while single nucleotide gains/losses are more likely to be of maternal origin. Additionally, it was found that most familial cases were due to maternally derived mutations, due to either gonadal mosaicism or carrier mothers with highly skewed X-inactivation. These mechanisms are discussed in the succeeding paragraphs.

Gonadal mosaicism refers to the presence of more than one distinct cell line in the germ cells (egg or sperm). Gonadal mosaicism may occur in one of two ways. In some cases, a mutation occurs in a germ cell which then continues to divide and creates a unique cell line. In other cases, a mutation can occur in a very early somatic cell, which then separates into somatic and germ cells, leading to both somatic and gonadal mosaicism. Mosaic individuals may exhibit mild symptoms of the condition or be completely asymptomatic (Zlotogora, 1998).

X-inactivation refers to the silencing of either the maternal or paternal X-chromosome in female cells. The selection of the silenced chromosome is often random and varies from cell to cell; one cell population expresses the maternal X chromosome, while the remainder of the cells express the paternal X chromosome. This is referred to as the "X-inactivation pattern" and can vary from female to female; some may express a 50:50 ratio, while others may express a more highly skewed ratio, such as 90:10 or 80:20 (Plenge et al., 2002). It has been proposed that unaffected mothers of children with an X-linked intellectual disability (XLID) syndrome, such as Rett syndrome, may have a highly skewed X-inactivation pattern. Plenge et al. (2002) suggest that skewed XCI patterns are more common in mothers of children with XLID syndromes due to selection

against the X-chromosome containing the mutation, leading to higher XCI patterns (≥80:20).

Zhang et al. (2012) suggests that there are important clinical implications for mutation interpretation. For example, when insertion/deletion is observed in a patient, this is more likely due to a maternally derived mutation. In this case, the mother has a higher chance of being a carrier of the mutation or having germline mosaicism, and the family may be counseled on a modified recurrence estimate from the typical 1%. In the case of an affected child born to apparently healthy parents, the chance of recurrence can be as high as 50% if one parent is mosaic (Wilbe et al., 2017). However, there are currently no accepted methods in practice for analysis of sperm or eggs for germline mosaicism.

Approximately 99% of cases of females with RTT are *de novo* and have been identified as almost exclusively arising from mutations on the paternal X chromosome (Trappe et al., 2001; Zhang et al., 2012). This is a likely explanation for the high female: male ratio of individuals with RTT. It was previously thought that RTT was male lethal; however, males with *MECP2*-related conditions have been reported and may have inherited the mutation either through a maternal *de novo* mutation or through carrier mothers (Trappe et al., 2001). Additionally, after determining that *de novo MECP2* mutations were primarily paternally derived, Trappe et al. (2001) concluded that these mutations were not in fact male lethal, and the paucity of affected males could be attributed to the fact that boys do not receive an X chromosome from their fathers. Venâncio et al. (2007) describe a case report of a young female with classic RTT found to have a mutation in *MECP2.* The same mutation was found in her younger brother who

presented with severe growth and developmental delays early in life. He was found to have severe neurological abnormalities and died at 21 months of age due to complications from infection. However, the mutation was not present in blood from either parent, thus suggesting a case of maternal germline mosaicism (Venâcio et al., 2007). Typically affected males present with a severe phenotype, as all cells will express the mutated copy of *MECP2*. Four boys with progressive encephalopathy were described by Kankirawatana et al. (2006) All four patients had *de novo* mutations in *MECP2.* Symptoms included failure to thrive, respiratory insufficiency, microcephaly, and abnormal movements (Kankirawatana et al., 2006). This suggests that *MECP2* analysis should be considered in males with these presenting features and speaks to the wide phenotypic spectrum of Rett syndrome, especially in males.

Family Planning in The Presence of Genetic Conditions

In this present study, family planning refers to decisions related to reproduction. Reproductive stoppage, or the decision to discontinue having children after a diagnosis is made, is a known phenomenon among families with a child diagnosed with autism (Wood et al., 2014; Hoffmann et al., 2014). Autism, like RTT, is not often diagnosed until the affected child is a few years old. Reproductive decisions may change after this diagnosis is made; Hoffmann et al. (2014) found that the first few years after a child's diagnosis of autism, parents had subsequent children at a rate similar to controls. However, birth rates changed in the following years, and it was found that families had another child at a lower rate than controls (Hoffmann et al., 2014). This idea has yet to be studied in the RTT community. We hypothesize that families of children with RTT may make similar decisions, given that the diagnosis of RTT is often not made until early childhood after the child regresses. In addition, it is imperative to better understand the

factors that may contribute to these reproductive decisions, such as caregiver strain, interpretation of recurrence risk, and psychosocial aspects such as fear, guilt, and shame.

Upon receiving a diagnosis of Rett syndrome, caregivers and family members must learn to manage not only their child's health, but their own health as well. It is well understood that in general, parents of children with disabilities experience more adverse mental and physical health outcomes (Yamaoka et al., 2015). Laurvick et al. (2006) investigated this claim specifically in mothers of children with RTT. It was found that maternal physical and mental health is lower than the general population. Child behavior, caregiver demands, and family function were identified as the major predictors of maternal physical and mental health outcomes (Laurvick et al., 2006). Mothers of children with RTT are also at risk for adverse mental health outcomes such as severe depression (Sarajlija, Djuri, & Tepavcevi, 2013), and are also reported to have poorer mental health than physical health (Killian Jr. et al., 2016).

Other non-genetic factors may play a role in a family's decision to have more children after an RTT diagnosis. Because women with Rett syndrome now may live into middle age and beyond (Kirby et al., 2010), caregivers are responsible for ensuring their child receives lifelong care. This may be through dedicating themselves to the care of their child, but the question remains who will care for the child should he or she outlive the parents. Some parents may assume the siblings of their affected child will take charge of the care. There is currently no available research analyzing associations between caring for a child with RTT and the decision to have more children after the diagnosis is made. The strain on parents who are also caregivers coupled with the uncertainty of who will care for the child should they become unable and the uncertainty of having another

child with RTT are among many factors that may influence reproductive decisions. Being aware of these factors may provide the healthcare professionals caring for these families with increased knowledge of the impact a RTT diagnosis has on a family. By having a working knowledge of the possible questions and uncertainties parents of children with RTT face, healthcare professionals may be able to address them more accurately to provide more personal care for these families.

The quality of life measures for caretakers of individuals with RTT are similar to those who are caring for individuals with other neurodevelopmental disorders. Difficulties with common features of RTT such as poor feeding and ambulation may have an impact on the physical and mental health of the caregiver (Killian Jr. et al., 2016). RTT leads to a lifetime of disability, and caregiver demands can be great. Caregiver strain due to poor mental and physical health may certainly play a role in family planning; however, parents of children with RTT may not experience the same level of stress in caring for their children in early childhood as they may later in childhood and adulthood. It is not clearly understood if parents are making decisions based on their perceived future caregiving responsibilities, versus their current responsibilities when their child receives a diagnosis.

In many cases, the specialist making a RTT diagnosis is a neurologist, developmental pediatrician, or a geneticist (Tarquinio et al., 2015). It is currently unclear to what extent these families are receiving genetic counseling, if at all. It is also not clear whether families are receiving the most accurate and up-to-date information on the genetics of RTT. It is not yet known if recurrence risk, typically quoted at 1% except in cases of gonadal mosaicism or carrier mothers, is a major factor in these parents'

decision-making processes when it comes to future pregnancies. This present study aims to assess if families are receiving genetic counseling and by whom, as well as to determine what information they were given at the time of diagnoses that may have had an impact on family planning decisions.

Parents of children diagnosed with RTT face a unique challenge when it comes to chance of recurrence. While the majority of cases are *de novo,* mothers may be unrecognized carriers of a *MECP2* mutation due to skewed X-inactivation, and both maternal and paternal gonadal mosaicism are virtually impossible to rule out. This uncertainty may add a layer of complexity to families faced with making decisions about future pregnancies. The following quote is from the parent of a child with RTT regarding the diagnostic odyssey her family faced. (Knott, Leonard, & Downs, 2011):

> "Not long after diagnosis, I had a test to confirm that I was not a silent carrier of the mutation and when given the all clear, my husband and I decided to have another child. We had read that the chances of having a second child with RTT was miniscule, however, the concern was always present. (p.11)"

The author went on to have a healthy daughter without RTT after her first daughter was diagnosed with RTT, yet she does not specify that she received any kind of family or genetic counseling on the matter to reach that decision. Parents of newly diagnosed children have many questions, one of them being "How will this affect planning for future children?" (Downs & Leonard, 2016). Currently, there is little literature on family planning decisions after a diagnosis of a typically *de novo* condition is made.

There is, however, an abundance of literature regarding family planning decisions following a diagnosis of a condition in which the inheritance pattern is well-established.

Fragile X syndrome (OMIM #300624) is the most common cause of autism and intellectual disability in males (Hunter et al., 2014). Interviews of mothers with a known mutation in the gene *FMR1,* which incurs a 50% chance of passing on Fragile X syndrome to their children, revealed that 77% of women decided against having more children after learning their carrier status (Raspberry & Skinner, 2011). These women reported several factors that influenced their decision making, including reproductive risk, emotional/financial strain of another child with a disability, and the implication of "social judgments" should they choose to have more children knowing the risks involved.

Another survey of individuals with a diagnosis of Peutz-Jeghers syndrome, an autosomal dominant cancer predisposition syndrome, revealed that 29% of respondents reported that their diagnosis influenced their family planning decisions, and 19% of these reported that they do not wish to have children at all (van Lier et al., 2012). These questions have yet to be explored in the RTT community. Although it is now known that RTT is not typically inherited from a parent as in Peutz-Jeghers syndrome, it was initially thought to be inherited from the mother prior to the discovery that *MECP2* mutations were responsible. It is possible that healthcare professionals outside the field of genetics may not have the most up-to-date or accurate information on the recurrence risk of RTT.

In this study, we aim to assess to what extent a RTT diagnosis affects family planning decisions. We aim to identify factors such as parental interpretation of recurrence risk, the accuracy of recurrence information provided at the time of diagnosis, and the extent to which genetic counseling was received by families. In addition, we will investigate the rates at which parents elected prenatal diagnosis of RTT as well as the uptake of parental genetic studies to determine carrier status for RTT.

CHAPTER 2

FAMILY PLANNING DECISIONS AFTER A CHILD'S DIAGNOSIS OF RETT SYNDROME: A PILOT STUDY[1]

[1] Huggins, E.E., Baggett, L., Skinner, S.A., Ferrante, R. (2018) *Family Planning Decisions After a Child's Diagnosis of Rett Syndrome: A Pilot Study.* (to be submitted)

INTRODUCTION

Rett syndrome (RTT) is a rare neurodevelopmental genetic condition that affects about 1/10,000 girls and very rarely affects males (Laurvick et al., 2006). It is an X-linked dominant condition caused by mutations in the *MECP2* (methyl-CpG-binding protein 2, OMIM # 300005) gene, located at region Xq28 (Amir et al., 1999). RTT is characterized by a period of normal development between 6-18 months of age, followed by a period of regression. Though the clinical phenotype varies, the main diagnostic criteria for a diagnosis of RTT include partial or complete loss of acquired purposeful hand skills, partial or complete loss of acquired spoken language, gait abnormalities, and stereotypic hand movements (Neul et al., 2010). Other symptoms include seizures, vasomotor disturbances, scoliosis/kyphosis, and abnormal muscle tone.

RTT is considered sporadic in approximately 99% of cases. In the past, it was thought RTT was male lethal due to the paucity of affected males in the patient population. However, it was found that *de novo* cases typically arise due to mutations on the paternally inherited X chromosome (Trappe et al., 2001; Zhang et al., 2012). Few familial cases have been reported and are most likely maternally inherited. These cases are likely due to either parental gonadal mosaicism or carrier mothers with highly skewed X-inactivation (Zhang et al., 2012). While testing X-inactivation patterns in mothers is possible, it is nearly impossible to accurately screen either parent for gonadal mosaicism, making the assessment of recurrence risk a challenge.

In the event of an apparently sporadic case, parents of a child diagnosed with RTT are often told that recurrence is unlikely (i.e., < 1%), however; parental perception of this risk estimate has not been well-studied. Additionally, it is not clear to what extent parents

are receiving genetic counseling, and if they are informed of the possibility of an increased recurrence risk due to undetected gonadal mosaicism or carrier mothers. These uncertain risk assessments may alter family planning decisions after a child's diagnosis.

In this present study, family planning refers to decisions related to reproduction. Reproductive stoppage, or the decision to discontinue having children after a diagnosis is made, is a known phenomenon among families with a child diagnosed with autism (Wood et al., 2014; Hoffmann et al., 2014). As in RTT, autism is often undiagnosed until the affected child is a few years old and symptomatic. The inheritance of autism is variable and multifactorial, and many parents never receive an explanation of the cause of autism in their child. Due to the limits of genetic testing, recurrence risk for autism is often difficult to explain and interpret for families. Parents of children with autism may experience a similar type of uncertainty as parents of children with RTT. One study reported that, in the first few years after a child's diagnosis of autism, parents had subsequent children at a rate similar to controls. However, birth rates changed in the following years, and it was found that families had another child at a lower rate than controls (Hoffmann et al., 2014). This idea has yet to be studied in the RTT community. We hypothesize that families of children with RTT may make similar decisions, given that the diagnosis of RTT is often not made until early childhood after the child regresses and there is a similar level of uncertainty involved.

Reproductive decision-making in the wake of a genetic syndrome with an established inheritance pattern has been well-studied. While RTT is the leading cause of intellectual disability in females, Fragile X syndrome is the leading cause of autism and intellectual disability in males. Fragile X syndrome is caused by a trinucleotide repeat

expansion in the *FMR1* gene, also located on the X chromosome. Typically, mothers are carriers of an expansion, and have a 50% chance to pass on the full expansion to their sons in an X-linked recessive pattern. A study of known Fragile X carriers revealed that 77% of women decided against having more children after learning their carrier status (Raspberry & Skinner, 2011). While the inheritance pattern of Fragile X syndrome and RTT differ, there is phenotypic overlap between the two syndromes. Women in this study reported several factors that influenced their reproductive decision making, including reproductive risk, emotional/financial strain of another child with a disability, and the implication of "social judgments" should they choose to have more children knowing the risks involved. This present study aims to identify factors in this same decision- making process for both mothers and fathers of children with RTT.

RTT is a condition characterized by severe lifelong disability. As described by mothers in the above-mentioned study, there is both emotional and financial strain in caring for one child with a disability, let alone multiple children with disabilities. The mental and physical health of parent caregivers of children with special needs have been well-studied. In general, parents of children with disabilities experience increased adverse mental and physical health outcomes (Yamaoka et al., 2015). Specifically, mothers of children with RTT are known to have poorer mental and physical health than the general population (Laurvick et al., 2006) and are at an increased risk for depression (Sarajlija, Djuri, & Tepavcevi, 2013). Caregiver strain, along with uncertainty surrounding recurrence risk, must be considered when assessing a family's desire to alter their family planning strategy. It is hypothesized that a combination of genetic and non-genetic factors ultimately influences parental family planning decisions and strategies. Families

must navigate complex genetic information, manage the ongoing medical needs of their child with RTT, and deal with parenthood and the emotional distress that comes regardless of a genetic diagnosis. In addition, parents may experience feelings of guilt, shame, and fear, and may have little guidance in managing this distress. This present study aims to assess to what extent a RTT diagnosis affects family planning decisions, the accuracy of information given to parents at the time of diagnosis, and the measures parents have taken to manage these risks such as prenatal diagnosis of RTT and parental genetic studies.

MATERIALS AND METHODS

Participants

Approval for this study was obtained from the University of South Carolina Institutional Review Board. Biological parents of individuals of any age with a clinical and/or genetic diagnosis of RTT were eligible to participate in this study. Parents of children with known *MECP2* duplications, *FOXG1* alterations, or *CDKL5* variants were excluded from this study. Less information is widely available on these conditions, and although they are associated with RTT, they do not carry the same implications as a diagnosis of Rett syndrome presumed or known to be to be associated with a *MECP2* alteration. An invitation was sent to members of RettNet, a community for the family members of individuals with RTT, through an email listserv. The invitation was sent once a month for three months (October 2017-January 2018). In addition, an invitation to participate was posted on the public Facebook page RettSyndrome.org once a month for three months. There were 323 individuals that completed at least part of the online survey. Of these, 304 completed at least 80% of the survey and met the inclusion criteria.

Questionnaire Design

We developed an online questionnaire using SurveyMonkey. The questionnaire had four major sections. The first section included information on the diagnosis of the individual with RTT (age, age at diagnosis, diagnosis delivery, genetic status). The second section included questions related to subsequent pregnancies after their child was diagnosed with RTT (number of pregnancies, type of genetic screening/testing during pregnancy, genetic diagnosis for RTT). Participants were prompted to answer the same set of questions for up to 4 subsequent pregnancies. The first two sections consisted of primarily multiple-choice questions with few free-response questions. The third section included several in-depth free response questions (how their child's diagnosis impacted their family planning, what information they received about recurrence risk). The final section included participant demographics. The questionnaire was pilot tested by a staff member of RettSyndrome.org, who is also the mother of a daughter with RTT. The questionnaire took approximately ten minutes to complete.

Analysis

Descriptive statistics were used to describe quantitative questionnaire data. Chi-square analysis was used to analyze relationships between genetic counseling and family planning. Thematic analysis performed by the principal investigator was used to analyze free-response question data.

RESULTS

2.1 Demographics. A total of 323 participants completed all or part of the survey. Only biological mothers and fathers of children with RTT were eligible to participate in this study. Those that reported they were not biological parents and those that did not complete greater than 80% of the survey were excluded from analysis, resulting in a total of 304 responses. Participants could skip any question they did not want to answer; thus, there are variations in the number of responses per question analyzed. Of the participants that met inclusion criteria, 89.5% (272/304) were mothers and 10.5% (32/304) were fathers. Participants were primarily Caucasian, college-educated, and married. Demographics are outlined in Table 2.1.

2.2. Family Planning Decisions. Forty-three percent of participants (117/272) responded that having a child with RTT changed their family planning decisions, 50.4% (137/272) responded that it did not, and 6.6% (18/272) were unsure (Figure 2.1). Several major themes emerged when analyzing how a diagnosis of RTT impacted family planning. Detailed responses were provided by 194 participants regarding how their family planning decisions were or were not altered. Three major themes emerged: parental decisions to stop reproduction, parental decisions to continue reproduction, and parental decisions to make no changes to their reproductive plans based on their child's RTT diagnosis. Subthemes are presented in Figures 2.2-2.4. Analysis of these themes and their subthemes, as well as examples of participant responses, are outlined in Table 2.2.

2.2a. Decision to stop reproduction. There were 70 responses indicating that parents decided to stop having children after their child's diagnosis of RTT. Subthemes in this category include stoppage due to caregiver strain (38/70), the decision to dedicate their care to the affected child/did not wish to have more children (14/70), and fear/uncertainty of having another child with RTT or other special needs (18/70).

2.2b. Decision to continue reproduction. There were 37 responses indicating that parents decided to continue having children after their child's diagnosis of RTT. Subthemes in this category include continuation for the reason of having a sibling either for their child with RTT or for a typical child (12/37), continuation because the interpretation of the risk for future affected children was low (8/37), and a decision to have more children but with different age gaps between children than previously planned (17/37).

2.2c. No change in family planning decisions. There were 82 responses indicating that a diagnosis of RTT did not alter family planning decisions. One group of participants indicated that they were already done having children by the time their child was diagnosed (34/82). Ten participants were already pregnant at the time of the diagnosis, 16 participants responded that they were still planning to have the same number of children regardless of the diagnosis, 7 participants reported having either a tubal ligation or vasectomy prior to becoming aware of their child's diagnosis, and 5 participants were mothers who responded that their age was the major factor in stoppage. A group of 11 participants had miscellaneous reasons to stop reproduction that did not fit into categories including inability to get pregnant or did not give specific reasons.

2.2d. Undecided. A group of five participants reported that they were still unsure if their family planning decisions would change. Reasons include parental disagreement (2/5) and difficulty coming to a decision due to a variety of factors (3/5).

2.3 Future Pregnancies. Participants were asked questions regarding subsequent pregnancies after their child was diagnosed; 46.7% (136/291) reported that they had at least one subsequent pregnancy and 53.3% (155/291) reported that they had no subsequent pregnancies. Prenatal diagnostic testing via amniocentesis or chorionic villus sampling (CVS) for RTT was performed on 27 pregnancies, and prenatal diagnostic testing for other conditions such as chromosome abnormalities was performed for 37 pregnancies. Of note, none of the reported pregnancies tested for RTT prenatally were affected.

2.4. Recurrence Risk Information. Participants were asked questions regarding reproductive information they received prior to having future children; 55.5% (146/263) report that they were told their chances of having future children with RTT, 39.2% (103/263) reported they were not told their chances, and 5.3% (14/263) were unsure. Of the 263 that answered this question, 183 gave detailed responses regarding the information they received. Thematic analysis revealed five different categories of information participants received (Figure 2.5). Many respondents (78.7%, 144/183) reported that they were told their chances of having another affected child were 1% or less, or unlikely. Of note, 11 of these responses specifically mentioned being counseled on gonadal mosaicism (both maternal and paternal) and the possibility of carrier mothers. The second most common response (11.4%, 21/183) was by parents that were not told anything or did not have this discussion with their providers. A small subset (3.3%,

6/183) reported that this information was not known at the time of their child's diagnosis, and another small subset (3.3%, 6/183) were told a higher number, such as 5%, 25%, or 50% or more. Of note, one of these responses indicated that the mother was found to be an asymptomatic carrier for RTT; therefore, she was correctly informed her chance of having an affected child is 50%. Further, 3.3% (6/183) were told the chance was zero or impossible.

2.4a. Parental Genetic Testing. Participants were asked if either parent had received genetic testing to determine their carrier status for *MECP2* mutations. There were 272 responses to this question; 16.5% (45/272) report the mother only had genetic testing, 1.5% (4/272) report the father only had genetic testing, 22.8% (62/272) report both parents had testing, 58.5% (159/272) report neither had testing, and 0.7% (2/272) were unsure. Of the mothers that received testing, two were found to be asymptomatic carriers. There were no reports of carrier fathers.

2.5. Genetic Counseling. Participants were asked whether they had received genetic counseling. There were 272 responses to this question; 34.6% (94/272) reported they had received genetic counseling. Of those that chose to disclose who provided the genetic counseling, 82% (82/100) reported they were counseled by a genetic counselor, and 18% (18/100) were counseled by another physician or provider. Sixty-one percent of respondents (166/272) reported they did not receive genetic counseling, and 4.4% (12/272) were unsure. There was no significant association between families receiving genetic counseling and altering their family planning decisions ($p= 0.8$).

DISCUSSION

The themes identified in this present study allude to previous findings in the literature regarding family planning. As expected, there were more participants that made decisions to stop reproduction based on non-genetic factors (i.e., caregiver strain), versus stopping based on the recurrence risk of RTT. While only about half of participants reported being informed of their chances to have another affected child, participants more frequently reported that they stopped reproduction based on recurrence risk than continued despite recurrence risk. Families are often quoted a "1% or less" recurrence risk (except in situations of gonadal mosaicism or carrier mothers) when discussing the chances for their future children to have RTT. This study was the first to investigate how parents of children with RTT interpreted this chance and applied it to their family planning decisions.

Caregiver strain was the most commonly reported reason for reproductive stoppage. It is known that individuals with RTT have many complex medical issues, including seizures, feeding problems, mobility issues, and poor communication skills. Affected individuals require lifelong care and are surviving longer than they have been in the past; currently, survival into the fifth decade is common (Tarquinio et al., 2015). Parents must balance their care between their affected child and their other children; a commonly reported reason for reproductive stoppage included parents reporting wanting to dedicate all their time to their current children. For example, one participant responded, "After the diagnosis we choose to put all our efforts into giving our daughter the best possible life that we could." The second most common reason for reproductive stoppage was due to fear or uncertainty of RTT happening again in a future child. Of

note, some participants made the distinction that it was not solely another child with RTT that was concerning but having another child with any kind of special needs, as the needs of their affected child were so great. It is uncertain if recurrence of RTT or recurrence of disability was more strongly associated with reproductive stoppage.

There is literature regarding reproductive stoppage in the presence of known heritable genetic conditions, such a Fragile X syndrome. Mothers who are carries for Fragile X syndrome have a 50% risk to have an affected son with each male pregnancy and a 50% chance to have a carrier daughter with each female pregnancy. Raspberry & Skinner et al. (2011) found fear of "social judgments" was a reason for reproductive stoppage by Fragile X carrier mothers. While many parents of children with RTT reported stopping due to the strain of having a child with a disability as well as fearing having another child with similar needs, no participants reported feeling social pressure or judgements when making family planning decisions. This could be due in part to the fact that many parents are likely informing family and friends that RTT happened in their child sporadically and there is a low chance for it to occur again; however, it is possible there is pressure to stop having children to dedicate themselves to caring for their child with special needs. Although stopping reproduction to dedicate more care to their affected child was a commonly reported reason for stopping reproduction, no participants reported that this was due to any kind of pressure from family, friends, or society.

In terms of reproductive continuation, many participants reported wanting a sibling either for their child with RTT or for their typical child. A few reported wishing to have another child that could help with the care of their child with RTT as he/she aged and would require more care. Additionally, another set of participants wanted to have

another typical child so that the lone sibling of an affected individual would not face the burden of caring for him/her in adulthood alone. Adult siblings of individuals with intellectual disabilities are known to take on a variety of roles when their sibling reaches adulthood, including caregiver, friend, advocate, legal representative (such as a guardian), and informal service coordinator (Hall & Rosetti, 2017). This could be a possible motive behind parental desire for more typical children, but interpretations of those future sibling roles were not explicitly stated by any respondents.

When families receive a diagnosis of RTT, frequently from a neurologist, developmental pediatrician, or geneticist (Tarquinio et al., 2015), it is imperative they receive genetic counseling. Genetic counseling is defined as the "process of helping people understand and adapt to the medical, psychological and familial implications of genetic contributions to disease" (National Society of Genetic Counselors' Definition Task Force, 2006). In the case of RTT, parents should receive counseling based on up-to-date literature regarding the genetics of RTT, *de novo* vs. inherited RTT, recurrence risk in future pregnancies, and potential genetic testing in the event of a suspected carrier mother. Prior to this study, it was unclear to what extent parents of those with RTT were receiving genetic counseling. Typically, genetic counseling is provided by a genetic counselor. In areas where genetic counselors are not readily available, physicians or other practitioners may provide this counseling. In the event a diagnosis is made by a neurologist or developmental pediatrician, rather than a geneticist, there may be a delay in reception of genetic counseling due to wait times for genetics referrals, or the family may never receive genetic counseling at all. Less than half of respondents reported receiving genetic counseling. Of those, the majority did receive counseling from a genetic

counselor. It is possible, however, that participants received some degree of genetic counseling but may not have been aware of it at the time due to the misunderstanding that genetic counseling is only provided in a geneticist's office, or only by a genetic counselor. Even discussing recurrence risk with a physician qualifies as some degree of genetic counseling. Therefore, reception of genetic counseling may be underreported in this patient population.

Participants were invited to explain what they were told in general regarding recurrence risk of RTT. Thematic analysis of these responses revealed that the majority were told that there was a less than 1% chance, "minimal," or "negligible" chance that they would have another affected child. Of these responses, there were several participants that reported specific information on recurrence risk in the presence of gonadal mosaicism or carrier mothers. For example, one mother reported being told that "although I, [the] mother, did not have any abnormalities in my genetic makeup, my eggs may carry the genetic mutation." Another participant discussed the changes to risk based on carrier status: "If I (the mother) carry [the mutation] our chances go up. But if not it's like 1:100,000." As stated previously, this information may have been shared by a genetic counselor or another provider. Further investigation into information received by parents from genetic counselors and information received from other practitioners is warranted. However, no significant difference was reported regarding family planning decisions between those that received genetic counseling and those that did not.

Other participants reported being told the chance for recurrence was "zero" or impossible. It is unclear whether these participants were truly told the risk was zero, or if that was their interpretation of a "1% of less" risk estimate. To some, zero and "1% or

less" may be equivalent. Patients interpret risk estimates in a variety of ways, and it is not clear whether the responses to this question were their own interpretation or were the words of the genetic counselor or other provider. Many participants report not discussing recurrence risk with a healthcare provider for a variety of reasons. Some chose not to speak with a genetic counselor because they were not planning to have any more children, while others reported simply not having this discussion at all. For others, the chance of recurrence was simply not known at the time of their child's diagnosis, and they did not receive any information. A small subset of participants was told a risk number greater than 1%, such as 5%, 25%, or 50%. One respondent who was found to be an asymptomatic carrier of a *MECP2* mutation reported that she was informed that there was a 50% chance if the future baby was a boy and an unknown chance if the future baby was a girl. It is possible that this participant was referring to the uncertainty of whether a daughter would have highly skewed X-inactivation, but this is not clear based on her response. In the presence of gonadal mosaicism, there can be a risk of up to 50% to have another affected child; it is complicated to counsel on this number, however, since it is virtually impossible to test eggs or sperm to determine the level of mosaicism. It is unclear whether those that reported being informed of a 50% risk were told this in the context of inheritance or of gonadal mosaicism.

Further, one of the four participants that reported being told a 50% recurrence risk was found to be an asymptomatic carrier of a *MECP2* mutation. This participant, a mother of a male child with RTT, has a twin sister with RTT. In this case, she had a 50% chance of passing on her X chromosome with the *MECP2* mutation, and a 50% chance of passing on her X chromosome without the *MECP2* mutation, regardless of the sex of the

baby. This mother had unique insight into family planning decisions when the mother is an asymptomatic carrier. She reported

> "…we got pregnant when [son with RTT] was around 1 year old. But we terminated the pregnancy because we did not know what was wrong with him and we did not know what to test for. We couldn't risk having another child with such high needs. We got pregnant again and then the insurance company finally agreed to let us do a full DNA test and found out that I am an asymptomatic carrier. Praise the heavens that this second pregnancy did not have Rett syndrome."

Her experience entails many different themes surrounding family planning, such as fear and uncertainty of having another affected child, potential caregiver strain from having a child with complex medical needs and pursuing parental genetic testing to determine parental carrier status to gain a better understanding of their personal recurrence risk.

Limitations

This study had several limitations that were potential barriers to data analysis. Most of the participants were educated Caucasian females. Having a more diverse pool of participants may have given more insight into how different groups of people may interpret recurrence risk, and how different groups deal with the strain of having a child with a disability. Religion was not asked as a demographic question, but it is possible religious and spiritual beliefs played a role in family planning.

This questionnaire was only distributed electronically. It is possible more groups may have been reached with the use of a mailed paper survey, as many do not have access to Internet, email, or social media sites where the questionnaire was distributed.

Directions for Future Research

There has been little research in the field of genetic counseling regarding counseling families on recurrence risk in the presence of a typically *de novo* condition, primarily surrounding the possibility of gonadal mosaicism. Further, the RTT community may benefit from analysis of current genetic counseling practices of genetic counselors/geneticists, developmental pediatricians, and neurologists to assess their way of reporting recurrence risk to parents. It is also unclear to what extent counseling on the prognosis of RTT has to do with decisions regarding family planning. This could give more insight into parent perceptions of the roles siblings of their children with RTT may take on as children and as adults.

Conclusion

This present study was a pilot study into the perceptions of parents of children with RTT of recurrence risk and how their family planning was impacted. It was found that more participants altered their family planning based on non-genetic factors, such as caregiver strain and desire for their children (both with RTT and without) to have siblings, rather than their perception of the recurrence risk. Non-genetic factors were reported more frequently by both groups of parents, those who stopped reproduction and those who continued. Genetic counseling had no significant association with altering family planning decisions. More research is needed to determine the extent families are receiving genetic counseling and whether that includes the chance for the possibility of gonadal mosaicism or asymptomatic carrier mothers.

TABLES

Table 2.1 Demographics

		Percentage	Frequency
Relationship to Individual with RTT	Biological Mother	89.5	272
	Biological Father	10.5	32
	Total	100	304
Race/Ethnicity	Caucasian	84.5	229
	African-American	1.1	3
	Hispanic/Latino	5.9	16
	Native American	0.4	1
	Asian/Pacific Islander	0.7	2
	Multiethnic	3	8
	Prefer not to respond	1.1	3
	Other	3.3	9
	Total	100	271
Education Level	Less than high school	0.7	2
	High school diploma or equivalent	5.9	16
	Some college	20.2	55
	Associate's degree	13.2	36
	Bachelor's degree	29	79
	Graduate degree	27.6	75
	Prefer not to respond	3.3	9
	Total	100	272
Employment Status	Employed	55	149
	Unemployed	1.1	3
	Stay-at-home parent	28.8	78
	Student	0.4	1
	Retired	5.9	16
	Other	8.9	24
	Total	100	271
Marital Status	Single	1.5	4
	Married	83.1	226
	Divorced/Separated	8.8	24
	In a relationship but not married	6.6	18
	Total	100	272

Table 2.2 Thematic Analysis of Family Planning Decisions Based on a Child's Diagnosis of RTT

Theme	Subthemes	Example(s)
Affected Decision Making: Decided to have more children	Addition of another sibling for their child with RTT and/or for their typical child	"We wanted a sister for our Rett daughter." "We wanted the typical child to have a typical sibling."
	Delayed having more children/intentionally altered children's age gaps from original plan	"We delayed planning for a third child to adjust to our new life."
	Interpreted recurrence risk as low	"No, we were aware that Rett is caused by a random mutation and was unlikely to repeat."
Affected Decision Making: Decided to cease having children	Caregiver strain	"Rett syndrome consumes your entire life, not just severe complications with your child. Your marriage, relationships and you as the caretaker, your health." "Not for fear of Rett but because of the amount of time and energy we would have to devote to our daughter."
	Uncertainty or fear regarding recurrence/Fear of having another child with RTT or other special needs	"I felt I couldn't risk bringing another child into the world with Retts. I couldn't do that to another child…" "I could not take the risk of another child potentially having any type of special needs."
Did Not Affect Decision Making	Already pregnant when diagnosis received	"We were nine months pregnant with daughter #4 when we got the diagnosis."

	Finished having children/Had tubal ligation or vasectomy prior to diagnosis	"I was 47 when [child] was diagnosed." "My husband chose to have a vasectomy when our child with RTT was a newborn." "I was finished having children when she got her diagnosis."
	Planned to have more children regardless of diagnosis	"We wanted a third child in spite of our daughter's disability."
Unsure/Uncertain	Parental Disagreement	"After my Rett daughter passed in 2015, I have considered another child. My husband does not agree."
	Weighing Options	"I haven't fully decided yet. I'd like to have another but I'm worried about coping and giving my daughter with Rett syndrome enough time and input. I want her to achieve All she can within the sphere of her abilities. I want to give my all to this. At the same time I'd like my older daughter to have another sibling. I'm also just scared."

FIGURES

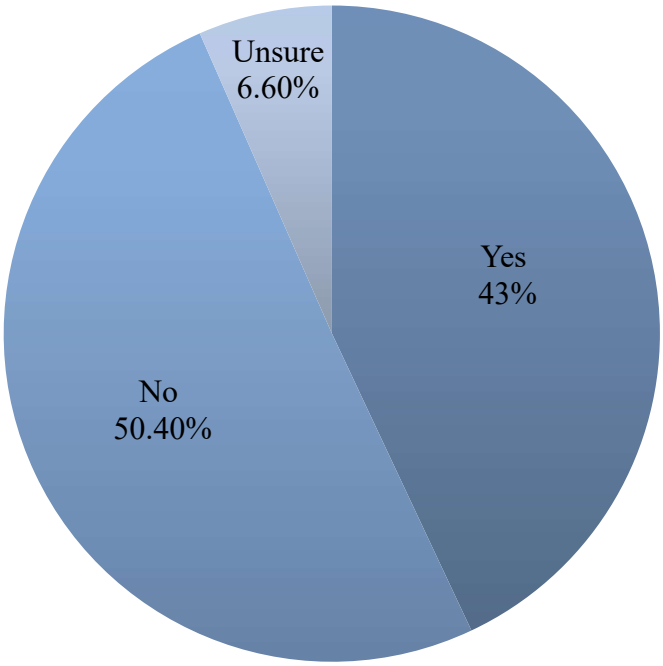

Figure 2.1 Parent Report: “Did your child's Rett syndrome diagnosis alter your family planning?” (n=272)

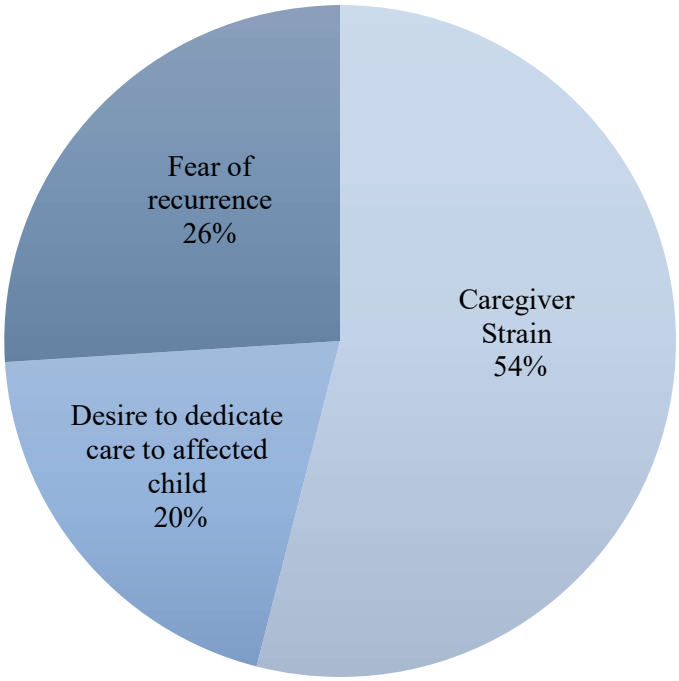

Figure 2.2 Factors Involved in Ceasing to Have Children (n=70)

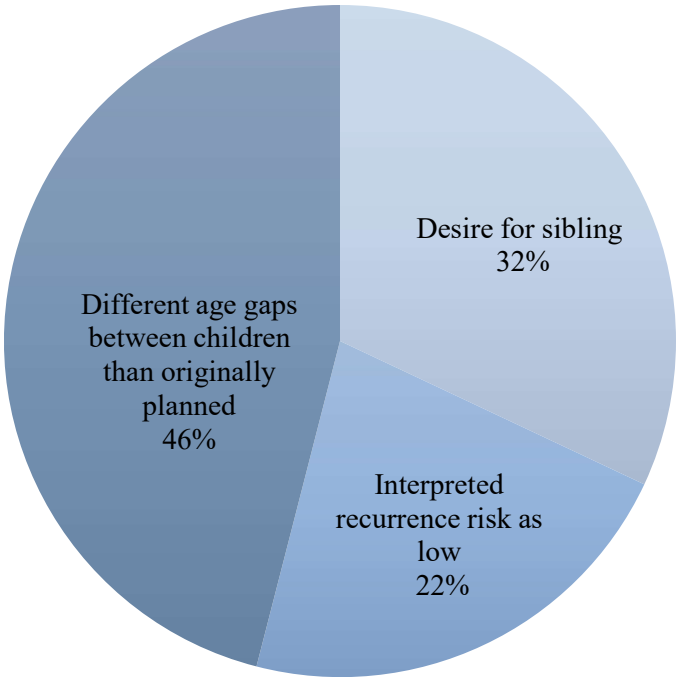

Figure 2.3 Factors Involved in Having More Children (n=37)

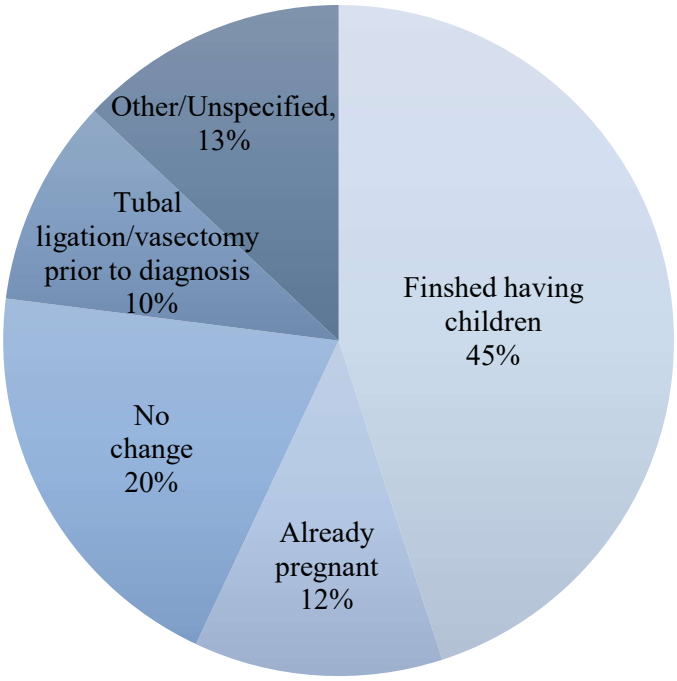

Figure 2.4 Factors Involved in Deciding Not to Alter Family Planning (n=82)

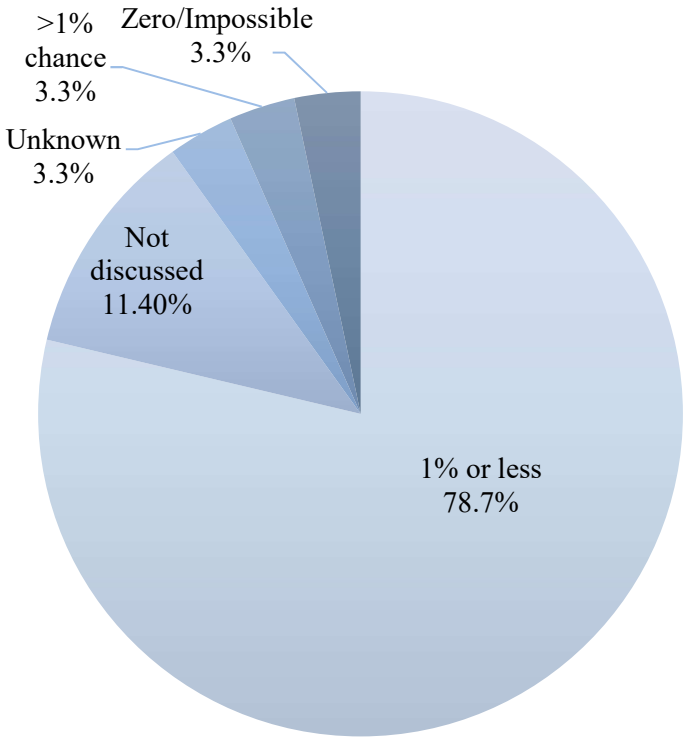

Figure 2.5 Parental Understanding of Recurrence Risk (n=183)

REFERENCES

Amir, R. E., Van den Veyver, I. B., Wan, M., Tran, C. Q., Francke, U., & Zoghbi, H. Y. (1999). Rett syndrome is caused by mutations in X-linked MECP2, encoding methyl-CpG-binding protein 2. *Nature Genetics*, 23(2), 185–188. https://doi.org/10.1038/13810

Archer, H. L., Evans, J., Edwards, S., Colley, J., Newbury-Ecob, R., O'Callaghan, F., … Osborne, J. (2006). CDKL5 mutations cause infantile spasms, early onset seizures, and severe mental retardation in female patients. *Journal of Medical Genetics*, 43(9), 729–734. https://doi.org/10.1136/jmg.2006.041467

Ariani, F., Hayek, G., Rondinella, D., Artuso, R., Mencarelli, M. A., Spanhol-Rosseto, A., … Renieri, A. (2008). FOXG1 Is Responsible for the Congenital Variant of Rett Syndrome. *American Journal of Human Genetics*, 83(1), 89–93. https://doi.org/10.1016/j.ajhg.2008.05.015

Christodoulou J, Grimm A, Maher T, Bennetts B. (2003). RettBASE: The IRSA MECP2 Variation Database - A New Mutation Database in Evolution. Hum Mutat 21:466-472. Pubmed: 12673788

Downs, J., & Leonard, H. (2016). Quantitative and qualitative insights into the experiences of children with Rett syndrome and their families. *Wiener Medizinische Wochenschrift (1946),* 166(11–12), 338–345. https://doi.org/10.1007/s10354-016-0494-6

Hagberg, B., Aicardi, J., Dias, K., & Ramos, O. (1983). A progressive syndrome of autism, dementia, ataxia, and loss of purposeful hand use in girls: Rett's syndrome: report of 35 cases. *Annals of Neurology*, 14(4), 471–479. https://doi.org/10.1002/ana.410140412

Hagberg, B., Hanefeld, F., Percy, A., & Skjeldal, O. (2002). An update on clinically applicable diagnostic criteria in Rett syndrome. Comments to Rett Syndrome Clinical Criteria Consensus Panel Satellite to European Paediatric Neurology Society Meeting, Baden Baden, Germany, 11 September 2001. *European Journal of Paediatric Neurology: EJPN: Official Journal of the European Paediatric Neurology Society*, 6(5), 293–297.

Hall S, A. & Rossetti, Z. (2017). The roles of adult siblings in the lives of people with severe intellectual and developmental disabilities. *Journal of Applied Research in Intellectual Disabilities*, 0(0), 1-12. https://doi.org/10.1111/jar.12421

Hoffmann, T. J., Windham, G. C., Anderson, M., Croen, L. A., Grether, J. K., & Risch, N. (2014). Evidence of Reproductive Stoppage in Families With Autism Spectrum Disorder: A Large, Population-Based Cohort Study. *JAMA Psychiatry*, 71(8), 943–951. https://doi.org/10.1001/jamapsychiatry.2014.420

Hunter Jessica, Rivero-Arias Oliver, Angelov Angel, Kim Edward, Fotheringham Iain, & Leal Jose. (2014). Epidemiology of fragile X syndrome: A systematic review and meta-analysis. *American Journal of Medical Genetics Part A*, 164(7), 1648–1658. https://doi.org/10.1002/ajmg.a.36511

Kankirawatana, P., Leonard, H., Ellaway, C., Scurlock, J., Mansour, A., Makris, C. M., … Percy, A. K. (2006). Early progressive encephalopathy in boys and MECP2 mutations. *Neurology*, 67(1), 164–166. https://doi.org/10.1212/01.wnl.0000223318.28938.45

Killian, J., Lane, J., Lee, H.-S., Pelham, J., Skinner, S., Kaufmann, W., … Percy, A. (2016). Caretaker Quality of Life in Rett Syndrome: Disorder Features and Psychological Predictors. *Pediatric Neurology*, 58, 67–74. https://doi.org/10.1016/j.pediatrneurol.2015.12.021

Kirby, R. S., Lane, J. B., Childers, J., Skinner, S. A., Annese, F., Barrish, J. O., … Percy, A. K. (2010). Longevity in Rett syndrome: analysis of the North American Database. *The Journal of Pediatrics*, 156(1), 135–138.e1. https://doi.org/10.1016/j.jpeds.2009.07.015

Knott, M., Leonard, H., & Downs, J. (2012). Genetic drift. The diagnostic odyssey to Rett syndrome: the experience of an Australian family. *American Journal of Medical Genetics Part A*, 158A(1), 10–12. https://doi.org/10.1002/ajmg.a.34372

Laurvick, C. L., de Klerk, N., Bower, C., Christodoulou, J., Ravine, D., Ellaway, C., … Leonard, H. (2006). Rett syndrome in Australia: a review of the epidemiology. *The Journal of Pediatrics*, 148(3), 347–352. https://doi.org/10.1016/j.jpeds.2005.10.037

Laurvick, C. L., Msall, M. E., Silburn, S., Bower, C., de Klerk, N., & Leonard, H. (2006). Physical and mental health of mothers caring for a child with Rett syndrome. *Pediatrics,* 118(4), e1152-1164. https://doi.org/10.1542/peds.2006-0439

National Society of Genetic Counselors' Definition Task Force, Resta, R., Biesecker, B.B., Bennett, R.L., Blum, S., Hahn, S.E., Strecker, M.N., Williams, J.L. (2006). A new definition of Genetic Counseling: National Society of Genetic Counselors' Task Force report. *Journal of Genetic Counseling,* 15(2):77-83.

Neul, J. L., Fang, P., Barrish, J., Lane, J., Caeg, E. B., Smith, E. O., … Glaze, D. G. (2008). Specific mutations in methyl-CpG-binding protein 2 confer different severity in Rett syndrome. *Neurology*, 70(16), 1313–1321. https://doi.org/10.1212/01.wnl.0000291011.54508.aa

Neul, J. L., Kaufmann, W. E., Glaze, D. G., Christodoulou, J., Clarke, A. J., Bahi-Buisson, N., … Percy, A. K. (2010). Rett Syndrome: Revised Diagnostic Criteria and Nomenclature. *Annals of Neurology*, 68(6), 944–950. https://doi.org/10.1002/ana.22124

Plenge, R. M., Stevenson, R. A., Lubs, H. A., Schwartz, C. E., & Willard, H. F. (2002). Skewed X-Chromosome Inactivation Is a Common Feature of X-Linked Mental Retardation Disorders. *American Journal of Human Genetics*, 71(1), 168–173. Retrieved from https://www.ncbi.nlm.nih.gov/pmc/articles/PMC384975/

Raspberry, K. A., & Skinner, D. (2011). Negotiating Desires and Options: How Mothers Who Carry the Fragile X Gene Experience Reproductive Decisions. *Social Science & Medicine* (1982), 72(6), 992–998. https://doi.org/10.1016/j.socscimed.2011.01.010

Renieri, A., Mari, F., Mencarelli, M. A., Scala, E., Ariani, F., Longo, I., … Zappella, M. (2009). Diagnostic criteria for the Zappella variant of Rett syndrome (the preserved speech variant). *Brain & Development*, 31(3), 208–216. https://doi.org/10.1016/j.braindev.2008.04.007

Rett, A. (1966). [On a unusual brain atrophy syndrome in hyperammonemia in childhood]. *Wiener Medizinische Wochenschrift* (1946), 116(37), 723–726.

Sarajlija, A., Djurić, M., & Tepavcević, D. K. (2013). Health-related quality of life and depression in Rett syndrome caregivers. *Vojnosanitetski Pregled*, 70(9), 842–847.

Tarquinio, D. C., Hou, W., Neul, J. L., Kaufmann, W. E., Glaze, D. G., Motil, K. J., … Percy, A. K. (2015). The Changing Face of Survival in Rett Syndrome and MECP2-Related Disorders. *Pediatric Neurology*, 53(5), 402–411. https://doi.org/10.1016/j.pediatrneurol.2015.06.003

Trappe, R., Laccone, F., Cobilanschi, J., Meins, M., Huppke, P., Hanefeld, F., & Engel, W. (2001). MECP2 mutations in sporadic cases of Rett syndrome are almost exclusively of paternal origin. *American Journal of Human Genetics*, 68(5), 1093–1101. https://doi.org/10.1086/320109

van Lier, M. G. F., Korsse, S. E., Mathus-Vliegen, E. M. H., Kuipers, E. J., van den Ouweland, A. M. W., Vanheusden, K., … Wagner, A. (2012). Peutz–Jeghers syndrome and family planning: the attitude towards prenatal diagnosis and pre-implantation genetic diagnosis. *European Journal of Human Genetics*, 20(2), 236–239. https://doi.org/10.1038/ejhg.2011.152

Venâncio, M., Santos, M., Pereira, S. A., Maciel, P., & Saraiva, J. M. (2007). An explanation for another familial case of Rett syndrome: maternal germline mosaicism. European Journal of Human Genetics, 15(8), 902–904. https://doi.org/10.1038/sj.ejhg.5201835

Wan, M., Lee, S. S., Zhang, X., Houwink-Manville, I., Song, H. R., Amir, R. E., … Francke, U. (1999). Rett syndrome and beyond: recurrent spontaneous and familial MECP2 mutations at CpG hotspots. American Journal of Human Genetics, 65(6), 1520–1529. https://doi.org/10.1086/302690

Wilbe, M., Gudmundsson, S., Johansson, J., Ameur, A., Stattin, E.-L., Annerén, G., … Bondeson, M.-L. (2017). A novel approach using long-read sequencing and ddPCR to investigate gonadal mosaicism and estimate recurrence risk in two families with developmental disorders. *Prenatal Diagnosis*, *37*(11), 1146–1154. https://doi.org/10.1002/pd.5156

Wood, C.L., Warnell, F., Johnson, M., Hames, A., Pearce, M.S., McConachie, H., Parr, J.R. (2014). Evidence for ASD Recurrence Rates and Reproductive Stoppage From Large UK ASD Research Family Databases. *Autism Research,* 8(1), 73-81.

Yamaoka, Y., Tamiya, N., Moriyama, Y., Sandoval Garrido, F. A., Sumazaki, R., & Noguchi, H. (2015). Mental Health of Parents as Caregivers of Children with Disabilities: Based on Japanese Nationwide Survey. *PLoS ONE*, *10*(12).

Zhang, J., Bao, X., Cao, G., Jiang, S., Zhu, X., Lu, H., … Wu, X. (2012). What does the nature of the MECP2 mutation tell us about parental origin and recurrence risk in Rett syndrome? *Clinical Genetics*, *82*(6), 526–533. https://doi.org/10.1111/j.1399-0004.2011.01838.x

Zlotogora, J. (1998). Germ line mosaicism. *Human Genetics*, *102*(4), 381–386.

APPENDIX A
ONLINE QUESTIONNAIRE

Dear Parent,

My name is Erin Huggins and I am a graduate student in the Genetic Counseling Training Program at the University of South Carolina. I am conducting a research study as part of the requirements of my degree in Genetic Counseling, and I would like to invite you to participate.

I am studying the impact a Rett syndrome diagnosis has on family planning. If you decide to participate, you will be asked to complete an electronic survey. In particular, you will be asked questions about your child's diagnosis, decisions regarding genetic testing, and your pregnancy history. Participation is voluntary, and you will be able to skip any questions that you prefer not to answer. Those eligible to participate include biological parents of an individual with a clinical and/or genetic diagnosis of Rett syndrome. If a genetic diagnosis has been made, parents of individuals with a MECP2 alteration are eligible. Those not eligible to participate includes parents of individuals with genetic diagnosis of MECP2 duplication, FOXG1 alteration, or CDKL5 alteration.

Survey responses are anonymous. Surveys will only be accessible by myself and the research team. So, please do not fill in your name, your child's name, or other identifying information on the survey. The results of the study may be published or presented at professional meetings, but your identity will not be revealed. Taking part in this study is your decision. Completion of all or part of the survey implies you have given your

consent to participate. However, participation is not required, and you may exit the survey at any time or decide not to answer any question with no penalty.

I will be happy to answer any questions you have about the study. You may contact me at rettsyndromestudy@gmail.com if you have study related questions or problems. If you have any questions about your rights as a research participant, you may contact the Office of Research Compliance at the University of South Carolina at 803-777-7095.

Your time is greatly valued and appreciated. If you would like to participate, please complete the following survey at your convenience. Thank you for your consideration.

Regards,

Erin Huggins

USC Genetic Counseling Student

rettsyndromestudy@gmail.com

1. What is your relationship to the individual with Rett syndrome?

- Biological mother
- Biological father
- Other (please specify)

2. Has anyone else in your family been diagnosed with a neurodevelopmental disorder (such as autism, ADD/ADHD, etc.)?

- Yes
- No
- Unsure

3. If yes, what is their relationship to you and their diagnosis?

Relationship:

Diagnosis:

4. How old was your child when she/he received a clinical diagnosis of Rett syndrome (i.e., how old was your child when you were first told she/he had Rett syndrome)?

5. Who diagnosed your child with Rett syndrome?

- Neurologist
- Geneticist
- Pediatrician
- Developmental Pediatrician
- Unsure
- Other (please specify)

6. Has your child had genetic testing (i.e., through a laboratory) to diagnose Rett syndrome?

- Yes
- No
- Unsure

7. If yes, at what age did your child receive a genetic diagnosis (from a laboratory test)?

8. Who informed you of the genetic diagnosis?

- Neurologist
- Geneticist
- Pediatrician
- Developmental Pediatrician
- Unsure
- Other (please specify)

9. Does your child have an alteration in the MECP2 gene?

- Yes
- No
- Unsure
- No, she/he has a change in a different gene (please specify)

10. During the pregnancy of your child who has Rett syndrome, was any prenatal genetic screening or testing performed? Select all that apply.

- ☐ First trimester screen (Blood test and ultrasound performed between 10 and 13 weeks gestation to screen for chromosomal conditions and some birth defects)
- ☐ Second trimester/quad screen (Blood test performed between 15 and 22 weeks gestation to screen for chromosomal conditions and major physical defects such as spina bifida)
- ☐ Non-Invasive Prenatal Screening (Blood test performed any time after 10 weeks gestation to screen for chromosomal conditions, including those involving sex chromosomes-not available prior to 2011)
- ☐ Chorionic Villus Sampling (CVS) (Procedure in which a catheter is inserted through the mother's cervix to take a small sample of cells from the placenta)
- ☐ Amniocentesis (Procedure in which a needle is inserted through the mother's abdomen to withdraw amniotic fluid from the sac surrounding the fetus)
- ☐ Unsure
- ☐ No

11. What were the reasons for this screening/testing during pregnancy? Select all that apply.

- ☐ Routine screening
- ☐ Advanced Maternal Age
- ☐ Suspected genetic condition
- ☐ Prefer not to respond
- ☐ Not applicable
- ☐ Other (please specify)

12. When your child was diagnosed with Rett syndrome, how many other children did you already have?

- o None
- o Number of children

13. What ages were your other children at the time of your child's diagnosis? Please list each child's age separated by a comma (ex. 5,7).

14. Did you/the child's mother have any more pregnancies after your child was diagnosed with Rett syndrome?

- o Yes
- o No
- o Prefer not to respond

15. Please indicate the number of pregnancies you/the child's mother had after your child was diagnosed with Rett syndrome

16. You will be prompted to answer these questions for up to four subsequent pregnancies. Only answer as many question sets as there were pregnancies after your child was diagnosed with Rett syndrome.

Please answer the questions below for the first pregnancy after your child was diagnosed with Rett syndrome.

- Was prenatal genetic screening for chromosome conditions, such as Down syndrome, performed via a blood draw on this pregnancy (Note: this is not the same as prenatal testing for Rett syndrome)?
- Yes
- No
- Unsure
- Prefer not to respond

17. If yes, please select all that apply.

- ☐ First trimester screen (Blood test and ultrasound performed between 10 and 13 weeks gestation to screen for chromosomal conditions and some birth defects)
- ☐ Second trimester/quad screen (Blood test performed between 15 and 22 weeks gestation to screen for chromosomal conditions and major physical defects such as spina bifida)
- ☐ Non-Invasive Prenatal Screening (Blood test performed any time after 10 weeks gestation to screen for chromosomal conditions, including those involving sex chromosomes-not available prior to 2011)
- ☐ Other (please specify)

18. What were the reasons behind the decision to receive prenatal screening? Please select all that apply.

- ☐ Routine screening
- ☐ Advanced Maternal Age
- ☐ Suspected genetic condition
- ☐ Other (please specify)

19. Was prenatal diagnostic testing for chromosome conditions, such as Down syndrome, performed for this pregnancy?

- Yes: Amniocentesis
- Yes: CVS
- No
- Unsure
- Prefer not to respond

20. If yes, what were the results of this test?

21. Was prenatal diagnostic testing for Rett syndrome performed on this pregnancy (via amniocentesis or CVS)?

- Yes
- No

22. If yes, what were the results of this test?

23. What influenced the decision to receive (or not receive) prenatal diagnostic testing for Rett syndrome? Your answer may be as detailed as you wish. You are not required to answer this question.

24. Did you/the child's mother have more than one pregnancy after your child was diagnosed with Rett syndrome?

- Yes
- No

(Questions 16-24 repeat for up to 4 subsequent pregnancies)

51. Did your child's Rett syndrome diagnosis alter your family planning (i.e., the decision to have more children)?

- Yes
- No
- Unsure

52. Please explain your response in as much detail as you wish.

53. Did either biological parent of your child with Rett syndrome receive genetic testing?

- Yes-biological mother received genetic testing
- Yes-biological father received genetic testing
- Yes-both parents received genetic testing
- No
- Unsure

54. If yes, what were the results of the test?

Mother's Genetic Test Results:

Father's Genetic Test Results:

55. If yes, please describe what influenced the biological parent's decision to have genetic testing.

Mother's Response:

Father's Response:

56. Please explain to the best of your knowledge how Rett syndrome is inherited.

57. Were you told your chances of your future children being born with Rett syndrome?

- Yes
- No
- Unsure

59. Has your family ever received genetic counseling?

- Yes
- No
- Unsure

60. If so, by whom?

- Certified genetic counselor
- Other physician or provider (please specify)

61. Have any of your child's siblings had genetic testing?

- Yes
- No
- Unsure

62. If yes, please fill in the results for each individual tested in the following format:

gender/age/test result

Sibling 1:

Sibling 2:

Sibling 3:

Sibling 4:

63. What is your current age?

64. What was your age at the time your child with Rett syndrome was born?

65. How old is your child with Rett syndrome currently?

66. Is your child with Rett syndrome female or male?

- o Female
- o Male

67. What is your race/ethnicity? Check all that apply.

- ☐ Caucasian
- ☐ African-American
- ☐ Hispanic or Latino
- ☐ Native American or American Indian
- ☐ Asian/Pacific Islander
- ☐ Prefer not to respond
- ☐ Other (please specify)

68. What is your highest degree of education?

- ☐ Less than high school
- ☐ High school diploma or equivalent
- ☐ Some college
- ☐ Associate's degree
- ☐ Bachelor's degree
- ☐ Graduate degree
- ☐ Prefer not to respond

69. What is your employment status?

- Employed
- Unemployed
- Stay-at-home parent
- Student
- Retired
- Prefer not to respond
- Other (please specify)

70. What is your marital status?

- Single
- Married
- Divorced/Separated
- In a relationship but not married
- Prefer not to respond