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# Developmental Regression Analysis and Investigation of Genotype Correlations in Individuals With Classic Rett Syndrome

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# Developmental Regression Analysis and Investigation of Genotype Correlations in Individuals with Classic Rett Syndrome

by

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## DEDICATION

This research is dedicated to girls and women with Rett syndrome and their families. Your stories of perseverance, joy, and hope have inspired me to pursue this project and continue to inspire me as I strive to support families now and in the future. I am especially grateful to the families and patients enrolled in the Rett Syndrome Natural History Study. This project would not have been possible without this database, and I know that you have given your time in order to be included in this data. I hope that this research can help families with current diagnoses, and families who receive a diagnosis in the future. From the bottom of my heart, thank you.

ACKNOWLEDGEMENTS

I would not be where I am today without the support of my family and friends. First, thank you to my parents, who have supported and encouraged me throughout my journey to becoming a genetic counselor. I am also forever grateful for my classmates who have been by my side for the past two years. I couldn't have done this without you and I'm so happy to say that I have made lifelong friends. I also want to thank my friends that were there for the late-night data analysis and last-minute phone calls. This project took a village and I'm glad I had support in so many forms.

I want to say a special thank you to Crystal Hill-Chapman for her assistance with statistics and data analysis. I appreciate you taking time to meet with me and to review my analysis and writing. I learned so much throughout this process and I cannot thank you enough for your guidance.

Finally, I want to thank my committee members and others at the University of South Carolina and Greenwood Genetic Center for their involvement in this project. I appreciate everyone who took time to review my project and offer suggestions and advice. Your input was invaluable and truly helped this project grow into what it is today.

ABSTRACT

Rett syndrome (RTT) is a neurodevelopmental disorder impacting 1 in 10,000 females worldwide, making it one of the most common causes of complex disability in girls. RTT is caused by pathogenic variants in the *MECP2* gene and is characterized by developmental regression, stereotypical hand movements, and an abnormal gait. Despite consistency in the presence of these core features, a wide range of features and varying severity can be observed in girls with RTT. Similarly, the particular type of *MECP2* variant present also differs between patients. Previous studies have assessed correlations between genotype and phenotype in patients with RTT. While past research revealed some correlations, they were limited by small sample size and inconsistencies in data collection methods. This study uses data from the Rett Syndrome Natural History Study (RSNHS), a nationwide study that has been enrolling patients for the past 15 years. The analysis focuses on further characterizing developmental regression and features present in patients with RTT. The study also elucidates genotype-phenotype correlations in RTT, including patients with less common variants in *MECP2.* Analysis of these correlations focuses not only on overall severity but also on details of development and the presence of particular features. Results are consistent with previous studies of genotype-phenotype correlations in RTT and suggest that multiple features of RTT, including overall severity, regression onset, motor skills, and head circumference, differ significantly based on the type of *MECP2* variant present. These correlations could provide important prognostic information for families with a new diagnosis of RTT.

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LIST OF ABBREVIATIONS

CSS ..... Clinical Severity Score

CTD..... C-terminal Domain

CTT .....C-terminal Truncation

HC ..... Head Circumference

ID ..... Intervening Domain

MBD ..... Methyl Binding Domain

MeCP2 .....Methyl CpG Binding Protein 2

NTD ..... N-terminal Domain

RSNHS.....Rett Syndrome Natural History Study

RTT ..... Rett Syndrome

SD ..... Standard Deviation(s)

SEM .....Standard Error of the Mean

SPSS .....Statistical Package for Social Sciences

TRD..... Transcription Repression Domain

# CHAPTER 1

# BACKGROUND

## 1.1 Rett Syndrome

Rett syndrome (RTT, MIM 312750) is a neurodevelopmental disorder that almost exclusively impacts girls. It is considered one of the most common causes of complex disability in females, with an estimated worldwide incidence of 1 in 10,000 females (Rett Syndrome, n.d.; Smeets et al., 2012). RTT was first described in 1966 by Dr. Andreas Rett, an Austrian pediatrician who recognized a pattern of symptoms in a number of his female patients (Rett, 1966). A short time later, Dr. Begnt Hagberg recognized similar symptoms in his patients in Sweden. In 1983, Dr. Hagberg published a report on the condition, naming it Rett's syndrome (Hagberg et al., 1983). This spurred a rapid increase in research into the disorder, and in the 55 years since its first identification, understanding of RTT has progressed rapidly (Percy, 2014; Percy, 2016).

RTT is characterized by developmental abnormalities and regression beginning between 6 and 18 months of age, following a period of seemingly normal development. In addition to regression, affected individuals experience both motor and cognitive symptoms. Patients diagnosed with classic RTT experience four main features: regression of fine motor skills, regression of communication skills, presence of hand stereotypies, and an abnormal or no gait. Other symptoms commonly observed in patients with RTT and atypical RTT are known as minor features. These characteristics include head growth

deceleration, bruxism, abnormal breathing, sleep disruption, and abnormal muscle tone. Head growth deceleration can result in acquired microcephaly, in which head circumference may be within the normal range at birth but later measures more than two standard deviations (SD) below the mean. Despite having a known genetic etiology, RTT remains a clinical diagnosis. The presence of all four main criteria indicates a diagnosis of classic RTT. In contrast, the presence of a subset of major and minor features suggests a diagnosis of atypical, or variant, RTT (Percy et al., 2010).

Classic RTT can be described in four main stages. Stage 1 is the "Early Onset Phase," which starts around 6 to 18 months when patients exhibit subtle differences in development and may begin to show abnormal hand movements and hypotonia. Stage 2 is referred to as the "Rapid Destructive Phase," usually observed between ages 1 and 4 years. During this time, developmental regression, hand stereotypies, and other Rett features become more apparent. Stage 3 is known as the "Plateau" or "Pseudo-Stationary Phase." During this time, patients may see improvements in behavior, although motor problems and seizures can begin to occur more frequently. Some limited research has shown that certain skills that are initially lost during developmental regression can be relearned during this stage (Monteiro et al., 2014). The final stage of Rett syndrome is known as the "Late Motor Deterioration Phase" and is characterized by reduced mobility, scoliosis, muscle weakness, and more. It has been reported that during this phase, girls who previously could walk may lose that ability (D'Souza, n.d.; Dunn, 2001; Hagberg & Witt-Engerström, 1986; Monteiro et al., 2014; *Rett Syndrome Fact Sheet | National Institute of Neurological Disorders and Stroke*, n.d.). It is thought that most patients with RTT survive into at least their 20s, and there are some women that survive to their 40s or 50s. However, survival

rates are not well characterized at these later ages. Research suggests that respiratory infections, asphyxiation, respiratory failure, and seizures are the most common causes of death in patients with RTT (Anderson et al., 2014).

RTT is most often caused by *de novo* variants in the *MECP2* gene, meaning that variants are new in the patient and are not inherited from a parent. The *MECP2* gene is found on the X-chromosome and encodes Methyl CpG Binding Protein 2 (MeCP2) (Amir et al., 1999). With advances in genetic technology, variants in other genes have also been recognized as causing certain features seen in RTT. This includes *CDKL5*, which is associated with epileptic encephalopathy (EEP2, MIM 300672), and *FOXG1*, which has been described as causing the congenital variant of RTT (MIM 613454). Variants in additional genes have also been observed in patients with overlapping features. However, pathogenic variants in *MECP2* account for over 95% of classic RTT cases and 75% of atypical RTT cases (Vidal et al., 2019).

MeCP2 functions as an epigenetic and transcriptional regulator and plays a role in the expression of many genes that are important for proper neuronal function. This protein consists of several major domains, including the N-terminal domain (NTD), methyl binding domain (MBD), intervening domain (ID), transcriptional repression domain (TRD), and C-terminal domains α and β (CTDα/CTDβ) (Claveria-Gimeno et al., 2017). It is reported that MeCP2 has at least 40 binding partners, including transcriptional co-repressors, transcriptional activators, chromatin remodelers, and splicing factors (Lyst & Bird, 2015). Research suggests that RTT-causing variants in *MECP2* interfere with the protein's ability to connect DNA to the NCoR-SMRT complex, which represses transcription when properly recruited (Tillotson et al., 2017). This is supported by the fact

that RTT-associated missense variants are seen primarily in the MBD and TRD, despite benign missense variants being observed throughout other regions of the *MECP2* gene. The C-terminal domain is also a region in which pathogenic variants are known to cluster (Adkins & Georgel, 2011; Halbach et al., 2012; Lyst et al., 2013). While there is still much to understand about the mechanisms underlying MeCP2 function, this research provides essential clues towards disease pathology in RTT. It may also aid in the development of therapeutic approaches in the future.

### 1.2 Advances in Early Diagnosis

More than 99% of RTT cases result from *de novo* variants, many of which are of paternal origin. In rare cases, a pathogenic variant can be passed from a parent with gonadal mosaicism, more commonly from mothers with ovarian mosaicism (Zhang et al., 2012). Since the majority of cases are sporadic, family history rarely gives specific clues towards a genetic diagnosis. Rather than family history, diagnosis relies on the patient's developmental history and features of the disease.

In early accounts of Rett syndrome, development was described as being initially normal, with the first concerns appearing as developmental abnormalities and regression at 6-18 months of age (Amir et al., 1999). However, more recent research suggests that early development might not be as typical as it appears in individuals with RTT. Neul et al. (2014) reported that patients with classic RTT were less likely to develop particular skills as the skills became more advanced. For example, patients were more likely to acquire the ability to crawl but less likely to learn to walk. The same study also found that even among skills acquired, patients with classic RTT experienced delays in skill development compared to the timing expected for typically developing children.

Despite the recognition of early developmental abnormalities, this period still makes early diagnosis challenging. Some improvement in the age of diagnosis has been noted; since diagnostic criteria were first established in 1985, the average age of diagnosis has decreased from about six years old to about 2.5 years old. This may be due to improved developmental screening and increased testing of the *MECP2* gene. However, the age of diagnosis has remained relatively steady since 2001, possibly due to the "wait-and-see" approach (Neul et al., 2014). Some literature calls for more rigorous screening during early development, in hopes of using these recently-understood early abnormalities to further improve the age of diagnosis (Cosentino et al., 2019).

With recent advances in genetic testing, there may be another method to shift the age at which patients with Rett syndrome are diagnosed. In 2017, Natera, Inc. announced the launch of a new Non-Invasive Prenatal Test called "Vistara" (*Natera, Inc. Announces Launch of Vistara Single-Gene Mutation NIPT*, 2017). This test can detect 25 single-gene disorders, including Rett syndrome, and Natera states that the detection rate for pathogenic *MECP2* variants is >78% (*Vistara NIPT Single Gene Test*, 2019). This test's availability may lead to increased screening and detection of Rett syndrome in the prenatal period.

With increased understanding of Rett syndrome and advances in genetic technology, early diagnosis is likely to become standard. This shift has many implications for patients and families, including earlier intervention, earlier enrollment into studies or clinical trials, and reduced psychosocial stress that may result from spending less time in the uncertain period between the onset of symptoms and diagnosis (Palacios-Ceña et al., 2018; Tarquinio et al., 2015). It also allows for genetic counseling to occur at an earlier stage of the disease. Families of patients in this position will likely have questions

regarding what to expect in terms of their child's development, including questions about regression and overall disease severity.

### 1.3 Genetic and Phenotypic Variation

There is significant genetic and phenotypic diversity among patients with pathogenic variants in *MECP2* and a classic RTT diagnosis. Although classic RTT is only diagnosed in the presence of the same four core features, patients with this diagnosis vary greatly regarding when these features arise and how they present. For example, developmental regression shows significant variation among patients, differing in age of onset and specific skills lost. When evaluating the age of onset, past studies have observed regression beginning between the ages of 12 to 19 months (Einspieler & Marschik, 2019). Research also shows that the exact skills lost during regression differ between individuals. Skills lost during fine motor regression include holding a bottle, pincer grasp, and finger feeding. According to one study, these skills were lost in 32.2%, 40.8%, and 40.1% of patients, respectively (Tarquinio et al., 2015). Language regression included loss of babbling, single words with meaning, and phrases. The same study found that, respectively, 37.9%, 58.7%, and 14.5% of patients lost these skills (Tarquinio et al., 2015). This illustrates how significantly regression varies among patients, even though it is a defining feature of classic RTT. Similarly, while all patients diagnosed with classic RTT experience stereotypical hand movements, the types of hand movements observed differ between patients. Individuals may experience hand wringing, mouthing, clapping/tapping, or other repetitive movements (Stallworth et al., 2019).

Head growth deceleration and other features described as supportive diagnostic criteria can also be seen in up to 80% of RTT cases but are not observed in all patients

(Percy et al., 2010). Given this variation, it is well-established that overall severity differs between patients diagnosed with classic RTT. Several scales are available to evaluate severity; each scale rates aspects of phenotype to give patients a numerical severity score. On each scale, a higher score represents greater severity (Archer et al., 2007; Neul et al., 2008).

Similarly, there is also significant diversity in pathogenic *MECP2* variants observed in patients with RTT. RettBASE, a variation database including major genes implicated in classic and atypical RTT, lists 925 unique *MECP2* variants. While this includes both benign and pathogenic variants, 55.8% of these are described as either pathogenic or likely pathogenic. These variants range from relatively common, with up to 420 cases reported, to very rare, with only one reported case (Christodoulou et al., 2003). RTT-causing variants include missense, nonsense, splice-site, and truncating variants. As previously discussed, pathogenic missense variants typically occur in either the MBD or the TRD, while truncating variants and large deletions can occur throughout the gene. C-terminal variants are also known to be common disease-causing variants. In the cohort included in this study, there are more than 43 unique missense variants. This group also includes individuals with C-terminal deletions, splice variants, frameshift variants, large deletions, and early truncations in the *MECP2* gene.

### 1.4 Understanding Genotype-Phenotype Correlations

With such a wide range of features, there is interest in understanding possible ways to better predict RTT phenotype. Some research has focused on determining whether the presence of some features in early development can predict the occurrence of others later in development. For example, recent research suggests that language skills developed early

in life may correlate with motor skills that are present later in development. In a recent publication, Saikusa et al. (2020) reported that walking ability over age ten was associated significantly with the acquisition of meaningful words, microcephaly, and crawling. Patients who acquired meaningful words, patients who learned to crawl, and patients without microcephaly were more likely to walk over age ten. Despite microcephaly being recognized even in early Rett syndrome reports, few studies have investigated this feature in great detail (Hagberg et al., 1983; Huppke et al., 2000). Some literature also suggests that microcephaly may be associated with an increased incidence of epilepsy in patients with Rett syndrome. However, this association is unclear and other research has reported no such correlation (Dolce et al., 2013).

Another approach to predicting RTT features lies in focusing on the underlying genetic cause of the condition. Since RTT is an X-linked disorder, X-chromosome inactivation has been shown to have some correlation with overall severity in girls (Archer et al., 2007). Several studies have also evaluated the effects of pathogenic *MECP2* variants on RTT features and on overall disease severity. These studies looked at specific pathogenic variants in *MECP2*, and some also grouped variants by their location in the MeCP2 protein. Past research has focused on variants in the MBD, TRD, and CTD when grouping variants since these are known "hotspots" for pathogenic variants in patients with RTT (Bebbington et al., 2008; Halbach et al., 2012).

This previous research has revealed general phenotypic patterns that can be observed in the presence of some pathogenic *MECP2* variants. Early research in this area showed significant differences in particular features when comparing missense and truncating variants (Monrós et al., 2001). Further research has revealed more trends, and

specific pathogenic *MECP2* variants have been shown to correlate with overall severity and the age of onset of particular features. In assessing overall severity, reduced severity is observed among patients with the variants p.R133C, p.R294X, and p.R306C. Increased severity has been observed more frequently in patients with p.R168X, p.R270X, and p.R255X variants. Studies evaluating overall severity also reported that particular variants are associated with increased incidence of features such as the delayed onset of regression and hand stereotypies, reduced severity of oro-motor difficulties, preservation of hand skills, and head circumference (Bebbington et al., 2008; Cuddapah et al., 2014; Halbach et al., 2012; Neul et al., 2008). A study focusing on C-terminal deletions found that this variant group is associated with a mild to moderate disease severity. These variants were associated with a more severe phenotype than milder variants like p.R133C but showed reduced severity compared to more severe variants such as p.R270X and large deletions (Bebbington et al., 2010).

Research focusing on genotype-phenotype relationships in RTT was overwhelmingly published before 2012. More recent publications sometimes include brief discussion of genotypic relationships but are confined to the most common pathogenic *MECP2* variants (Neul et al., 2014). While existing data suggests some relationships exist between genotype and phenotype in patients with RTT, variable severity observed in these studies remains a concern, making it difficult to use these correlations to inform prognosis (Halbach et al., 2012). However, since these studies were published, information available in databases has grown, making it possible to study a broader range of existing *MECP2* variants in a larger cohort of patients. A number of these studies also used the InterRett database, which provides a valuable patient data source from an international cohort.

However, this data relies on clinicians and families to input available data, meaning that the database is not population-based and may increase inconsistencies in available data (Bebbington et al., 2008, 2010; *InterRett*, n.d.). This suggests the need for updated investigation of these relationships, particularly through analysis of a large cohort of individuals that has been followed over time.

### 1.5 The Rett Syndrome Natural History Study

The Rett Syndrome Natural History Study (RSNHS) provides a valuable database for investigating developmental regression and genotype-phenotype relationships in patients with classic RTT. The study has been registered with ClinicalTrials.gov: NCT00299312 since March 3, 2006, and NCT02738281 since April 14, 2016. There are 14 sites involved in the study, located throughout the United States. The study includes data from individuals with classic RTT, atypical RTT, *MECP2* Duplication, disorders that involve pathogenic variants in *CDKL5* and *FOXG1*, and patients with other pathogenic *MECP2* variants that do not meet the clinical criteria for RTT. Data were collected during clinic visits that were scheduled every six months until age six and annually after that point. After nearly 15 years of data collection, this study includes a substantial amount of longitudinal data on individuals with classic RTT, including the type of *MECP2* variant, developmental milestones, and additional disease features. It also provides consistency in data collection, and includes regular assessments by trained physicians ("Natural History Study," n.d.).

### 1.6 The Value of Genotype-Phenotype Correlations in Genetic Counseling

With the potential for earlier diagnosis, the value and need for prognostic data in RTT is increasing. This information could be beneficial given that the condition has such

a wide range of features and limited indications in early development. While research suggests that some relationships exist, there is little information regarding possible correlations between genotype and phenotype in Rett syndrome. Existing studies call for future investigation in this area, particularly in a cohort of patients that has been followed over time. The Rett Syndrome Natural History Study serves as a valuable source of this data, following many patients over an extended period. Though the use of this data, this study confirms previously observed relationships and expands on current knowledge of genotype-phenotype relationships in RTT with a focus on developmental regression.

### 1.7 Objectives

1. Assess characteristics of regression in patients with Rett syndrome, including age of onset, skills lost, and skills maintained or relearned.
2. Determine whether correlations exist between specific pathogenic *MECP2* variants and: features of Rett syndrome, regression onset, skills lost during regression, and overall disease severity.

### 1.8 Hypothesis

Specific pathogenic variants in the *MECP2* gene will correlate with the presence of particular features of Rett syndrome and aspects of developmental regression including age of onset, skills learned, skills lost, and skills relearned. Specific *MECP2* variants will also correlate with differences in overall disease severity.

# CHAPTER 2

# DEVELOPMENTAL REGRESSION ANALYSIS AND INVESTIGATION OF GENOTYPE CORRELATIONS IN INDIVIDUALS WITH CLASSIC RETT SYNDROME[1]

[1] Rose, A., Zvejnieks, D., Moore, H., Skinner, S. To be submitted to *Neurology.*

## 2.1 Abstract

Rett syndrome (RTT) is a neurodevelopmental disorder impacting 1 in 10,000 females worldwide, making it one of the most common causes of complex disability in girls. RTT is caused by pathogenic variants in the *MECP2* gene and is characterized by developmental regression, stereotypical hand movements, and an abnormal gait. Although these core features are observed in all patients, a wide range of features and varying severity can be observed in girls with classic RTT. Similarly, the particular type of *MECP2* variant present also differs between patients. Some previous studies have assessed correlations between genotype and phenotype in patients with RTT. While past research revealed some correlations, they were limited by small sample size and inconsistencies in data collection methods. This study uses data from the Rett Syndrome Natural History Study (RSNHS), a nationwide study that has been enrolling patients for the past 15 years. The analysis focuses on characterizing developmental regression and features present in patients with RTT. The study also elucidates genotype-phenotype correlations in RTT, including patients with less common variants in *MECP2.* Analysis of these correlations focuses not only on overall severity but also on details of development and the presence of particular features. Results are consistent with previous analyses of genotype-phenotype correlations in RTT. They suggest that multiple features of RTT, including overall severity, regression onset, motor skills, and head circumference, differ significantly based on the type of *MECP2* variant present. These correlations could provide important prognostic information for families with a new diagnosis of RTT.

## 2.2 Introduction

Rett syndrome (RTT, MIM 312750) is a neurodevelopmental disorder that almost exclusively impacts girls. It is considered one of the most common causes of complex disability in females, with an estimated worldwide incidence of 1 in 10,000 females (*Rett Syndrome*, n.d.; Smeets et al., 2012). This disorder is characterized by developmental abnormalities and regression beginning between 6 and 18 months of age, following a period of seemingly normal development. In addition to regression, affected individuals experience both motor and cognitive symptoms. Patients diagnosed with classic RTT experience four main features: regression of fine motor skills, regression of communication skills, presence of hand stereotypies, and an abnormal or no gait. Other features often include head growth deceleration, bruxism, periodic breathing, sleep disruption, and abnormal muscle tone. Despite having a known genetic etiology, RTT remains a clinical diagnosis. The presence of all four main criteria indicates a diagnosis of classic RTT. In contrast, the presence of a subset of major and minor features suggests a diagnosis of atypical, or variant, RTT (Percy et al., 2010).

RTT is often described in 4 main stages. Stage 1 is the "Early Onset Phase," which starts around 6 to 18 months when patients exhibit subtle differences in development and may begin to show abnormal hand movements and hypotonia. Stage 2 is referred to as the "Rapid Destructive Phase," usually observed between ages 1 and 4. During this time, developmental regression, hand stereotypies, and other Rett features become more apparent. Stage 3 is known as the "Plateau" or "Pseudo-Stationary Phase." During this time, patients may see improvements in behavior, although motor problems and seizures can begin to occur more frequently. The final stage is known as the "Late Motor

Deterioration Phase," and is characterized by reduced mobility, scoliosis, muscle weakness, and more. It has been reported that during this phase, girls who previously could walk may lose that ability (D'Souza, n.d.; *Rett Syndrome Fact Sheet | National Institute of Neurological Disorders and Stroke*, n.d.).

RTT is most often caused by *de novo* variants in the *MECP2* gene, which is found on the X-chromosome and encodes Methyl CpG Binding Protein 2 (MeCP2) (Amir et al., 1999). Variants in additional genes have also been observed in patients with overlapping features. However, pathogenic variants in *MECP2* account for over 95% of classic RTT cases and 75% of atypical RTT cases (Vidal et al., 2019). The MeCP2 protein functions as an epigenetic and transcriptional regulator and plays a role in the expression of several genes that are important for proper neuronal function. This protein consists of several major domains, including the N-terminal domain (NTD), methyl binding domain (MBD), intervening domain (ID), transcriptional repression domain (TRD), and C-terminal domains α and β (CTDα/CTDβ) (Claveria-Gimeno et al., 2017). Research suggests that RTT-causing variants in *MECP2* interfere with the protein's ability to connect DNA to the NCoR-SMRT complex, which represses transcription when properly recruited (Tillotson et al., 2017). This is supported by the fact that RTT-associated missense variants are seen primarily in the MBD and TRD, despite benign missense variants being observed throughout other regions of the *MECP2* gene. The C-terminal domain is also a region in which pathogenic variants are known to cluster (Adkins & Georgel, 2011; Halbach et al., 2012; Lyst et al., 2013).

There is significant genetic and phenotypic diversity among patients with pathogenic variants in *MECP2* and a classic RTT diagnosis. Although classic RTT is only

diagnosed in the presence of the same four core features, patients with this diagnosis vary greatly regarding when these features arise and how they present. For example, developmental regression shows significant variation among patients, differing in age of onset and specific skills lost. Patients also differ in terms of which features may be present in addition to the core features of the disease. Head growth deceleration, bruxism, and other features described as supportive diagnostic criteria can be seen in up to 80% of RTT cases, but are not observed in all patients. Given this variation, it is well-established that overall severity differs between patients diagnosed with classic RTT. Several scales are available to evaluate severity. Each scale rates aspects of phenotype to give patients a numerical severity score. On each scale, a higher score represents greater severity (Archer et al., 2007; Neul et al., 2008; Percy et al., 2010).

Similarly, there is also significant diversity in pathogenic *MECP2* variants observed in patients with RTT. RettBASE, a variation database including major genes implicated in classic and atypical RTT, lists 925 unique *MECP2* variants. While this includes both benign and pathogenic variants, 55.8% of these are described as either pathogenic or likely pathogenic. These variants range from relatively common, with up to 420 cases reported, to very rare, with only one reported case (Christodoulou et al., 2003). RTT-causing variants include missense, nonsense, splice-site, and truncating variants. In this study cohort, there are more than 43 unique missense variants (Figure 2.1). This group also includes individuals with C-terminal deletions, splice variants, frameshift variants, large deletions, and early truncations in the *MECP2* gene.

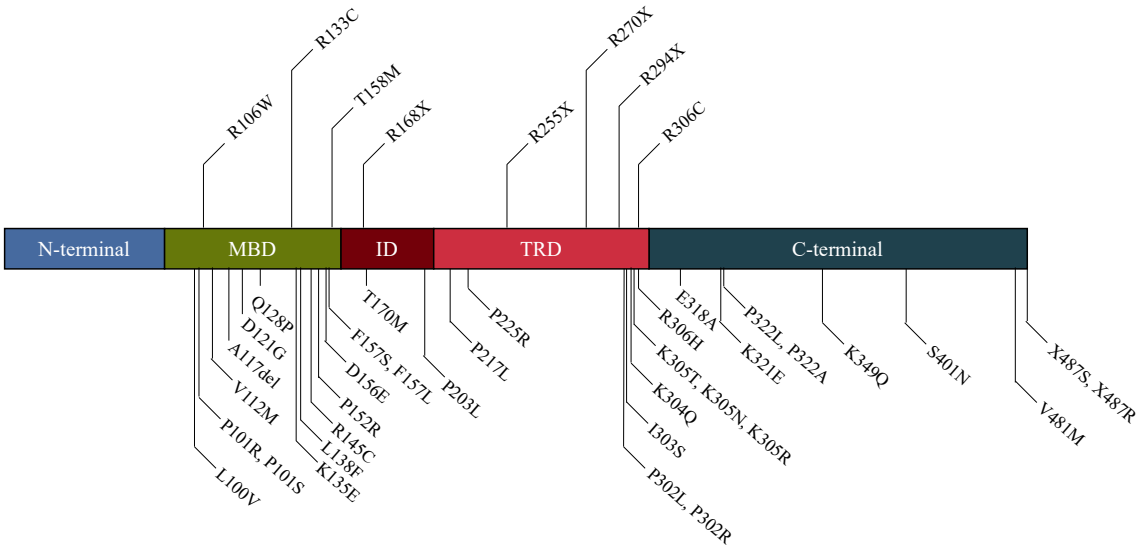



**Figure 2.1. MeCP2 protein and amino acid variants observed in the study cohort.** Functional domains of the MeCP2 protein are pictured. MBD = methyl binding domain, ID = intervening domain, TRD = transcriptional repression domain.

With such a wide range of features, there is interest in understanding possible causes of phenotypic variation in RTT. Since RTT is an X-linked disorder, X-chromosome inactivation has been shown to have some correlation with overall severity in girls (Archer et al., 2007). Several studies have also evaluated the effects of pathogenic *MECP2* variants on RTT features and overall disease severity (Bebbington et al., 2008; Halbach et al., 2012). Specific pathogenic *MECP2* variants have been shown to correlate with overall severity and the age of onset of particular features. In assessing overall severity, reduced severity is observed among patients with the variants p.R133C, p.R294X, and p.R306C. Increased severity has been observed more frequently in patients with p.R168X, p.R270X, and p.R255X variants. Studies evaluating overall severity also reported that particular variants are associated with increased incidence of features such as delayed onset of

regression and hand stereotypies, reduced severity of oro-motor difficulties, preservation of hand skills, and head circumference (Bebbington et al., 2008; Halbach et al., 2012; Neul et al., 2008).

Research focusing on genotype-phenotype relationships in RTT was overwhelmingly published before 2012. More recent publications sometimes include a brief discussion of genotypic relationships but are confined to the most common pathogenic *MECP2* variants (Neul et al., 2014). While existing data suggests some relationships exist between genotype and phenotype in patients with RTT, variable severity observed in these studies remains a concern, making it difficult to use these correlations to inform prognosis (Halbach et al., 2012).

With the potential for earlier diagnosis, the value and need for prognostic data in RTT is increasing. This information could be beneficial given that the condition has such a wide range of features and limited indications in early development. While research suggests that some relationships exist, there is little information regarding possible correlations between genotype and phenotype in Rett syndrome. Existing studies call for future investigation in this area, particularly in a cohort of patients that has been followed over time. The Rett Syndrome Natural History Study (RSNHS) serves as a valuable source of this data, following many patients over an extended period. Through the use of this data, this study confirms previously observed relationships and expands on current knowledge of genotype-phenotype relationships in RTT with a focus on developmental regression.

## 2.3 Methods

### *2.3.1 Participants*

There were 1,591 participants enrolled in the RSNHS when data was obtained in June 2020. Patients included in the analysis are female, have a diagnosis of classic Rett Syndrome, and have a pathogenic variant in *MECP2*. Males, patients with a diagnosis other than classic Rett Syndrome, patients with a *MECP2* duplication, and patients with missing data were excluded from analysis (Figure 2.2).

### *2.3.2 Research Methods*

This study is a retrospective review of data collected as part of the RSNHS. Relevant data were collected from available records, including detailed information about onset and presence of disease features and skills, head circumference, overall disease severity, specific *MECP2* variant, and relevant demographic information (Appendix A). Responses and measurements were collected at multiple appointments throughout patients' enrollment in the RSNHS.

For analysis, patients were divided into groups based on *MECP2* variant. Common groups used in the RSNHS included eight common missense and nonsense variants, C-terminal truncations, early truncations, large deletions, pathogenic exon one variants, and pathogenic splice variants. A subset of the study participants had rare pathogenic missense variants and was not included in the groups listed above. These patients were grouped into three categories based on the location of the pathogenic missense variant in the MeCP2 protein: C-terminal, MBD, and TRD (Figure 2.3).

Some participants were initially grouped in study forms as having rare variants, but were regrouped based on the type of variant reported. This included several frameshift

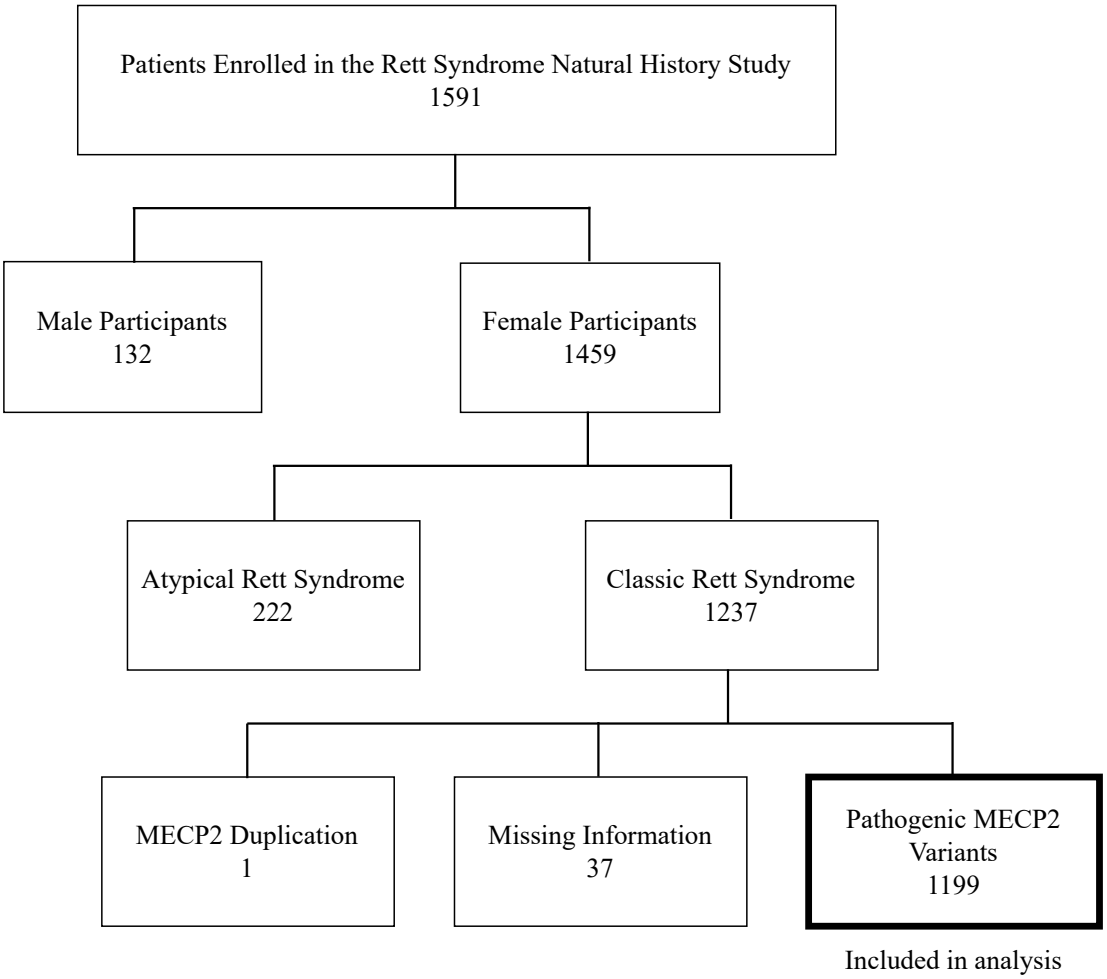
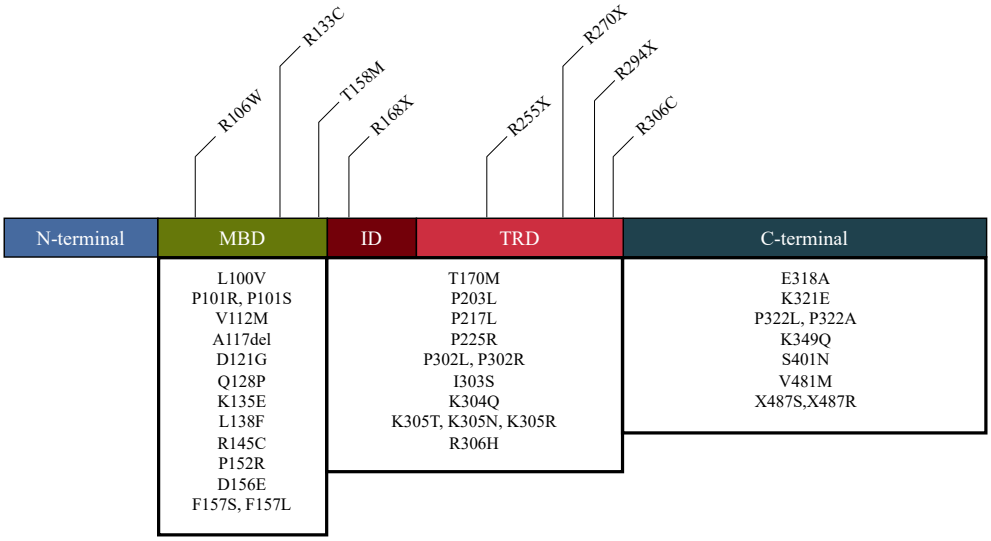



**Figure 2.2. Cohort distribution and exclusion criteria.**

**A.**




**B.**

| Variant Grouping | N |
|---|---|
| R106W | 44 |
| R133C | 76 |
| T158M | 129 |
| R168X | 130 |
| R255X | 116 |
| R270X | 76 |
| R294X | 75 |
| R306C | 97 |
| Methyl Binding Domain Pathogenic Missense Variants (MBD) | 43 |
| Transcription Repression Domain Pathogenic Missense Variants (TRD) | 17 |
| C-Terminal Pathogenic Missense Variants (C-Terminal) | 17 |
| C-Terminal Truncations (CTT) | 129 |
| Early Truncations (EarlyTrunc) | 107 |
| Large Deletions (LargeDel) | 111 |
| Pathogenic Exon 1 Variants (Exon1) | 16 |
| Pathogenic Splice Variants (Splice) | 16 |
| **TOTAL** | **1199** |

**Figure 2.3. Categories of pathogenic variants used for analysis.**

variants and one nonsense variant included in the early truncation group, one variant (p.Ala2Val) included in the group of pathogenic exon 1 variants, and one 3' UTR variant that was grouped with pathogenic splice variants.

Data was collected and organized using Microsoft Excel and exported to Statistical Package for Social Sciences (SPSS) version 25 for quantitative analysis. Microsoft Excel was used for descriptive statistics. Figures and tables were constructed using Microsoft Office Products and SPSS.

### *2.3.3 Visit Dates*

The number of visits for each patient was determined using entries in both the Developmental History Log and the Clinical Severity Score Log. If the same patient had a different number of entries between these two forms, the larger number was used to determine the patient's number of visits. The time between appointments varied, but visits were typically scheduled either every six months or once a year.

The number of years that patients were seen as part of the RSNHS was calculated using the first recorded entry and the last recorded entry between the Developmental History and Clinical Severity Score Logs. The age at which patients were last seen was also calculated using each participant's date of birth and the last recorded entry in the logs listed above.

### *2.3.4 Clinical Severity*

Clinical severity score (CSS) is a quantitative scale used to determine the severity of overall clinical presentation in individuals with Rett syndrome. CSS was calculated using clinical observations made by experienced physicians involved in the RSNHS. The CSS is calculated using clinical features observed during an appointment and reported by

parents. Each feature is scored on a scale of 0-4 or 0-5, for a possible total of 53 points. A higher score corresponds with increased disease severity. For patients with multiple reported CSS scores, the mean CSS was calculated and used for analysis.

### *2.3.5 Rett Features*

The protocol for the RSNHS was updated in 2016, and this update included changes to forms filled out during appointments and how data was collected for the study. Of data analyzed in this study, only changes to the Rett Features Log impacted how data was recorded and stored. To combine data for patients, responses to questions regarding the same information between the two forms were combined, and only features included in both questionnaires were analyzed. For patients who were seen multiple times throughout the study, responses were combined to represent whether a given skill was present at any visit. If a skill was present at any visit, the skill was recorded as present in the patient's history regardless of the frequency at which the skill was observed. If a skill was never present, it was recorded as absent.

### *2.3.6 Developmental History*

The presence of developmental skills and details of regression were categorized using the Developmental History Log. Responses were coded to represent skills that each participant never learned, learned+retained, learned+lost, and learned+lost+relearned. These categories were used for analysis. If a skill was marked as "lost" but had not been marked "gained", the patient was included in the "learned+lost" group for this skill. Likewise, if a skill was marked as "relearned" but had not been marked "lost", the patient was included in the "learned+lost+relearned" group for this skill. Ages that skills were learned, lost, or relearned were also included for some participants. In some cases,

participants were not recorded as having learned a skill, but had an age at which they learned the skill. In these cases, participants were recorded as having learned the given skill. This was also true for recording skills as lost or relearned.

For analysis of skills assessed as part of developmental history, only patients who were seen as part of the RSNHS at age 12 or older were included in the analysis. This was done to help increase the chance that lost skills or relearned skills were not missed due to lack of follow-up. A total of 614 study participants were included in these analyses.

Ages at which skills were learned, lost, and relearned were evaluated for ten skills: sat without support, cruised furniture while holding someone, walked independently, raking grasp, pincer grasp, finger fed, respond to familiar words, identified body parts, babbled, and words with meaning. These skills were chosen based on their importance in the clinical setting. Analysis of ages also included only participants seen at or after age 12. The additional analysis assessed skills lost after the age of 18. This analysis included only patients who were seen as part of the RSNHS after age 18 (n=369).

Age of onset of regression was obtained using the clinical severity score form since this is one of the features used to score severity. Age of onset was recorded in one of six groups: <6 months, 6-12 months, 12-18 months, 18-30 months, >30 months, or unknown. Patients with an unknown age of onset of regression were excluded from the analysis.

### *2.3.7 Head Circumference*

Head circumference (HC) was recorded in centimeters for patients at various ages. For this study, the HC z-score was calculated using estimated mean and SD values from the Nellhaus curve. The Nellhaus curve was selected since it is a widely-accepted curve used by neurologists to determine the HC percentile for patients up to age 18. Mean and

SD values were estimated from the curve between ages 1 to 18 at 0.5-year intervals. Ages for each HC measurement were rounded to the nearest 0.5 years, and estimated mean and SD values at that age were used to calculate a z-score for each HC measurement. Z-scores for measurements taken above age 18 were predicted using estimated mean and SD at age 18. The accuracy of this calculation was confirmed using a random sample of 18 measurements which were plotted using software from Epic Systems. Calculated z-score values were within 0.5 SD of the mean when compared to values determined using Epic, with a mean difference in z-score of ±0.174 (SD 0.148, Range 0.491).

Calculated z-scores were also used to characterize the frequency of acquired microcephaly among study participants. If any HC measurement taken during a participant's time in the RSNHS was greater than two SD below the mean for the patient's age at the time of the measurement, the patient was recorded as having microcephaly. If no HC measurements fell greater than two SD below the mean for each age that a measurement was recorded, a patient was recorded as not having microcephaly.

The analysis only included patients who were seen as part of the RSNHS at age 12 or older for analysis of correlations between microcephaly and skills lost during regression. Patients were included in the analysis if they had either learned+retained or learned+lost skills. Analysis was performed for the same ten skills for which ages were analyzed in Developmental History.

### *2.3.8 Statistical Analysis*

Descriptive statistics (frequencies and percentages) were calculated for categorical data. Binary logistic regression was used for variables with a binary output, including head circumference and data from the Rett Features Log. Differences in developmental skills

gained and/or lost, as reported in the Developmental History Log, were assessed using Pearson's chi-square test for independence. Differences in age of onset of regression by *MECP2* variant were also assessed using Pearson's chi-square test for independence. Differences in head circumference and average clinical severity by *MECP2* variant were assessed using linear regression.

### *2.3.9 Human Studies Approval*

The University of South Carolina's Institutional Review Board approved this study in June 2020. The RSNHS (ClinicalTrials.gov: NCT00299312, NCT02738281) has been enrolling patients since 2006. There are 14 participating sites: University of Alabama at Birmingham, UCSF Oakland Benioff Children's Hospital, University of California San Diego, University of Colorado Denver, Rush University Medical Center, Children's Hospital Boston, Gillete Children's Specialty Healthcare, Washington University School of Medicine & St. Louis Children's Hospital, Cincinnati Children's Hospital Medical Center, Cleveland Clinic, Children's Hospital of Philadelphia, Greenwood Genetic Center, Vanderbilt University, and Baylor College of Medicine. Every clinic obtained and maintained IRB approval throughout the study. Parental consent for study conduct and publication of results was obtained before entry into the study.

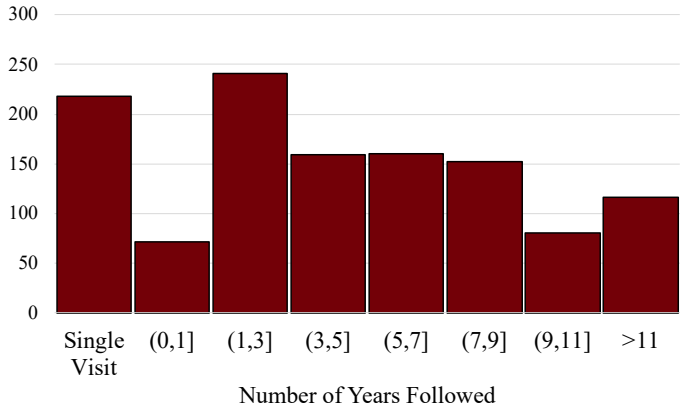
## 2.4 Results

### *2.4.1 Demographic Information*

The average age of participants at the time of the study was 19.64 years, and most (910/1,199; 75.90%) were followed as part of the RSNHS for a year or more. Participants were seen an average of 5.46 times (SD 3.81), and the number of visits ranged from 1 to

16. Patients who were seen more than once were followed for an average of 5.67 years (SD 3.67), ranging from less than a year to over 14 years (Figure 2.4).

**A.**




**B.**

| Number of Years Followed | N | % |
|---|---|---|
| Single Visit | 218 | 18.18% |
| 0-1 years | 72 | 6.01% |
| 1-3 years | 241 | 20.1% |
| 3-5 years | 159 | 13.26% |
| 5-7 years | 160 | 13.34% |
| 7-9 years | 152 | 12.68% |
| 9-11 years | 81 | 6.76% |
| 11+ years | 116 | 9.67% |
| **TOTAL** | **1199** | **100%** |

**Figure 2.4. Number of years followed in the RSNHS.**

Of participants with available information regarding racial background, a majority (73.14%) were Non-Hispanic White. Racial demographic information is presented in Table 2.1.

**Table 2.1. Demographic Information.**

| Race | N | % |
|---|---|---|
| Non-Hispanic White | 874 | 73.14% |
| Hispanic or Latino | 173 | 14.48% |
| Two or more races | 55 | 4.60% |
| Black | 49 | 4.10% |
| Asian | 40 | 3.35% |
| Native American/Alaskan Native | 3 | 0.25% |
| Native Hawaiian/Pacific Islander | 1 | 0.08% |
| Unknown | 4 | -- |
| **TOTAL** | **1199** | **100%** |

### *2.4.2 Clinical Severity*

Differences in average clinical severity between groups were assessed using a linear regression model. *MECP2* variant group predicted average clinical severity, $F(15)=13.619$, $p<0.001$, and accounted for 13.7% of the variation in clinical severity (Adjusted $R^2=0.137$). Average clinical severity scores and standard error of the mean (SEM) for each variant group are reported in Figure 2.5.

A.
Average CSS
0
10
20
30
40
50
Exon 1
R133C
R306C
TRD
R294X
C-Terminal
CTT
MBD
T158M
R106W
Early Trunc
Splice
Large Del
R255X
R270X
R268X

**B.**

| Variant Grouping | N | Average CSS (SEM) |
|---|---|---|
| Exon 1 | 16 | 17.82 (1.43) |
| R133C | 75 | 18.88 (0.71) |
| R306C | 97 | 19.14 (0.63) |
| TRD | 17 | 19.55 (1.89) |
| R294X | 75 | 20.08 (0.68) |
| C-Terminal | 17 | 20.29 (1.86) |
| CTT | 129 | 20.78 (0.57) |
| MBD | 43 | 22.92 (1.02) |
| T158M | 127 | 23.94 (0.59) |
| R106W | 44 | 24.02 (0.87) |
| Early Truncation | 107 | 24.58 (0.63) |
| Splice Variants | 16 | 25.40 (1.82) |
| Large Deletions | 111 | 25.59 (0.73) |
| R255X | 116 | 25.90 (0.62) |
| R270X | 75 | 26.11 (0.83) |
| R168X | 130 | 26.26 (0.59) |
| **TOTAL** | **1,195** | |

**Figure 2.5. Average clinical severity score by *MECP2* variant.**

### *2.4.3 Rett Features*

The most common feature observed among all participants was hand stereotypies, followed by constipation, drooling, bruxism, breath-holding, and cold/hot hands. Each of these features was observed in over 80% of participants. The least commonly observed features included gallbladder dysfunction, spitting, and hyperactivity, which were observed in less than 30% of participants. Frequencies of each Rett Feature are presented in Figure 2.6 and Supplemental Table B.1.

Logistic regression models were used to assess whether *MECP2* variant predicted the frequency at which features were present in participants. The logistic regression model was statistically significant for the following skills: hyperventilation, aerophagia, spitting, bruxism, chewing problems, gastroesophageal reflux, constipation, sleep difficulty, waking at night, self-abusive behavior, and hyperactivity. A summary of logistic regression results for each Rett feature is presented in Table 2.2. Observed frequencies of selected skills by *MECP2* variant are reported in Supplemental Figure B.1.

### *2.4.4 Developmental History*

Frequencies at which skills were never learned, learned, learned+lost, and learned+lost+relearned among all study participants are presented in Figure 2.7 and Supplemental Tables B.2-B.4. A chi-square test of independence was used to assess whether observed frequencies of patients that never learned, learned, learned+lost, and learned+lost+relearned skills differed significantly from expected values based on developmental skill. All expected cell frequencies were greater than five. There was a statistically significant association between developmental skill and frequencies learned, lost, and relearned, $X^2(150) = 25004.25$, $p<0.001$.

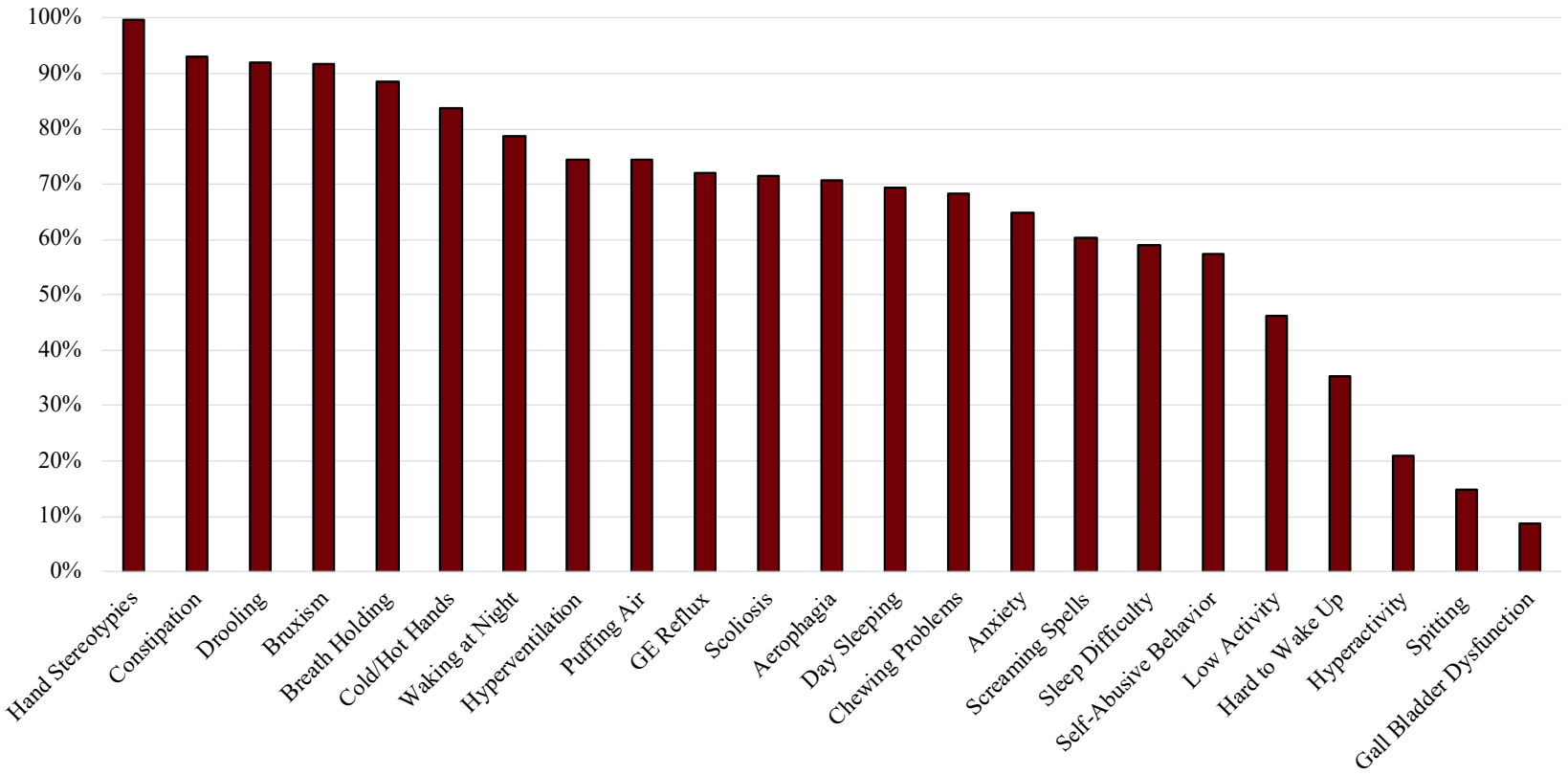


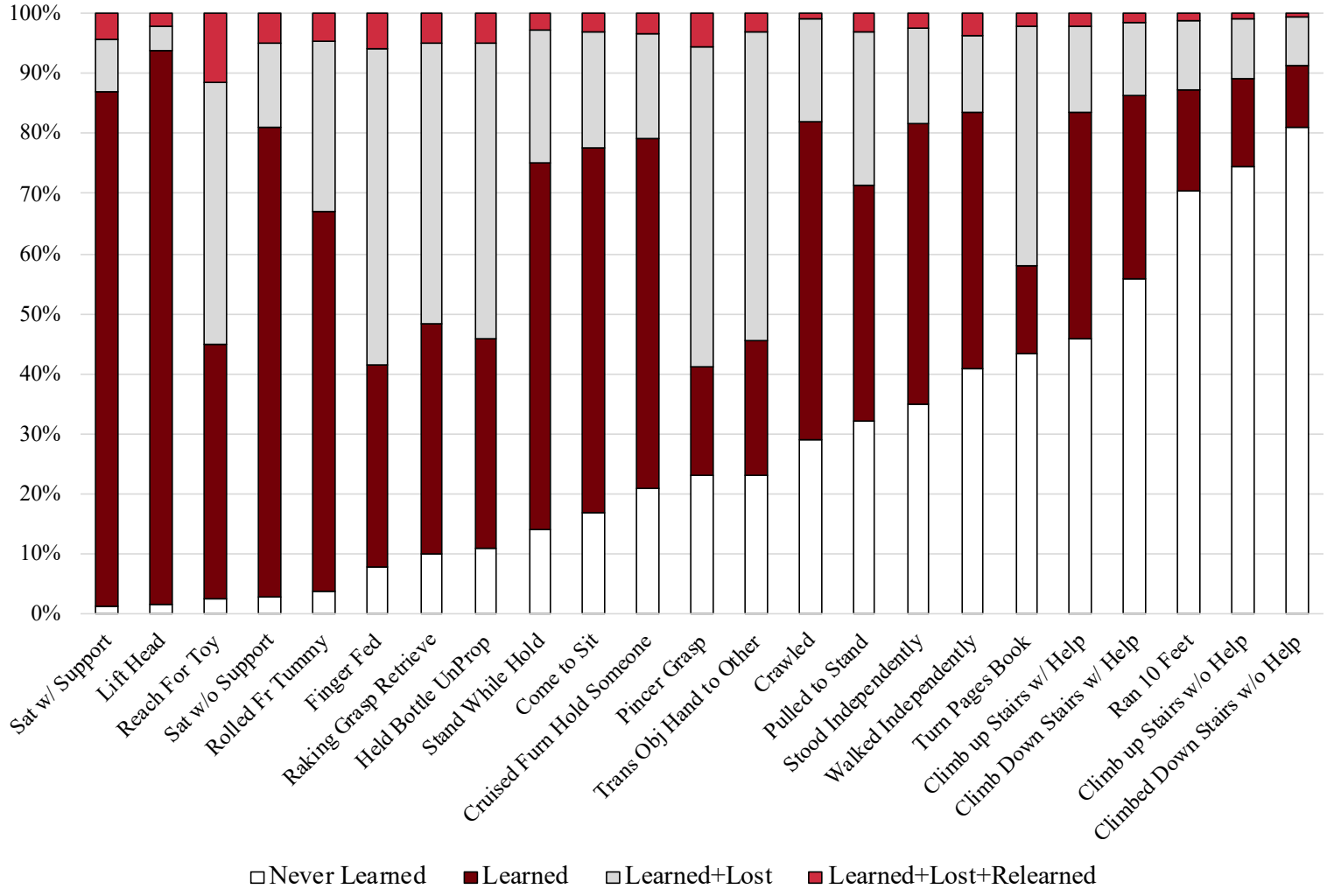

**Figure 2.6. Frequencies of Rett features.**

**Table 2.2. Logistic regression results – Rett features.** Analyses with statistical significance ($p<0.05$) are reported in bold font.

| Rett Feature | X2 | df | Cox & Snell $R^2$ | p-value |
|---|---|---|---|---|
| Hand Stereotypies | 10.911 | 15 | 0.009 | 0.759 |
| **Hyperventilation** | **27.870** | **15** | **0.023** | **0.022** |
| Breath Holding | 18.866 | 15 | 0.016 | 0.220 |
| Cold/Hot Hands | 17.436 | 15 | 0.014 | 0.293 |
| Drooling | 16.640 | 15 | 0.014 | 0.341 |
| **Aerophagia** | **25.878** | **15** | **0.021** | **0.039** |
| Puffing Air | 21.316 | 15 | 0.018 | 0.127 |
| **Spitting** | **26.717** | **15** | **0.042** | **0.031** |
| **Bruxism** | **35.954** | **15** | **0.030** | **0.002** |
| **Chewing Problems** | **43.613** | **15** | **0.068** | **<0.001** |
| **GE Reflux** | **31.916** | **15** | **0.026** | **0.007** |
| **Constipation** | **36.772** | **15** | **0.030** | **0.001** |

| | | | | |
|---|---|---|---|---|
| Gall Bladder Dysfunction | 23.645 | 15 | 0.020 | 0.071 |
| **Sleep Difficulty** | **34.898** | **15** | **0.029** | **0.003** |
| **Waking at Night** | **25.470** | **15** | **0.021** | **0.044** |
| Hard to Wake Up | 20.404 | 15 | 0.017 | 0.157 |
| Day Sleeping | 22.021 | 15 | 0.018 | 0.107 |
| Screaming Spells | 19.522 | 15 | 0.031 | 0.191 |
| **Self-Abusive Behavior** | **28.573** | **15** | **0.024** | **0.018** |
| **Hyperactivity** | **38.449** | **15** | **0.060** | **0.001** |
| Low Activity | 15.240 | 15 | 0.024 | 0.434 |
| Anxiety | 24.214 | 15 | 0.038 | 0.062 |
| Scoliosis | 16.438 | 15 | 0.026 | 0.354 |

**A.**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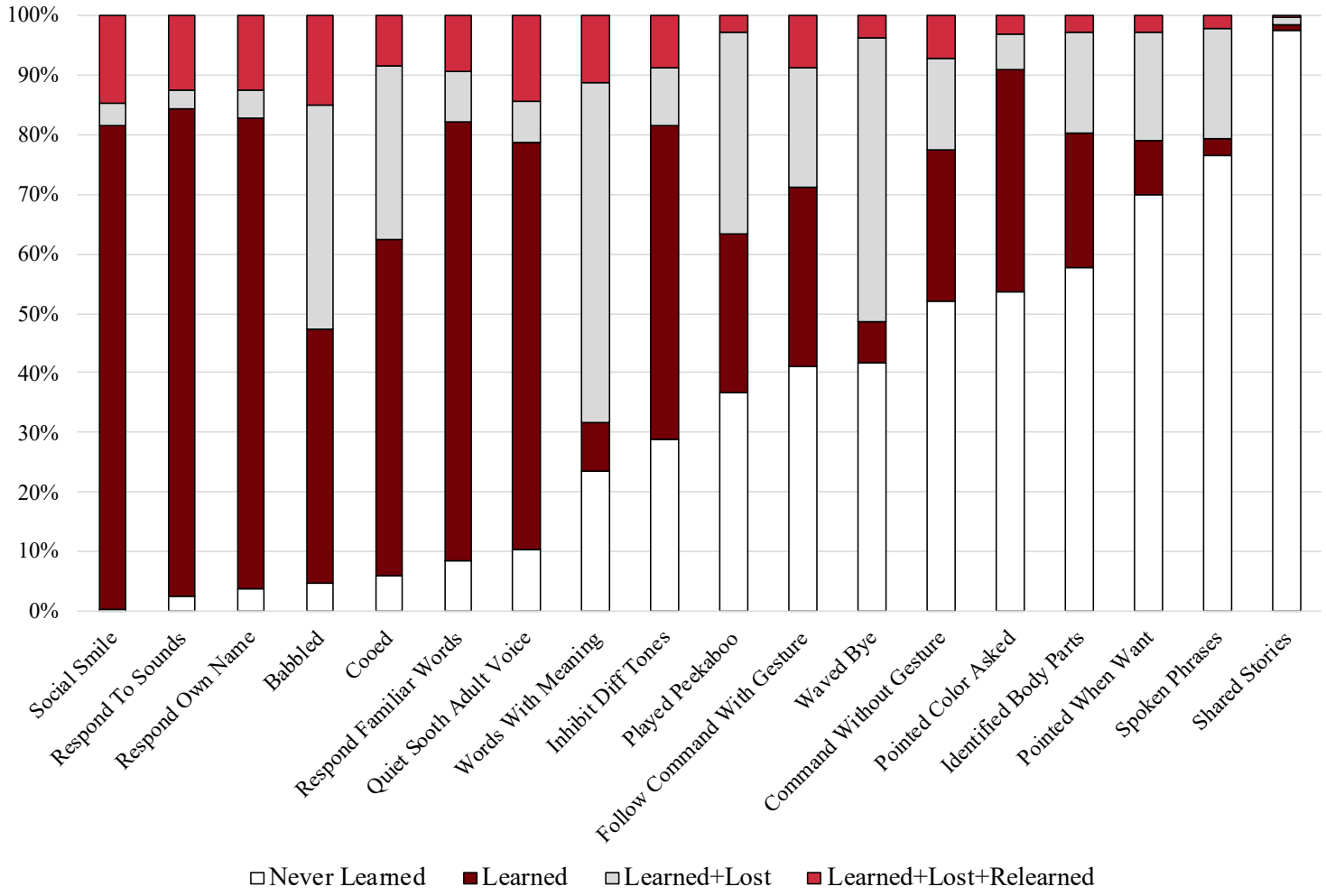
Sat w/ Support
Lift Head
Reach For Toy
Sat w/o Support
Rolled Fr Tummy
Finger Fed
Raking Grasp Retrieve
Held Bottle UnProp
Stand While Hold
Come to Sit
Cruised Furn Hold Someone
Pincer Grasp
Trans Obj Hand to Other
Crawled
Pulled to Stand
Stood Independently
Walked Independently
Turn Pages Book
Climb up Stairs w/ Help
Climb Down Stairs w/ Help
Ran 10 Feet
Climb up Stairs w/o Help
Climbed Down Stairs w/o Help
Never Learned
Learned
Learned+Lost
Learned+Lost+Relearned

**B.**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Cooed
Respond Familiar Words
Quiet Sooth Adult Voice
Words With Meaning
Inhibit Diff Tones
Played Peekaboo
Follow Command With Gesture
Waved Bye
Command Without Gesture
Pointed Color Asked
Identified Body Parts
Pointed When Want
Spoken Phrases
Shared Stories
Never Learned
Learned
Learned+Lost
Learned+Lost+Relearned

**C.**

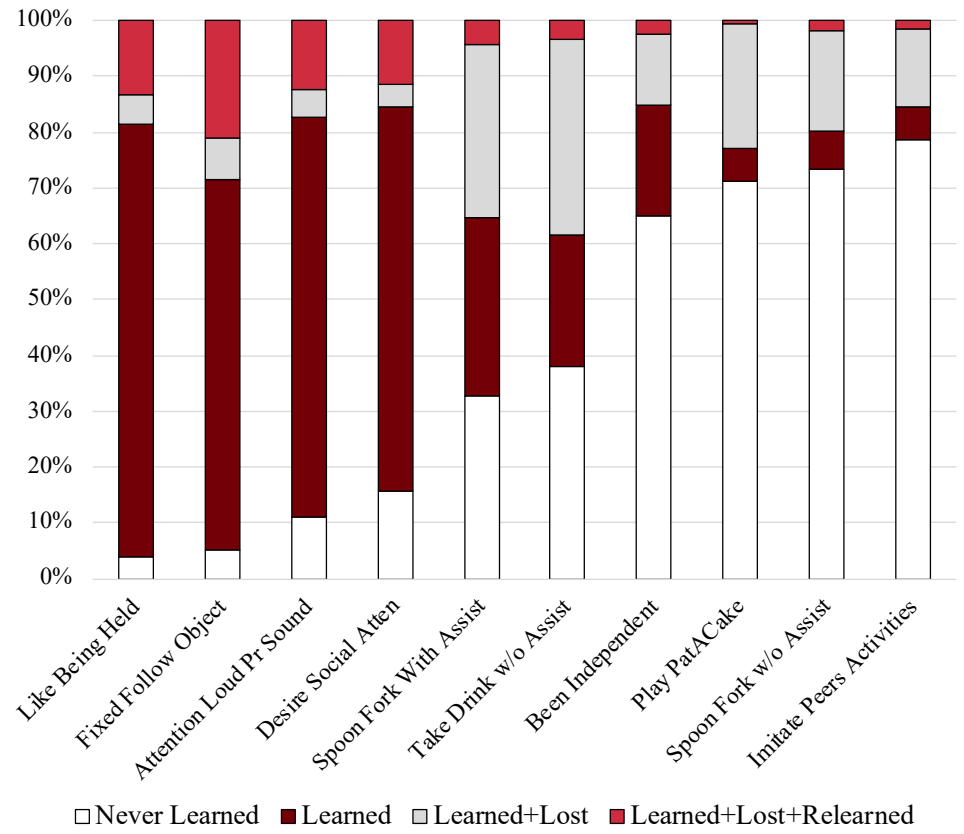



**Figure 2.7. Frequencies of developmental skills learned, lost, and relearned.**
A) Motor skills. B) Language skills. C) Personal-social/adaptive skills

Chi-square tests of independence were used to assess whether observed frequencies of patients that never learned, learned, learned+lost, and learned+lost+relearned individual skills differed significantly from expected values based on *MECP2* variant. Not all expected cell frequencies were greater than five. Observed values differed significantly from expected values for 34 of 51 skills. Results of all chi-square analyses are summarized in Tables 2.3 through 2.7. Observed frequencies of skills learned by *MECP2* variant are reported for selected skills in Supplemental Figures B.2 through B.4. These figures show frequencies of patients who learned a skill compared to patients that never learned a skill. For presentation of this data, patients were included in calculation of these frequencies even if some later lost, or lost and relearned the skill. For selected graphs reporting frequencies of skills learned, learned+lost, and learned+lost+relearned, see Supplemental Figure B.5.

The difference in regression onset by *MECP2* variant was also assessed with a chi-square test of independence. Thirty-five percent of cells had an expected count of less than five. There was a statistically significant association between variant group and age of onset of regression, $X^2(60) = 108.34$, $p<0.001$. Graphs showing the age of onset of regression by *MECP2* variant are shown in Figure 2.8. Observed values are reported in Supplemental Table B.5.

Ages at which developmental skills were learned, lost, or relearned were assessed for ten skills: sat without support, cruised furniture while holding someone, walked independently, raking grasp, pincer grasp, finger fed, respond to familiar words, identified body parts, babbled, and words with meaning. Average ages learned and lost are shown in Figure 2.9. Average ages and SEM values are reported in Supplemental Table B.6.

**Table 2.3. Chi-square analysis of gross motor skills.** Analyses with statistical significance ($p<0.05$) are reported in bold font.

| Developmental Skill | Chi-Square Value | df | Asymptotic Significance |
|---|---|---|---|
| **Lift Head** | **61.771** | **45** | **0.049** |
| **Rolled From Tummy** | **83.835** | **45** | **<0.001** |
| Sat with Support | 43.335 | 45 | 0.543 |
| **Sat without Support** | **68.960** | **45** | **0.012** |
| **Come to Sit** | **93.317** | **45** | **<0.001** |
| **Crawled** | **86.320** | **45** | **<0.001** |
| **Stand while Hold** | **67.582** | **45** | **0.016** |
| **Pulled to Stand** | **144.667** | **45** | **<0.001** |
| **Cruised Furniture Holding Someone** | **106.701** | **45** | **<0.001** |
| **Stood Independently** | **111.825** | **45** | **<0.001** |
| **Walked Independently** | **151.955** | **45** | **<0.001** |
| **Ran 10 Feet** | **73.006** | **45** | **0.005** |
| **Climb up Stairs with Help** | **80.980** | **45** | **0.001** |
| **Climb up Stairs without Help** | **103.168** | **45** | **<0.001** |
| **Climb down Stairs with Help** | **90.389** | **45** | **<0.001** |
| **Climb down stairs without Help** | **104.480** | **45** | **<0.001** |

**Table 2.4. Chi-square analysis of fine motor skills.** Analyses with statistical significance ($p<0.05$) are reported in bold font.

| Developmental Skill | Chi-Square Value | df | Asymptotic Significance |
|---|---|---|---|
| **Held Bottle Unpropped** | **109.859** | **45** | **<0.001** |
| **Reach for Toy** | **101.731** | **45** | **<0.001** |
| Raking Grasp | 60.804 | 45 | 0.058 |
| **Transfer Object One Hand to Other** | **96.875** | **45** | **<0.001** |
| **Pincer Grasp** | **87.171** | **45** | **<0.001** |
| **Finger Fed** | **90.144** | **45** | **<0.001** |
| **Turn Pages Book** | **77.010** | **45** | **0.002** |

**Table 2.5. Chi-square analysis of receptive language skills.** Analyses with statistical significance ($p<0.05$) are reported in bold font.

| Developmental Skill | Chi-Square Value | df | Asymptotic Significance |
|---|---|---|---|
| Soothe Adult Voice | 31.473 | 45 | 0.937 |
| Respond To Sound | 60.734 | 45 | 0.059 |
| **Played Peek-a-Boo** | **62.460** | **45** | **0.043** |
| Respond Familiar Words | 45.635 | 45 | 0.446 |
| Respond Own Name | 38.003 | 45 | 0.761 |

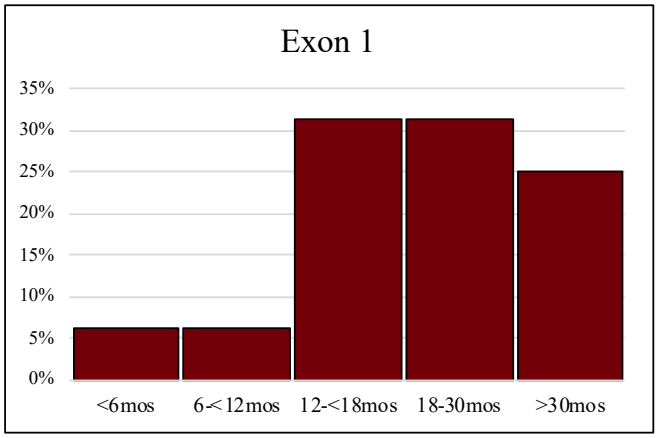
| | | | |
|---|---|---|---|
| **Inhibit to ”No” or Different Tones** | **66.919** | **45** | **0.019** |
| **Follow Command with Gesture** | **73.986** | **45** | **0.004** |
| Follow Command w/o Gesture | 56.679 | 45 | 0.114 |
| **Identified Body Parts** | **74.329** | **45** | **0.004** |
| Pointed to Color when Asked | 40.414 | 45 | 0.666 |

**Table 2.6. Chi-square analysis of expressive language skills.** Analyses with statistical significance ($p<0.05$) are reported in bold font.

| Developmental Skill | Chi-Square Value | df | Asymptotic Significance |
|---|---|---|---|
| Social Smile | 49.729 | 45 | 0.291 |
| Cooed | 56.462 | 45 | 0.117 |
| **Babbled** | **79.422** | **45** | **0.001** |
| Words with Meaning | 52.362 | 45 | 0.210 |
| **Spoken Phrases** | **86.377** | **45** | **<0.001** |
| **Waved Bye** | **68.574** | **45** | **0.013** |
| **Pointed When Want** | **109.926** | **45** | **<0.001** |
| Shared Stories | 39.255 | 45 | 0.713 |

**Table 2.7. Chi-square analysis of personal-social/adaptive skills.** Analyses with statistical significance ($p<0.05$) are reported in bold font.

| Developmental Skill | Chi-Square Value | df | Asymptotic Significance |
|---|---|---|---|
| Like Being Held | 52.460 | 45 | 0.207 |
| Attention Loud Sound | 56.505 | 45 | 0.117 |
| Fixed Follow Object | 33.964 | 45 | 0.886 |
| **Play Pat-a-Cake** | **73.930** | **45** | **0.004** |
| Desire Social Attention | 59.748 | 45 | 0.069 |
| **Imitate Peers Activities** | **87.266** | **45** | **<0.001** |
| Been Independent | 61.215 | 45 | 0.054 |
| **Take Drink w/o Assistance** | **77.455** | **45** | **0.002** |
| **Spoon Fork with Assistance** | **73.230** | **45** | **0.005** |
| **Spoon Fork without Assistance** | **78.210** | **45** | **0.002** |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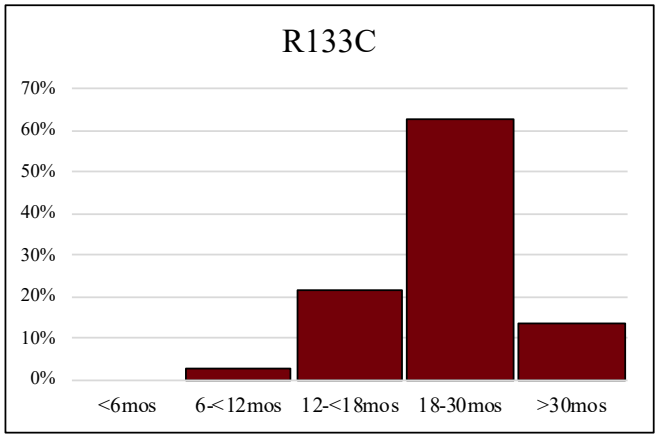
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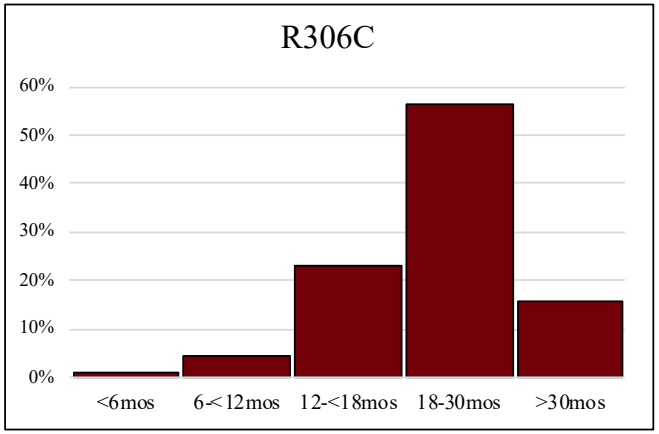
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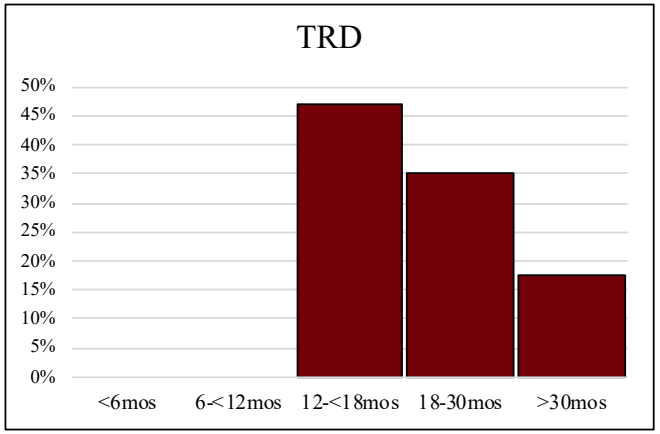
TRD
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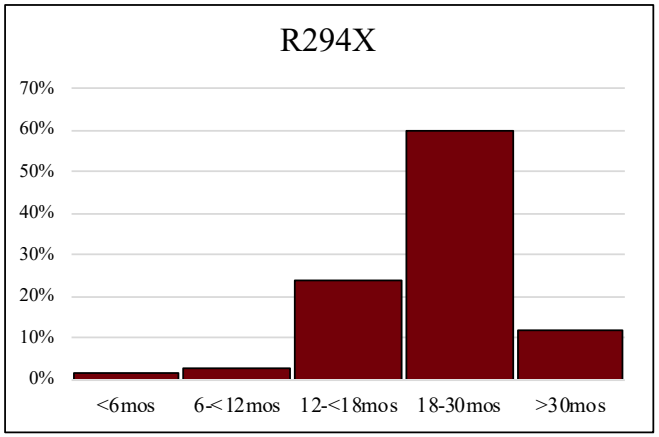
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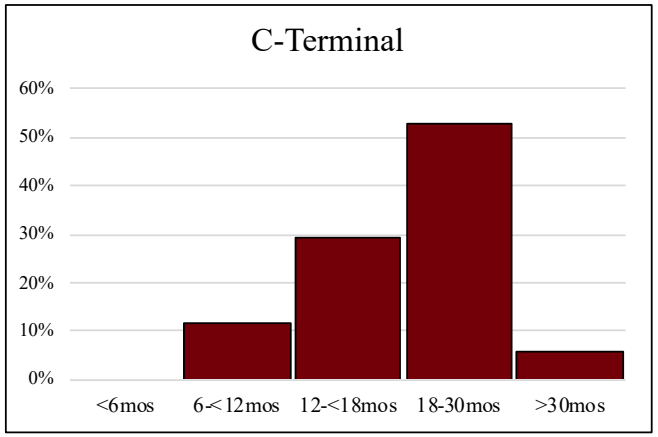
C-Terminal
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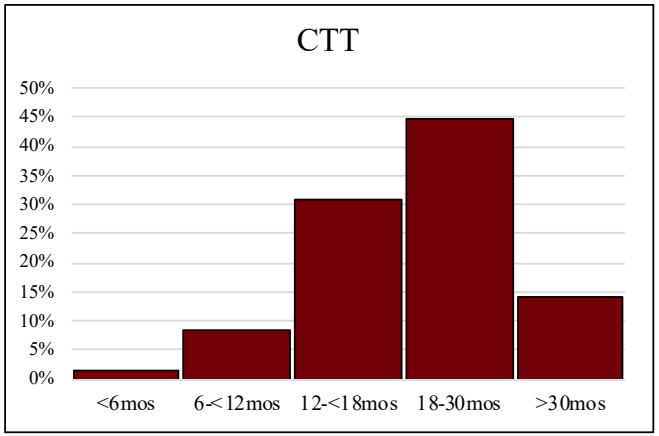
CTT
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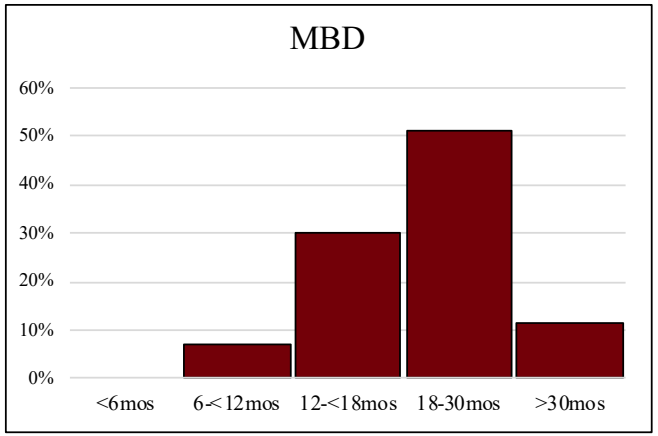
MBD
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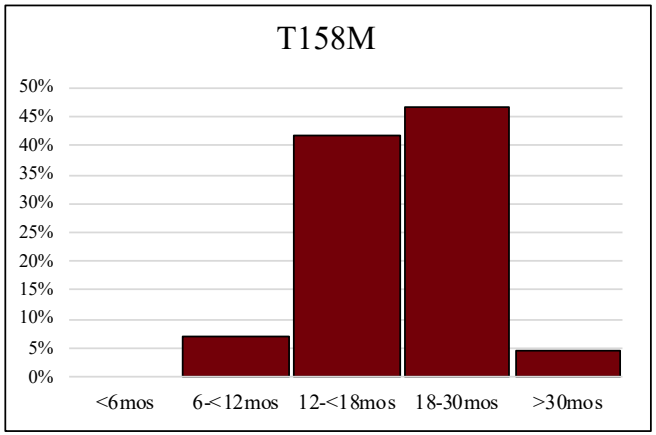
T158M
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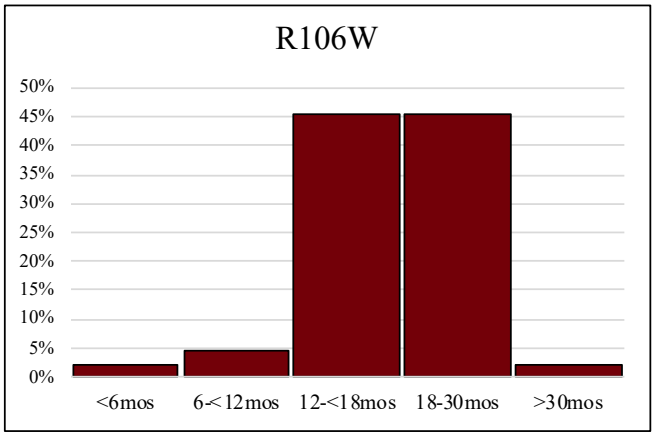
R106W
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35%
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5%
0%
<6mos
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12-<18mos
18-30mos
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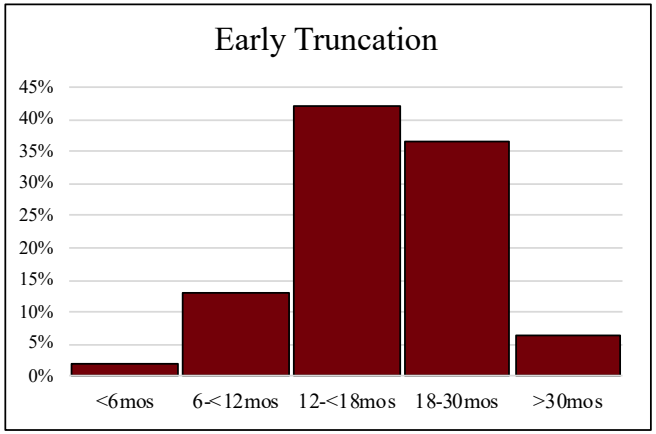
Early Truncation
45%
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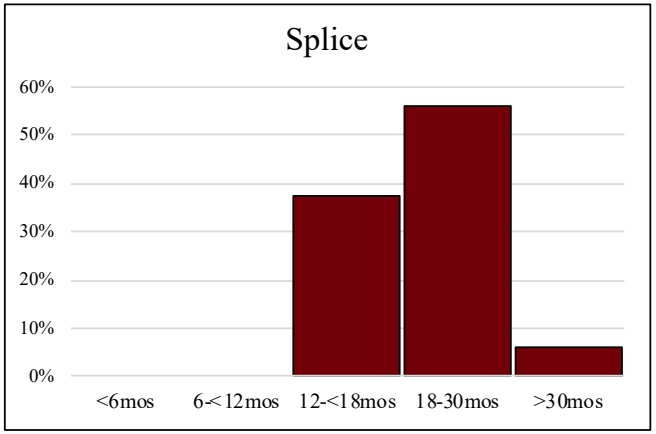
Splice
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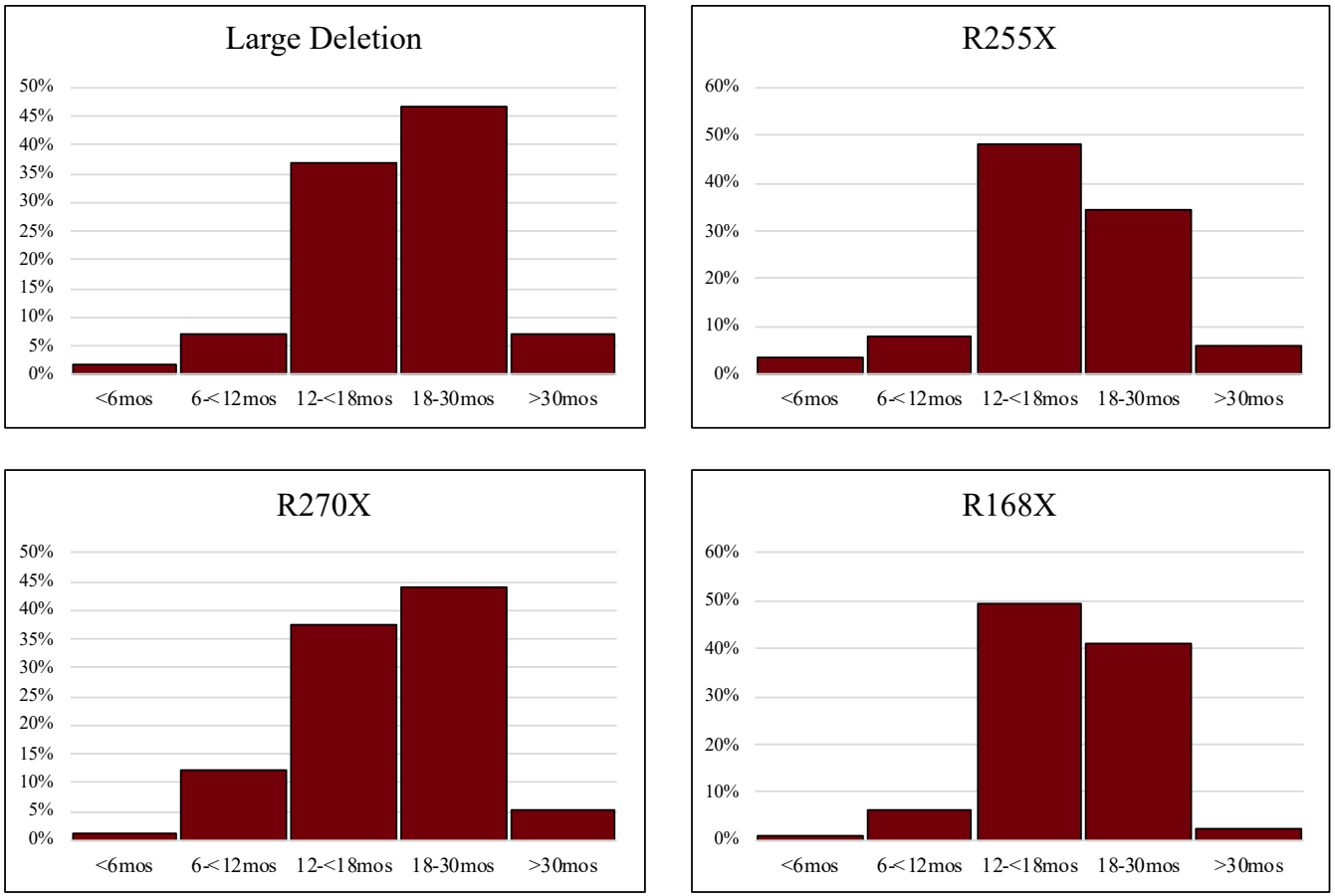
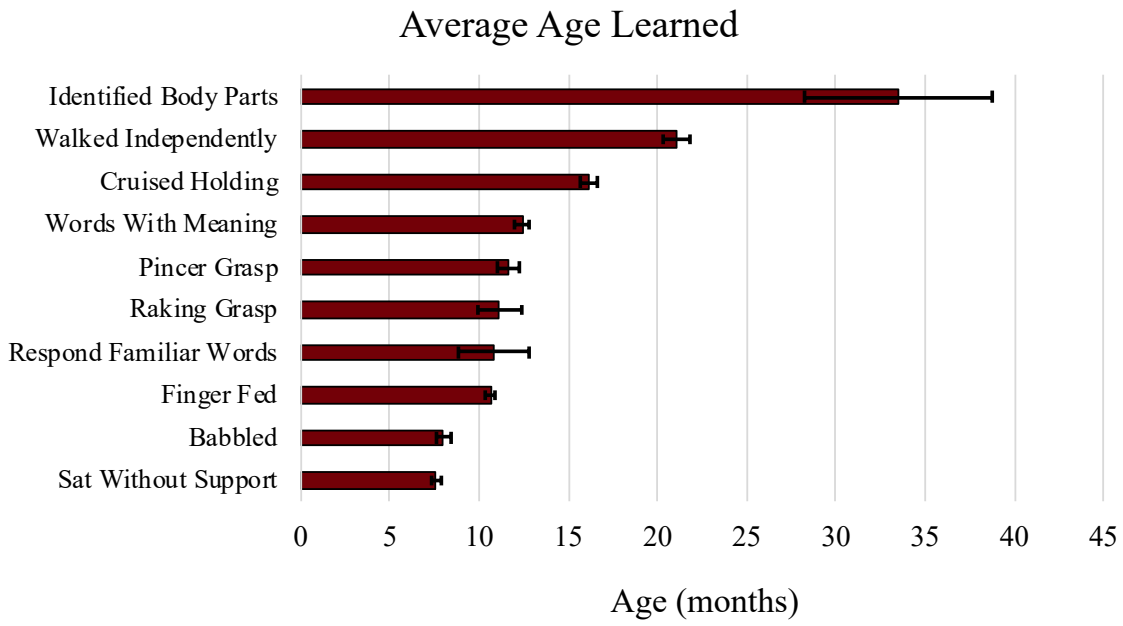



**Figure 2.8. Age of onset of regression by *MECP2* variant.**

**A.**




**B.**

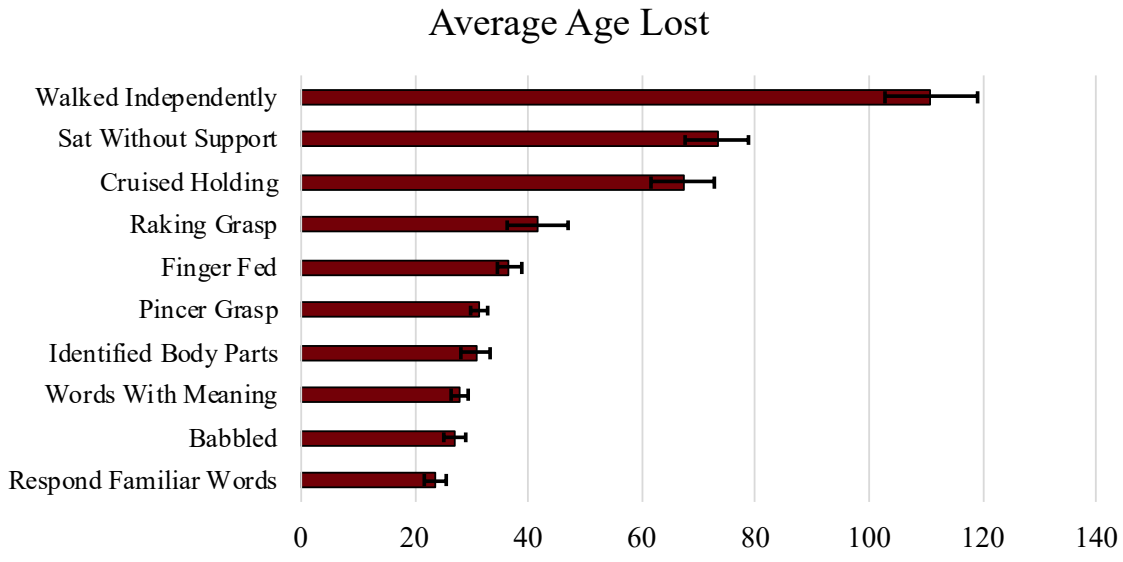



**Figure 2.9. Developmental skills – average ages learned and lost.**

Of 369 participants seen as part of the study after age 18, 29 had skills lost at age 18 or later. A majority of participants that lost skills after age 18 (11, 37.93%) lost only one of 51 skills. The number of skills lost by participants in this period ranged from one to as many as 13 skills. Frequencies of patients who lost skills after age 18 are presented in Figure 2.10.

Thirty of 51 skills had patients who were reported to have lost the skill after age 18. The skill most commonly lost during this period was walking independently (14 patients). Frequencies of skills lost after age 18 are reported in Table 2.8

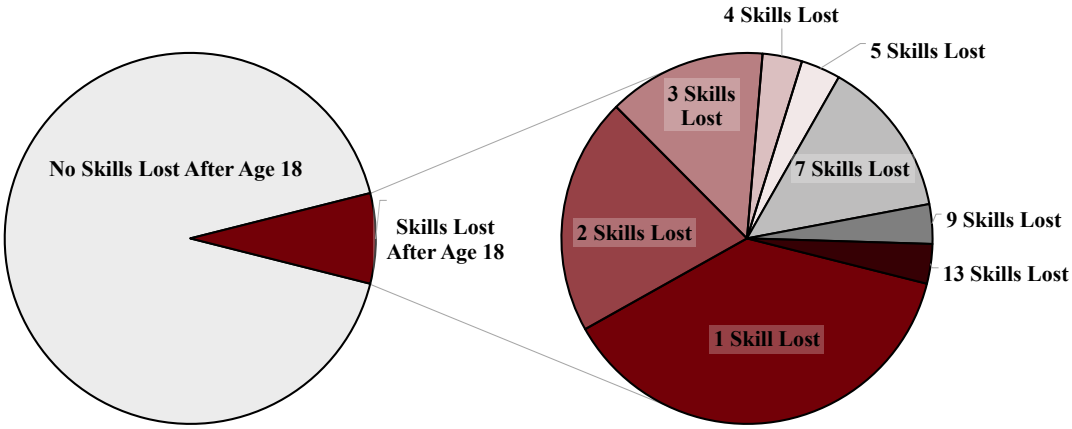
### *2.4.5 Head Circumference*

A linear regression model was used to assess whether *MECP2* variant accounted for any variation in the average HC z-score. This linear regression established that *MECP2* variant group could significantly predict average HC z-score, $F(15)=4.758$, $p<0.001$, and *MECP2* variant accounted for 4.5% of the variation (Adjusted $R^2 = 0.045$). The average HC z-score and SEM by *MECP2* variant is presented in Figure 2.11.

A logistic regression model was used to assess whether *MECP2* variant predicted the frequency at which microcephaly was observed in patients at any point in their enrollment in the Natural History Study. The logistic regression model was statistically significant, $X^2(15)=56.919$, $p<0.001$. The model accounted for 4.7% of the variance (Cox & Snell $R^2=0.047$) and correctly predicted 62.1% of cases. The frequency of acquired microcephaly by *MECP2* variant group is presented in Figure 2.12. Frequency of acquired microcephaly is also included in Supplemental Figure B.1.

A logistic regression model was also used to assess whether the average HC z-score predicted whether patients learned+retained or learned+lost a subset of skills from the

**A.**




**B.**

| Number of Skills Lost After Age 18 | Number of Patients | |
|---|---|---|
| No Skills Lost After Age 18 | | 340 (92.14%) |
| 1 Skill Lost | 11 (37.93%) | 29 (7.86%) |
| 2 Skills Lost | 6 (20.69%) | |
| 3 Skills Lost | 4 (13.79%) | |
| 4 Skills Lost | 1 (3.45%) | |
| 5 Skills Lost | 1 (3.45%) | |
| 7 Skills Lost | 4 (13.79%) | |
| 9 Skills Lost | 1 (3.45%) | |
| 13 Skills Lost | 1 (3.45%) | |
| **TOTAL** | | **369** |

**Figure 2.10. Regression after age 18.**

**Table 2.8. Frequencies of skills lost after age 18.**

| Developmental Skill | Number of Patients Who Lost After Age 18 |
|---|---|
| Lift Head | 1 |
| Climb down Stairs Without Help | 1 |
| Transfer Object One Hand to Other | 1 |
| Inhibit to ”No” or Different Tones | 1 |
| Cooed | 1 |
| Waved Bye | 1 |
| Pointed When Want | 1 |
| Been Independent | 1 |
| Spoon Fork without Assistance | 1 |
| Sat with Support | 2 |
| Ran 10 Feet | 2 |
| Climb up Stairs Without Help | 2 |
| Reach for Toy | 2 |
| Babbled | 2 |
| Take Drink w/o Assistance | 2 |
| Cruised Furniture Holding Someone | 3 |

| | |
|---|---|
| Climb up Stairs with Help | 3 |
| Held Bottle Unpropped | 3 |
| Social Smile | 3 |
| Words with Meaning | 3 |
| Sat without Support | 4 |
| Come to Sit | 4 |
| Climb Down Stairs with Help | 4 |
| Raking Grasp | 4 |
| Finger Fed | 4 |
| Rolled From Tummy | 5 |
| Stand while Hold | 5 |
| Pulled to Stand | 7 |
| Stood Independently | 7 |
| Walked Independently | 14 |

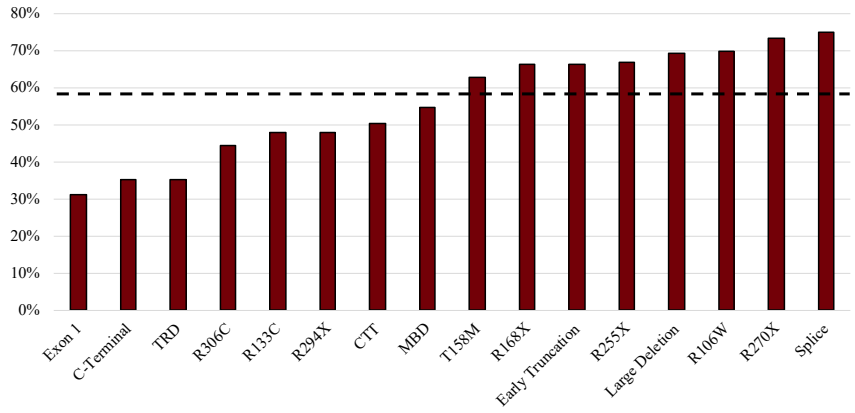
A.
Average HC z-score
4
2
0
-2
-4
-6
-8
Exon 1
TRD
R306C
CTT
C-Terminal
R133C
MBD
R294X
Early Trunc
T158M
R106W
R255X
R168X
Large Del
R270X
Splice

**B.**

| Variant Grouping | N | Average Head Circumference z-score (SEM) |
|---|---|---|
| Exon 1 | 16 | -0.92 (0.28) |
| TRD | 17 | -1.39 (0.31) |
| R306C | 97 | -1.45 (0.12) |
| CTT | 127 | -1.53 (0.12) |
| C-Terminal | 17 | -1.58 (0.39) |
| R133C | 75 | -1.66 (0.16) |
| MBD | 42 | -1.84 (0.23) |
| R294X | 75 | -1.86 (0.15) |
| Early Truncation | 107 | -2.08 (0.13) |
| T158M | 126 | -2.09 (0.13) |
| R106W | 43 | -2.13 (0.22) |
| R255X | 114 | -2.13 (0.11) |
| R168X | 130 | -2.23 (0.11) |
| Large Deletions | 110 | -2.25 (0.14) |
| R270X | 75 | -2.37 (0.16) |
| Splice Variants | 16 | -2.51 (0.31) |
| **TOTAL** | **1,187** | |

**Figure 2.11. Average head circumference z-score by *MECP2* variant.**

**A.**




**B.**

| Variant Grouping | Present (%) | Absent (%) | TOTAL |
|---|---|---|---|
| Exon1 | 5 (31.3%) | 11 (68.8%) | 16 |
| C-Terminal | 6 (35.3%) | 11 (64.7%) | 17 |
| TRD | 6 (35.3%) | 11 (64.7%) | 17 |
| R306C | 43 (44.3%) | 54 (55.7%) | 97 |
| R133C | 36 (48.0%) | 39 (52.0%) | 75 |
| R294X | 36 (48.0%) | 39 (52.0%) | 75 |
| CTT | 64 (50.4%) | 63 (49.6%) | 127 |
| MBD | 23 (54.8%) | 19 (45.2%) | 42 |
| T158M | 79 (62.7%) | 47 (37.3%) | 126 |
| R168X | 86 (66.2%) | 44 (33.8%) | 130 |
| earlytrunc | 71 (66.4%) | 36 (33.6%) | 107 |
| R255X | 76 (66.7%) | 38 (33.3%) | 114 |
| LargeDel | 76 (69.1%) | 34 (30.9%) | 110 |
| R106W | 30 (69.8%) | 13 (30.2%) | 43 |
| R270X | 55 (73.3%) | 20 (26.7%) | 75 |
| Splice | 12 (75.0%) | 4 (25.0%) | 16 |
| **TOTAL** | 704 (59.3%) | 483 (40.7%) | **1187** |

**Figure 2.12. Frequency of acquired microcephaly by *MECP2* variant.** A) Graph showing frequency (by percentage) of microcephaly in patients in each variant group. Dotted line shows the average frequency for all patients. B) Table reporting frequencies of microcephaly for each variant.

Developmental History Log. Skills analyzed include: sat without support, cruised furnature while holding someone, walked independently, raking grasp, pincer grasp, finger fed, respond to familiar words, identified body parts, babbled, and words with meaning. Of the ten skills assessed, the logistic regression model was significant for seven skills: sat without support, cruised furniture while holding someone, walked independently, raking grasp, pincer grasp, finger fed, and babbled. Logistic regression results are summarized in Table 2.9. Selected graphs are shown in Supplemental Figure B.6.

**Table 2.9. Logistic regression analysis – head circumference and loss of developmental skills.** Analyses with statistical significance ($p<0.05$) are reported in bold font.

| Developmental Skill | $X^2$ | df | Cox & Snell $R^2$ | p-value |
|---|---|---|---|---|
| **Sat Without Support** | **50.305** | **1** | **0.085** | **<0.001** |
| **Cruised Furniture Holding Someone** | **58.721** | **1** | **0.113** | **<0.001** |
| **Walked Independently** | **20.399** | **1** | **0.055** | **<0.001** |
| **Raking Grasp** | **51.476** | **1** | **0.163** | **<0.001** |
| **Pincer Grasp** | **41.566** | **1** | **0.088** | **<0.001** |
| **Finger Fed** | **71.543** | **1** | **0.123** | **<0.001** |
| Respond Familiar Words | 2.112 | 1 | 0.007 | 0.146 |
| Identified Body Parts | 1.765 | 1 | 0.014 | 0.184 |
| **Babbled** | **10.342** | **1** | **0.020** | **0.001** |
| Words With Meaning | 0.178 | 1 | 0.000 | 0.673 |

## 2.5 Discussion

### *2.5.1 Clinical Severity*

This study aimed to evaluate to what extent differences in the type of pathogenic *MECP2* variant contribute to variation in patients' average clinical severity score. Results support the hypothesis that differences in *MECP2* variant correlate significantly with overall disease severity. Previous investigations of genotype-phenotype correlations in Rett syndrome reported reduced severity in patients with *MECP2* variants p.R133C, p.R294X, and p.R306C. In contrast, increased severity was observed in patients with p.R168X, p.R270X, and p.R255X variants (Bebbington et al., 2008; Halbach et al., 2012; Neul et al., 2008). Patients with C-terminal truncations have been reported to have a more "moderate" phenotype, with severity falling between the more and less severe *MECP2* variants (Bebbington et al., 2010).

This study's results were consistent with these previous observations, confirming correlations between *MECP2* variant and average clinical severity score in a larger cohort of patients followed over time. Patients with exon 1, p.R133C, p.R306C, transcription repression domain, and p.R294X variants had the lowest average clinical severity scores, with average scores of 20.08 or less. Patients with p.R168X, p.R270X, or p.R255X variants had the highest average clinical severity scores, with average scores of 25.90 or above. C-terminal truncations and C-terminal missense variants fell between these with average clinical severity scores of 20.78 and 20.29, respectively.

In addition to confirming previously observed correlations, this study investigated clinical severity in patients with less common *MECP2* variants. Patients with large deletions, early truncations, and splice variants had higher average clinical severity scores,

with average scores between 24.58 and 25.59. Patients with pathogenic missense variants in the TRD had a milder phenotype, with a lower average clinical severity of 19.55. In contrast, patients with pathogenic missense variants in the MBD had a more moderate severity, with an average clinical severity score of 22.92. Previous studies investigating genotype-phenotype correlations have observed a higher clinical severity in patients with early truncations in the *MECP2* gene, which is consistent with what was observed in this analysis (Monrós et al., 2001). However, previous research has been limited by study size and by the *MECP2* variants present in their cohort, and this study is unique in its assessment of clinical severity in individuals with rare variants.

### *2.5.2 Rett Features*

This study also characterized the frequency of RTT features and assessed how occurence of RTT features differed between patients with different pathogenic *MECP2* variants. Of the 23 features assessed as part of this study, hand stereotypies, constipation, drooling, bruxism, and breath holding occurred most frequently. The least common features included hyperactivity, spitting, and gallbladder dysfunction. In a study from 2014 using data from the InterRett Database, some of the most common features were breath holding (88%), scoliosis (86%), constipation (83%), hyperventilation (74%) and sleep disturbance (63%). Screaming spells were observed in 46% of patients, and gallbladder dysfunction was observed in only 5% (Anderson et al., 2014). Many of these frequencies are consistent with observations from this study. In particular, the current study identified constipation as one of the most common features (93%) and gallbladder dysfunction as one of the least common (8.6%). Frequencies of hyperventilation (74.5%), breath holding (88.6%), and sleep difficulties (59%) were all similar to observations from the previous

study. Compared to previous research, scoliosis was observed less frequently in this group (71.4%), and screaming spells were observed more frequently (60.4%). Importantly, this study expanded on the number of features characterized and benefits from the use of consistent questionnaires administered to all participants.

Results of logistic regression analysis support the hypothesis that type of *MECP2* variant correlates with the presence of particular RTT features. Specifically, a significant correlation was found for nearly half of the features assessed as part of this study. Hyperventilation, aerophagia, spitting, bruxism, chewing problems, constipation, gastroesophageal reflux, sleep difficulty, waking at night, self-abusive behavior, and hyperactivity were all found to differ significantly based on *MECP2* variant. There is no previous research investigating association of *MECP2* variant and presence of particular features in patients with RTT. One study did find a correlation with certain *MECP2* variants and the severity of anxiety, suggesting that anxiety was more severe in patients with p.R133C variants compared to patients with p.T158M and p.R168X variants (Anderson et al., 2014). However, results of this study suggest that there are no significant differences in the frequency of anxiety with *MECP2* variant, and severity of features was not assessed.

### *2.5.3 Developmental History*

Developmental regression was evaluated in several ways as part of this study. First, the study evaluated frequencies at which skills were learned, lost, or relearned. As reported in previous research, patients were less likely to learn more advanced skills as compared to skills that are less advanced (Neul et al., 2014). For example, the majority of participants in this study learned to sit independently, while only about 70% learned how to crawl. Just over half of patients learned to walk independently, and even fewer were ever able to run

10 feet. Acquisition of language skills followed similar trends. For example, while most patients learned to babble, fewer acquired meaningful words, and only about a quarter of patients ever used spoken phrases. It is unsurprising that results of this study are consistent with previous research in this area, given that data reported in 2014 by Neul et al. were collected as part of the same RSNHS. However, this study includes additional data that have been collected in the past 6 years since the data were initially published.

This study expanded on the understanding of developmental regression in RTT by identifying trends in skills that were more commonly lost by patients after being learned. Many fine motor skills, such as finger feeding, raking grasp, pincer grasp, reaching for a toy, and transferring objects between hands were learned by as many as 92% of patients, but were far more commonly lost compared to other motor skills. This is consistent with the understanding that patients with RTT typically learn these earlier motor skills but lose purposeful hand movements during the regression period (Percy, 2016). Similarly, many patients who learned to babble and use words with meaning also lost these skills during the regression period. This is reflective of the loss of communication skills that is included as one of the four major criteria for patients diagnosed with RTT (Percy et al., 2010).

Although evidence of patients with RTT relearning skills after regression has not previously been well categorized, each skill had a subset of patients who relearned the skill after losing it. Compared to motor skills, language and social/adaptive skills were more commonly relearned. Only one motor skill, reaching for a toy, was relearned in over 10% of participants, and even this skill was not relearned in the majority of the patients that lost it. In contrast, 6 language skills and 4 social skills were relearned by more than 10% of participants. A number of these skills, including responding to sounds, responding to one's

name, and desiring social attention were relearned by the majority of patients that lost the skill.

Additionally, this study assessed differences in whether skills were learned, lost, or relearned based on the type of *MECP2* variant present in patients. Skills learned, lost, and relearned during development differ significantly based on pathogenic *MECP2* variant. Of 51 developmental skills assessed in this study, a majority showed statistically significant differences with the type of *MECP2* variant. Most skills with significant findings were motor skills. Of 16 gross motor and seven fine motor skills assessed in this study, observed values were found to differ significantly from expected values for 15 skills and six skills, respectively. This is compared to significant differences in only about half of receptive language, expressive language, and personal-social adaptive skills. These findings indicate that, for patients with Rett syndrome, the specific *MECP2* variant may have more of an impact on acquisition and loss of motor skills, and by comparison contributes less significantly to the variation observed in language and social skills.

Even among skills used to assess language and social skills, many skills that showed significant differences based on *MECP2* variant require particular motor abilities. For example, playing pat-a-cake is considered a personal-social skill in this study, but requires a level of motor coordination. This suggests that differences in motor skills may have confounded some measurements of language or social skills. This suggests the possibility that, when considering the effects of motor skills, *MECP2* variant may have a smaller effect on certain language and social skills than what was determined in this study.

This study also investigated variation in age of onset of regression with *MECP2* variant. For each variant, families reported regression onset occurring most commonly

between 12 and 30 months of age. However, observed values differed significantly from expected values when analyzed by *MECP2* variant. Notably, several variants, particularly less severe variants such as Exon 1 variants, p.R133C, and transcription repression domain variants had a higher percentage of patients with regression onset later than 30 months (over 2.5 years old). Other variants, such as early truncations, large deletions, and more severe nonsense variants like p.R255X, p.R270X, and p.R168X had a number of patients with regression onset between 6 and 12 months, and even some patients with onset before six months. This is consistent with data from a previous study which reported significant differences in age of onset of regression with *MECP2* variant (Bebbington et al., 2008). It also builds on these findings, supporting the hypothesis that age of onset of regression differs based on *MECP2* variant in an analysis including patients with less common variants.

Ages at which skills were learned, lost, and relearned were assessed for a subset of ten developmental skills. Skills such as sitting without support, finger feeding, babbling, responding to familiar words, and raking and pincer grasp were all learned on average before one year of age. Walking independently was learned at an average of 21 months. As has been previously reported, average ages that skills were learned by patients with RTT are somewhat delayed compared to expectations for typically-developing children as defined by the Centers for Disease Control and Prevention (CDC, 2020). For example, children are expected to babble beginning between four to six months of age. However, participants in this study learned to babble at an average age of nearly eight months old. Similarly, children are expected to learn how to cruise furniture at about 12 months and can typically walk by the age of 18 months old. In this study, participants learned these

skills at an average age of about 16 months and 21 months, respectively. These types of delays have been observed in previous research using data from the RSNHS (Neul et al., 2014).

Some of the earliest skills lost included responding to familiar words, identifying body parts, babbling, pincer grasp, and finger feeding. On average, these skills were lost by around age three years. On average, gross motor skills such as sitting, walking, and cruising furniture were lost later. Walking independently was lost the latest, with an average age of over nine years old. Walking independently was also the skill that patients relearned the latest, at an average age of about eight years old. However, ages that skills were relearned varied widely. For example, among patients who relearned how to walk independently, one participant lost the skill at about a year old and relearned how to walk at 15 months. Another participant who lost the ability to walk independently at age 30 years relearned the skill at 34 years old.

These observations of developmental delays and age of onset of regression may provide some explanation for why age of diagnosis of RTT has remained consistent at about 2.5 years old. While there are delays observed in ages that skills are learned, delays are subtle and parents may be encouraged to "wait and see" whether their children learn skills before being referred to specialists (Neul et al., 2014). The age that skills are lost may also help to explain the overall later age of diagnosis for patients with RTT, since concerns may not be noted or acted upon until regression onset at around age 1.5 to three years.

It is widely acknowledged that patients with Rett syndrome experience a "Late Motor Deterioration Phase", during which they lose additional skills, particularly motor skills (Hagberg & Witt-Engerström, 1986; Monteiro et al., 2014). This study aimed to

assess skills lost after age 18 years to more thoroughly characterize this later stage of developmental regression in patients with Rett syndrome. Of patients seen as part of the RSNHS after age 18, 29 patients (7.86%) lost skills in this later period. Most of these patients (37.93%) lost only one skill after age 18, but some patients lost as many as 13 skills. The most common skill lost at later ages was walking independently, with 14 patients losing this skill after age 18. Other skills commonly lost during this period were most often motor skills, including standing independently, pulling to stand, finger feeding, raking grasp, and climbing stairs. Fewer patients did lose skills such as cooing, babbling, and social smile. However, most language skills and social skills were not lost by any patients after the age of 18 years.

Specific characterizations of this late stage of RTT have not been provided by previous studies. These results may indicate that fewer patients experience late regression than what was previously believed. However, it may also represent limitations in data collection and analysis. It may be that some skills are lost later than age 18, and patients included in analysis have not yet entered this disease phase. There were also limitations in the amount of data recorded regarding ages that skills were lost. It is possible that some patients who lost skills after age 18 are not represented in this data.

### *2.5.4 Head Circumference*

Although acquired microcephaly is a feature commonly associated with Rett syndrome, there have been limited previous studies assessing head circumference, head growth deceleration, and acquired microcephaly in a large cohort like that of the RSNHS. This study aimed to determine the frequency of microcephaly in this cohort and evaluated how head circumference varied with the type of *MECP2* variant present in patients.

Previous research using data from the RSNHS has found that over 80% of patients experience head growth deceleration, but few studies have assessed how often this results in acquired microcephaly (Percy et al., 2010). One study found that only 46% of patients had head circumference measurements falling below the 3rd percentile (Pini et al., 2016). Results from this study are consistent with the idea that, while head growth deceleration has been observed in over 80% of patients with RTT, fewer patients develop acquired microcephaly. Only 59% of patients in this cohort had a head circumference measurement greater than 2 SD below the mean at any point during their enrollment in the RSNHS.

Additionally, results of this study support the hypothesis that differences in *MECP2* variant correlate with differences in average head circumference and frequency of microcephaly. This is consistent with results from a previous study that suggested the presence of significant differences in head growth between patients with a p.R294X variant and a p.R270X variant, where patients with a p.R294X variant were less likely to have a head circumference below the 3rd percentile in the first year of life (Halbach et al., 2012). This study found that only 48% of patients with a p.R294X variant had microcephaly occur during their enrollment in the study, compared to 73% of patients with a p.R270X variant. These differences were observed even when evaluating head circumference over a wider range of ages. Furthermore, this study was able to include other *MECP2* variants in this analysis that have not been previously investigated.

Some recent research has looked more closely at the impact of microcephaly on skills developed in patients with Rett syndrome. Most notably, a recent study suggests that walking ability over age ten is correlated with several early indicators, including microcephaly (Saikusa et al., 2020). Results of the Saikusa et al. (2020) study suggested

that patients with microcephaly were less likely to acquire the ability to walk by age 10. Similarly, this study assessed whether variation in head circumference correlated with whether selected developmental skills were lost or retained during the regression period. Results show that head circumference correlates with whether participants lost or retained certain skills, particularly motor skills. Of four language skills selected for assessment with head circumference, only babbling showed significance in the logistic regression model. However, 6 motor skills were found to have significant differences in frequency depending on head circumference z-score. For each of these seven skills, patients with a smaller head size were more likely to lose skills after initially learning them. This is consistent with observations reported by Saikusa et al. (2020) and furthers understanding of how presence of additional motor skills later in development may correlate with head circumference. Given these findings, head circumference could be another important predictor of future outcomes for patients with RTT.

### *2.5.5 Limitations*

While a diverse population was sampled for this study, the population distribution is not representative of the general US population, as it includes more Non-Hispanic White individuals and fewer Black individuals. Incidence of RTT is not known to differ by race, so differences in this study cohort likely represent an ascertainment bias. White individuals in the population may be more likely to receive the proper diagnosis of RTT and enroll in this type of natural history study. Additionally, this study does not account for loss to follow-up, right-censoring, and the impact of age on features of RTT. When assessing skills learned, lost, and relearned, age was accounted for in that only patients above age 12 years

were included in analysis. However, differences in features and skills by age were not investigated.

Although consistent surveys were used for collecting data in the RSNHS, there could still be challenges with interrater reliability given that there are 14 participating sites. As discussed in the methods section, adjustments were made in data analysis to correct for data recording errors that may have impacted results of this study. Several surveys also rely on parents' ability to recall developmental milestones and early changes in development such as onset of regression. Previous studies have accounted for this by including participants in analysis only if they were seen at an earlier age when parents' recall may be more reliable. However, this study did not use this method since one goal was to capture more information about loss and regain of skills later in life.

Finally, while this study benefited from a large number of participants, some analyses still had low sample sizes, particularly when participants were assigned to different *MECP2* variant categories. Specifically, chi-square analyses used in this study for analysis of development and age of onset of regression may be limited since some expected values were lower than 5. Analysis of average ages that skills were lost and relearned may also be limited by the number of ages recorded.

### *2.5.6 Future Directions*

Areas for future research include investigating impacts of age on the presence of particular features and developmental skills. Studies could investigate more details of disease progression and may be able to evaluate differences in severity over time with particular *MECP2* variants. Future research could also assess differences in severity of features based on *MECP2* variant, particularly since previous studies have shown some

promise in this area but investigations have been limited. Additionally, given evidence of patients' capacity to relearn skills after the regression period, it could be beneficial to pursue additional research into which therapies may be best suited for these individuals in order to maximize that capacity. Another area for future exploration may be determining families' preferences regarding what information they want to receive at the time of diagnosis and at subsequent visits. This could be helpful in evaluating parents' feelings regarding uncertainties in prognosis, and whether genotype-phenotype information would be considered important by families after an initial diagnosis given the variation that can still exist within these groups.

# CHAPTER 3

# CONCLUSIONS

This study aimed to assess genotype-phenotype correlations in patients with RTT and investigated these correlations with regards to overall disease severity, particular features of RTT, and attainment and/or loss of developmental skills. The results of this study are consistent with previous investigations into genotype-phenotype correlations in RTT but confirmed these correlations in a much larger cohort of patients compared to those that have been used before in this type of analysis. Additionally, it expanded this analysis to include patients with rare mutations and expanded some phenotypic investigations to include more specific features, particularly when looking at development.

For patients with a new diagnosis of RTT, these results may help answer questions regarding prognosis and provide families with more information about what to expect moving forward. As advancements in technology may allow for earlier diagnosis of RTT, this conversation will become increasingly common following diagnosis, particularly in a genetic counseling setting. Given the availability of Natera's "Vistara" test for single-gene conditions via NIPT, genetic counselors may be more frequently asked to discuss these questions in the prenatal setting with no patient-specific clinical information other than *MECP2* variant.

When discussing these types of correlations, it is important to consider differences present even among patients with the same *MECP2* variant. Although we describe

significant differences between categories of *MECP2* variant, there is still substantial variation within groups that could impact predictive power in a clinical setting. There are undoubtedly other factors that contribute to phenotypic variation in patients with RTT. For example, X-chromosome inactivation has been previously found to play a role in severity (Archer et al., 2007). It is also likely that social factors and even other genetic factors contribute to differences in phenotype among patients. Because of this variability, it is essential not to overstate the importance of *MECP2* variant in determining prognosis for patients with RTT. However, it may be a tool that can be used as part of this conversation with parents who are curious about what is to come.

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APPENDIX A

INCLUSION CRITERIA AND SURVEY QUESTIONS

**Inclusion Criteria**
Diagnosis: Classic Rett Syndrome
Gender: Female
Genetic Testing: Pathogenic variant in MECP2

**Survey Questions**
Clinical Assessment

- A.1.e. Head circumference (cm)
- A.1.f. Head circumference percentile

Physician Initial History Form

Diagnostic information

- 6. Diagnosis
- 8. Age at Diagnosis

Genetic Testing Results

- 2a. Common MECP2 Point Mutations
- 2b. Common MECP2 Deletions
- 2c. MECP2 Large Deletions
- 2d. Other MECP2 Mutations not listed above

Demographic and Birth History Form

- 1. Child's date of birth?
- 2. Child's gender?
- 4. Child's ethnicity?
- 5. Child's race?

Clinical Severity Scale Form 3

- Age of Onset of Regression
- Total Score

Development Log
(see below)

Rett Features Log
(see below)

# Development Log

**IMPORTANT INSTRUCTIONS FOR COMPLETION:**
**"Learned", "Lost", "Relearned":** For each of the developmental skills below, please give the age in months that the skill was **learned**, the age when she/he **began to lose** the skill, and the age that the skill was **relearned.** If the skill has not yet been learned, lost or relearned, please check "No".
**Determining "Age": Record all ages in months.** Multiply years x 12. For example, 5 years 3 months is 5x12+3 = 63 months. If your child gained, lost or relearned a skill in a certain time period but you are not exactly sure on the month, take the average age. For example, "My daughter learned to crawl from 21 to 25 months." The correct answer is 23 months. If you are unsure on the age learned, lost or relearned, please check the, "Age unknown" box.
**Determining "Loss": A "lost" skill is defined as a loss that persists for more than one month**. If your child did not lose a skill completely, but the skill has declined significantly in the **quality** or **quantity**, please consider this as a loss. For example, if she/he could hold a cup or a bottle to drink easily, but now she/he is clumsy or can only hold it for 2-3 seconds that is considered a loss even though the skill is not completely gone.
**Questions:** If you are unsure how to respond to a question, make note on this form. These can be reviewed at the time of the assessment.

## A. Overall Developmental Course

***1. When did you first become concerned about your child's development?***

Age (in months): ○ Not applicable (no concern)

***2. What was your first concern?***

Specify briefly:

○ Not applicable (no concern)

***3. Did your child experience any of the following during the first year of life? (check all that apply)***

| ***Feeding Difficulties*** | ***Low Muscle Tone*** | ***Developmental Delay*** |
|---|---|---|
| ○ Yes Age: ○ No ☐Age Unknown | ○ Yes Age: ○ No ☐Age Unknown | ○ Yes Age: ○ No ☐Age Unknown |
| | | |
| ***Decreased Vision*** | ***Seizures*** | ***Other (specify)*** |
| ○ Yes Age: ○ No ☐Age Unknown | ○ Yes Age: ○ No ☐Age Unknown | ○ Yes Age: ○ No ☐Age Unknown |

## B. Gross Motor Skill Development

**1. *Lifted head while lying down***

| *Learned* | *.Lost* | *.Relearned* |
|---|---|---|
| ○ Yes Age: ○ No | ○ Yes Age: ○ No | ○ Yes Age: ○ No |
| ☐Age Unknown | ☐Age Unknown | ☐Age Unknown |

**2. *Rolled from tummy to back***

| *Learned* | *Lost* | *Relearned* |
|---|---|---|
| ○ Yes Age: ○ No | ○ Yes Age: ○ No | ○ Yes Age: ○ No |
| ☐Age Unknown | ☐Age Unknown | ☐Age Unknown |

**3. *Sat with support when placed***

| *Learned* **(age must be entered)** | *Lost* **(age must be entered)** | *Relearned* **(age must be entered)** |
|---|---|---|
| ○ Yes Age: ○ No | ○ Yes Age: ○ No | ○ Yes Age: ○ No |
| | | |

4. ***Sat without support when placed***

| *Learned* | *Lost* | *Relearned* |
|---|---|---|
| ○ Yes Age: ○ No | ○ Yes Age: ○ No | ○ Yes Age: ○ No |
| ☐Age Unknown | ☐Age Unknown | ☐Age Unknown |

**5. *Come to sitting***

| *Learned* | *Lost* | *Relearned* |
|---|---|---|
| ○ Yes Age: ○ No | ○ Yes Age: ○ No | ○ Yes Age: ○ No |
| ☐Age Unknown | ☐Age Unknown | ☐Age Unknown |

**6.*Crawled***

| *Learned* | *Lost* | *Relearned* |
|---|---|---|
| ○ Yes Age: ○ No | ○ Yes Age: ○ No | ○ Yes Age: ○ No |
| ☐Age Unknown | ☐Age Unknown | ☐Age Unknown |

**7. *Stood while holding on***

| *Learned* | *Lost* | *Relearned* |
|---|---|---|
| ○ Yes Age: ○ No | ○ Yes Age: ○ No | ○ Yes Age: ○ No |
| ☐Age Unknown | ☐Age Unknown | ☐Age Unknown |

**8. *Pulled to standing***

| *Learned* | *Lost* | *Relearned* |
|---|---|---|
| ○ Yes Age: ○ No | ○ Yes Age: ○ No | ○ Yes Age: ○ No |
| ☐Age Unknown | ☐Age Unknown | ☐Age Unknown |

**9. *Cruised around furniture or holding on to someone***

| *Learned* | *Lost* | *Relearned* |
|---|---|---|
| ○ Yes Age: ○ No | ○ Yes Age: ○ No | ○ Yes Age: ○ No |
| ☐Age Unknown | ☐Age Unknown | ☐Age Unknown |

**10. *Stood independently***

| *Learned* | *Lost* | *Relearned* |
|---|---|---|
| ○ Yes Age: ○ No | ○ Yes Age: ○ No | ○ Yes Age: ○ No |
| ☐Age Unknown | ☐Age Unknown | ☐Age Unknown |

**11. *Walked independently***

| *Learned* (***age must be entered***) | *Lost* (***age must be entered***) | *Relearned* (***age must be entered***) |
|---|---|---|
| ○ Yes Age: ○ No | ○ Yes Age: ○ No | ○ Yes Age: ○ No |
| | | |

**12. *Ran 10 feet without falling***

| *Learned* | *Lost* | *Relearned* |
|---|---|---|
| ○ Yes Age: ○ No | ○ Yes Age: ○ No | ○ Yes Age: ○ No |
| ☐Age Unknown | ☐Age Unknown | ☐Age Unknown |

**13. *Climbed up stairs <u>with</u> help (holding a rail or a person)***

| *Learned* | *Lost* | *Relearned* |
|---|---|---|
| ○ Yes Age: ○ No | ○ Yes Age: ○ No | ○ Yes Age: ○ No |
| ☐Age Unknown | ☐Age Unknown | ☐Age Unknown |

**14. *Climbed up stairs <u>without</u> help***

| *Learned* | *Lost* | *Relearned* |
|---|---|---|
| ○ Yes Age: ○ No | ○ Yes Age: ○ No | ○ Yes Age: ○ No |
| ☐Age Unknown | ☐Age Unknown | ☐Age Unknown |

***15. Climbed down stairs <u>with</u> help (holding a rail or a person)***

| *Learned* | *Lost* | *Relearned* |
|---|---|---|
| ○ Yes Age: ○ No | ○ Yes Age: ○ No | ○ Yes Age: ○ No |
| ☐Age Unknown | ☐Age Unknown | ☐Age Unknown |

***16. Climbed down stairs <u>without</u> help***

| *Learned* | *Lost* | *Relearned* |
|---|---|---|
| ○ Yes Age: ○ No | ○ Yes Age: ○ No | ○ Yes Age: ○ No |
| ☐Age Unknown | ☐Age Unknown | ☐Age Unknown |

***17. Overall, how sudden was your child's loss in gross motor function?***

○ Not lost ○ Overnight ○ Days or weeks ○ 1-6 months ○ 6-12 months ○ >1 year ○ Unknown

## C. <u>Fine Motor Development</u>

***1. Held bottle or cup un-propped***

| *Learned* ***(ages must be entered)*** | *Lost* ***(ages must be entered)*** | *Relearned* ***(ages must be entered)*** |
|---|---|---|
| ○ Yes Age: ○ No | ○ Yes Age: ○ No | ○ Yes Age: ○ No |
| | | |

***2. Reached for toy***

| *Learned* | *Lost* | *Relearned* |
|---|---|---|
| ○ Yes Age: ○ No | ○ Yes Age: ○ No | ○ Yes Age: ○ No |
| ☐Age Unknown | ☐Age Unknown | ☐Age Unknown |

***3. Used raking grasp to retrieve an object***
*(Raking grasp is using all fingers to scoop an object into the palm of the hand)*

| *Learned* | *Lost* | *Relearned* |
|---|---|---|
| ○ Yes Age: ○ No | ○ Yes Age: ○ No | ○ Yes Age: ○ No |
| ☐Age Unknown | ☐Age Unknown | ☐Age Unknown |

***4. Transferred an object from one hand to the other***

| *Learned* | *Lost* | *Relearned* |
|---|---|---|
| ○ Yes Age: ○ No | ○ Yes Age: ○ No | ○ Yes Age: ○ No |
| ☐Age Unknown | ☐Age Unknown | ☐Age Unknown |

***5. Used a pincer grasp (either refined or modified)?***

| *Learned* | *Lost* | *Relearned* |
|---|---|---|
| ○ Yes Age: ○ No | ○ Yes Age: ○ No | ○ Yes Age: ○ No |
| ☐Age Unknown | ☐Age Unknown | ☐Age Unknown |

***6. Finger fed***

| *Learned* | *Lost* | *Relearned* |
|---|---|---|
| ○ Yes Age: ○ No | ○ Yes Age: ○ No | ○ Yes Age: ○ No |
| ☐Age Unknown | ☐Age Unknown | ☐Age Unknown |

***7. Turned pages in a book***

| *Learned* | *Lost* | *Relearned* |
|---|---|---|
| ○ Yes Age: ○ No | ○ Yes Age: ○ No | ○ Yes Age: ○ No |
| ☐Age Unknown | ☐Age Unknown | ☐Age Unknown |

***8. Overall, how sudden was your child's loss in fine motor development?***

○ Not lost ○ Overnight ○ Days or weeks ○ 1-6 months ○ 6-12 months ○ >1 year

## D. Receptive Language Development

***1. Quieted or been soothed by the sound of a familiar adult's voice***

| *Learned* | *Lost* | *Relearned* |
|---|---|---|
| ○ Yes Age: ○ No | ○ Yes Age: ○ No | ○ Yes Age: ○ No |
| ☐ Age Unknown | ☐ Age Unknown | ☐ Age Unknown |

***2. Responded to sounds***

| *Learned* | *Lost* | *Relearned* |
|---|---|---|
| ○ Yes Age: ○ No | ○ Yes Age: ○ No | ○ Yes Age: ○ No |
| ☐ Age Unknown | ☐ Age Unknown | ☐ Age Unknown |

***3. Played Peekaboo***

| *Learned* | *Lost* | *Relearned* |
|---|---|---|
| ○ Yes Age: ○ No | ○ Yes Age: ○ No | ○ Yes Age: ○ No |
| ☐ Age Unknown | ☐ Age Unknown | ☐ Age Unknown |

***4. Responded to familiar names/words such as mama or doggy***

| *Learned* | *Lost* | *Relearned* |
|---|---|---|
| ○ Yes Age: ○ No | ○ Yes Age: ○ No | ○ Yes Age: ○ No |
| ☐ Age Unknown | ☐ Age Unknown | ☐ Age Unknown |

***5. Responded to own name***

| *Learned* | *Lost* | *Relearned* |
|---|---|---|
| ○ Yes Age: ○ No | ○ Yes Age: ○ No | ○ Yes Age: ○ No |
| ☐ Age Unknown | ☐ Age Unknown | ☐ Age Unknown |

***6. Inhibited to "no" or responded to different tones of voice***

| *Learned* | *Lost* | *Relearned* |
|---|---|---|
| ○ Yes Age: ○ No | ○ Yes Age: ○ No | ○ Yes Age: ○ No |
| ☐ Age Unknown | ☐ Age Unknown | ☐ Age Unknown |

***7. Followed a command with a gesture (e.g. Give me the toy [name and point])***

| *Learned* | *Lost* | *Relearned* |
|---|---|---|
| ○ Yes Age: ○ No | ○ Yes Age: ○ No | ○ Yes Age: ○ No |
| ☐ Age Unknown | ☐ Age Unknown | ☐ Age Unknown |

***8. Followed a command without a gesture (e.g. Give me the toy [name])***

| *Learned* | *Lost* | *Relearned* |
|---|---|---|
| ○ Yes Age: ○ No | ○ Yes Age: ○ No | ○ Yes Age: ○ No |
| ☐ Age Unknown | ☐ Age Unknown | ☐ Age Unknown |

***9. Identified body parts (pointed with eyes or fingers)***

| *Learned* | *Lost* | *Relearned* |
|---|---|---|
| ○ Yes Age: ○ No | ○ Yes Age: ○ No | ○ Yes Age: ○ No |
| ☐ Age Unknown | ☐ Age Unknown | ☐ Age Unknown |

***10. Pointed to 1 color when asked (with fingers or eyes)***

| *Learned* | *Lost* | *Relearned* |
|---|---|---|
| ○ Yes Age: ○ No | ○ Yes Age: ○ No | ○ Yes Age: ○ No |
| ☐ Age Unknown | ☐ Age Unknown | ☐ Age Unknown |

***11. Overall, how sudden was your child's loss in receptive language development?***

○ Not lost ○ Overnight ○ Days or weeks ○ 1-6 months ○ 6-12 months ○ >1 year

## E. Expressive Language Development

**1. *Social smile***

| *Learned* | *Lost* | *Relearned* |
|---|---|---|
| ○ Yes Age: ○ No | ○ Yes Age: ○ No | ○ Yes Age: ○ No |
| ☐Age Unknown | ☐Age Unknown | ☐Age Unknown |

**2. *Cooed (making vowel sounds like "ooh," "ah")***

| *Learned* | *Lost* | *Relearned* |
|---|---|---|
| ○ Yes Age: ○ No | ○ Yes Age: ○ No | ○ Yes Age: ○ No |
| ☐Age Unknown | ☐Age Unknown | ☐Age Unknown |

**3. *Babbled ("bu, bu, bu")***

| *Learned* | *Lost* | *Relearned* |
|---|---|---|
| ○ Yes Age: ○ No | ○ Yes Age: ○ No | ○ Yes Age: ○ No |
| ☐Age Unknown | ☐Age Unknown | ☐Age Unknown |

**4. *Used words with meaning***

| *Learned* | *Lost* | *Relearned* |
|---|---|---|
| ○ Yes Age: ○ No | ○ Yes Age: ○ No | ○ Yes Age: ○ No |
| ☐Age Unknown | ☐Age Unknown | ☐Age Unknown |

**5. *Spoken in phrases (2 words or more with meaning)***

| *Learned* | *Lost* | *Relearned* |
|---|---|---|
| ○ Yes Age: ○ No | ○ Yes Age: ○ No | ○ Yes Age: ○ No |
| ☐Age Unknown | ☐Age Unknown | ☐Age Unknown |

**6. *Waved bye-bye***

| *Learned* | *Lost* | *Relearned* |
|---|---|---|
| ○ Yes Age: ○ No | ○ Yes Age: ○ No | ○ Yes Age: ○ No |
| ☐Age Unknown | ☐Age Unknown | ☐Age Unknown |

**7. *Pointed for something they want***

| *Learned* | *Lost* | *Relearned* |
|---|---|---|
| ○ Yes Age: ○ No | ○ Yes Age: ○ No | ○ Yes Age: ○ No |
| ☐Age Unknown | ☐Age Unknown | ☐Age Unknown |

**8. *Shared stories***

| *Learned* | *Lost* | *Relearned* |
|---|---|---|
| ○ Yes Age: ○ No | ○ Yes Age: ○ No | ○ Yes Age: ○ No |
| ☐Age Unknown | ☐Age Unknown | ☐Age Unknown |

**9. *Overall, how sudden was your child's loss in expressive language development?***

○ Not lost ○ Overnight ○ Days or weeks ○ 1-6 months ○ 6-12 months ○ >1 year

## F. Personal-social/Adaptive Development

**1. *Liked being held***

| *Learned* | *Lost* | *Relearned* |
|---|---|---|
| ○ Yes Age: ○ No | ○ Yes Age: ○ No | ○ Yes Age: ○ No |
| ☐Age Unknown | ☐Age Unknown | ☐Age Unknown |

**2. *Paid attention to a loud or prolonged sound***

| *Learned* | *Lost* | *Relearned* |
|---|---|---|
| ○ Yes Age: ○ No | ○ Yes Age: ○ No | ○ Yes Age: ○ No |
| ☐Age Unknown | ☐Age Unknown | ☐Age Unknown |

**3. *Fixed and followed an object with eyes***

| *F.3.a. Learned* | *F.3.b.Lost* | *F.3.c.Relearned* |
|---|---|---|
| ○ Yes Age: ○ No | ○ Yes Age: ○ No | ○ Yes Age: ○ No |
| ☐Age Unknown | ☐Age Unknown | ☐Age Unknown |

**4.. *Played pat-a-cake***

| *Learned* | *Lost* | *Relearned* |
|---|---|---|
| ○ Yes Age: ○ No | ○ Yes Age: ○ No | ○ Yes Age: ○ No |
| ☐Age Unknown | ☐Age Unknown | ☐Age Unknown |

**5. *Desired social attention***

| *Learned* | *Lost* | *Relearned* |
|---|---|---|
| ○ Yes Age: ○ No | ○ Yes Age: ○ No | ○ Yes Age: ○ No |
| ☐Age Unknown | ☐Age Unknown | ☐Age Unknown |

**6. *Imitated peers in play activities***

| *Learned* | *Lost* | *Relearned* |
|---|---|---|
| ○ Yes Age: ○ No | ○ Yes Age: ○ No | ○ Yes Age: ○ No |
| ☐Age Unknown | ☐Age Unknown | ☐Age Unknown |

**7. *Been independent – wanted to do things by themselves***

| *Learned* | *Lost* | *Relearned* |
|---|---|---|
| ○ Yes Age: ○ No | ○ Yes Age: ○ No | ○ Yes Age: ○ No |
| ☐Age Unknown | ☐Age Unknown | ☐Age Unknown |

**8. *Taken a drink from a cup held without assistance***

| *Learned* | *Lost* | *Relearned* |
|---|---|---|
| ○ Yes Age: ○ No | ○ Yes Age: ○ No | ○ Yes Age: ○ No |
| ☐Age Unknown | ☐Age Unknown | ☐Age Unknown |

**9. *Used a spoon/fork to eat <u>with</u> assistance?***

| *Learned* | *Lost* | *Relearned* |
|---|---|---|
| ○ Yes Age: ○ No | ○ Yes Age: ○ No | ○ Yes Age: ○ No |
| ☐Age Unknown | ☐Age Unknown | ☐Age Unknown |

**10. *Used a spoon/fork to eat <u>without</u> assistance?***

| *Learned* | *Lost* | *Relearned* |
|---|---|---|
| ○ Yes Age: ○ No | ○ Yes Age: ○ No | ○ Yes Age: ○ No |
| ☐Age Unknown | ☐Age Unknown | ☐Age Unknown |

**11. *Overall, how sudden was your child's loss in adaptive development?***

○ Not lost ○ Overnight ○ Days or weeks ○ 1-6 months ○ 6-12 months ○ >1 year

## G. <u>Other Developmental Skills</u>

**1. *Has your child gained or lost any other skills? If so, what skill?***

☐ ***NOT APPLICABLE, HAS NOT LOST ANY OTHER SKILLS***

| *Learned – Specify skill:* | *Lost* | *Relearned* |
|---|---|---|
| ○ Yes Age: ○ No | ○ Yes Age: ○ No | ○ Yes Age: ○ No |
| ☐Age Unknown | ☐Age Unknown | ☐Age Unknown |

## H. <u>Relationship of New Onset Seizures to Regression</u>

**1. *Did the regression or loss of skills occur at the same time your child first developed seizures?***

○ Yes ○ No ○ Not applicable (no loss of skills **and/or** no seizures)

## Rett Features Log

| *Feature* | *Ever occurred?* | *If yes, date started (DD/MMM/YYYY)* | *If start date unknown, enter age* | *Currently occurring?* | *Stopped?* | *Date stopped (DD/MMM/YYYY)* | *If stop date unknown, enter age* |
|---|---|---|---|---|---|---|---|
| Hand stereotypies | | | | | | | |
| Hyperventilation when awake | | | | | | | |
| Breath holding when awake | | | | | | | |
| Cold or hot hand and/or feet | | | | | | | |
| Drooling when awake | | | | | | | |
| Aerophagia | | | | | | | |
| Puffing air/saliva | | | | | | | |
| Spitting | | | | | | | |
| Bruxism when awake | | | | | | | |
| Chewing/swallowing problems | | | | | | | |
| GE reflux | | | | | | | |
| Constipation | | | | | | | |
| Gall bladder dysfunction | | | | | | | |
| Difficulty falling asleep at night | | | | | | | |
| Waking during the night | | | | | | | |
| Hard to wake in the morning | | | | | | | |
| Sleeping during the day | | | | | | | |
| Screaming spells | | | | | | | |
| Self-abusive behaviors | | | | | | | |
| Hyperactivity | | | | | | | |
| Low activity | | | | | | | |
| Anxiety | | | | | | | |
| Scoliosis | | | | | | | |



**Ever Occurred?**

***Rett Features Log***

*Version date 2.19.15*

*Clinician Completed*

Yes
No
**If yes, date started?**
Date
Unknown
**If date unknown, enter Age**
Free text
N/A
**Currently occurring?**
Yes
No
**Stopped?**
Yes
No
**Date stopped?**
Date
Unknown
**If stop date not known, enter age:**
Free text
N/A

APPENDIX B

SUPPLEMENTAL FIGURES

**Table B.1. Frequencies of Rett features.**

| Rett Feature | % Present |
|---|---|
| Hand Stereotypies | 99.75% |
| Constipation | 93.07% |
| Drooling | 91.89% |
| Bruxism | 91.81% |
| Breath Holding | 88.63% |
| Cold/Hot Hands | 83.78% |
| Waking at Night | 78.60% |
| Hyperventilation | 74.50% |
| Puffing Air | 74.33% |
| GE Reflux | 71.99% |
| Scoliosis | 71.43% |
| Aerophagia | 70.65% |
| Day Sleeping | 69.48% |
| Chewing Problems | 68.38% |
| Anxiety | 64.85% |
| Screaming Spells | 60.35% |
| Sleep Difficulty | 59.03% |

| | |
|---|---|
| Self-Abusive Behavior | 57.44% |
| Low Activity | 46.23% |
| Hard to Wake Up | 35.37% |
| Hyperactivity | 20.87% |
| Spitting | 14.77% |
| Gall Bladder Dysfunction | 8.61% |

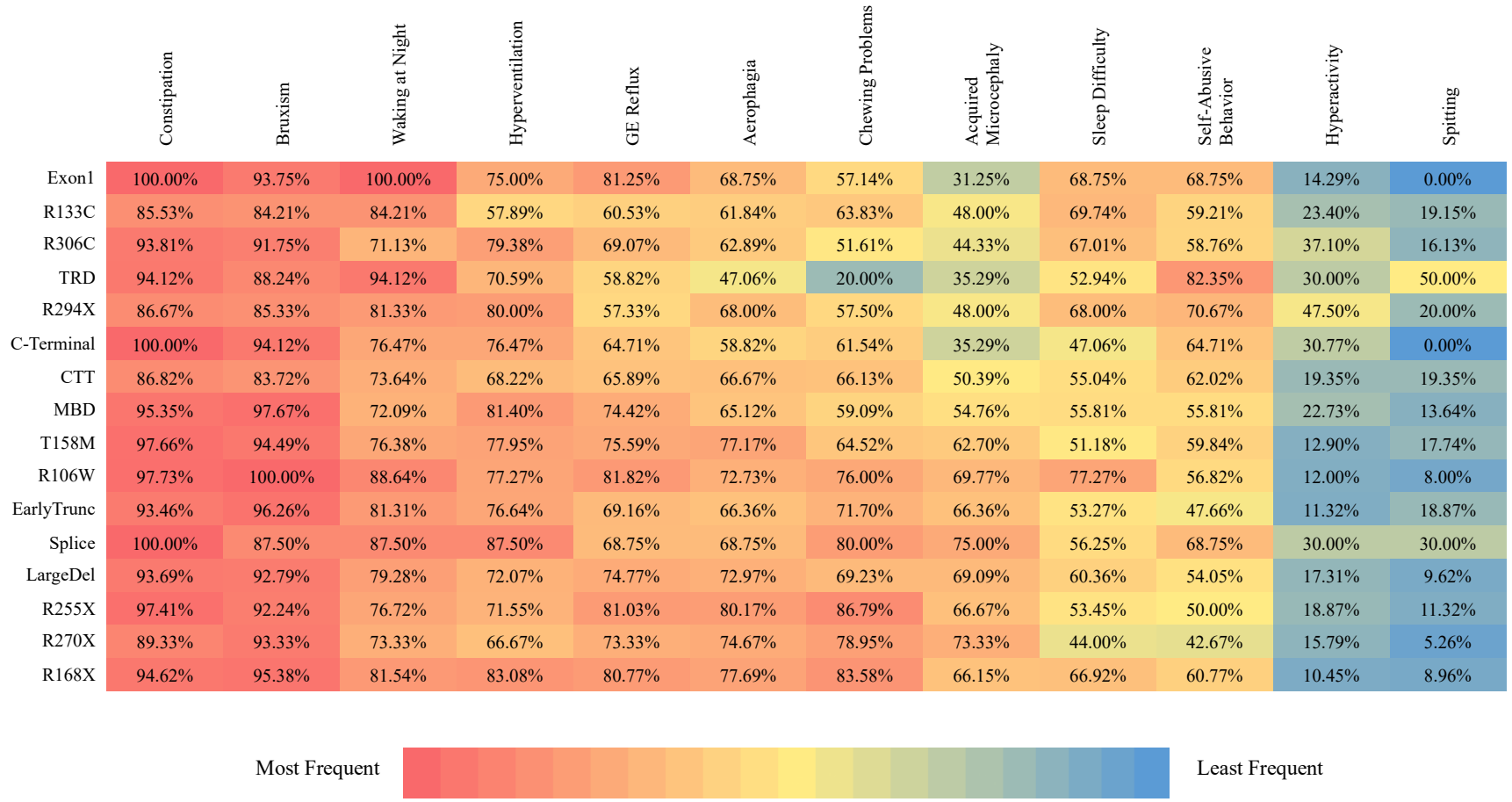



**Figure B.1. Frequencies of Rett features by *MECP2* variant.**

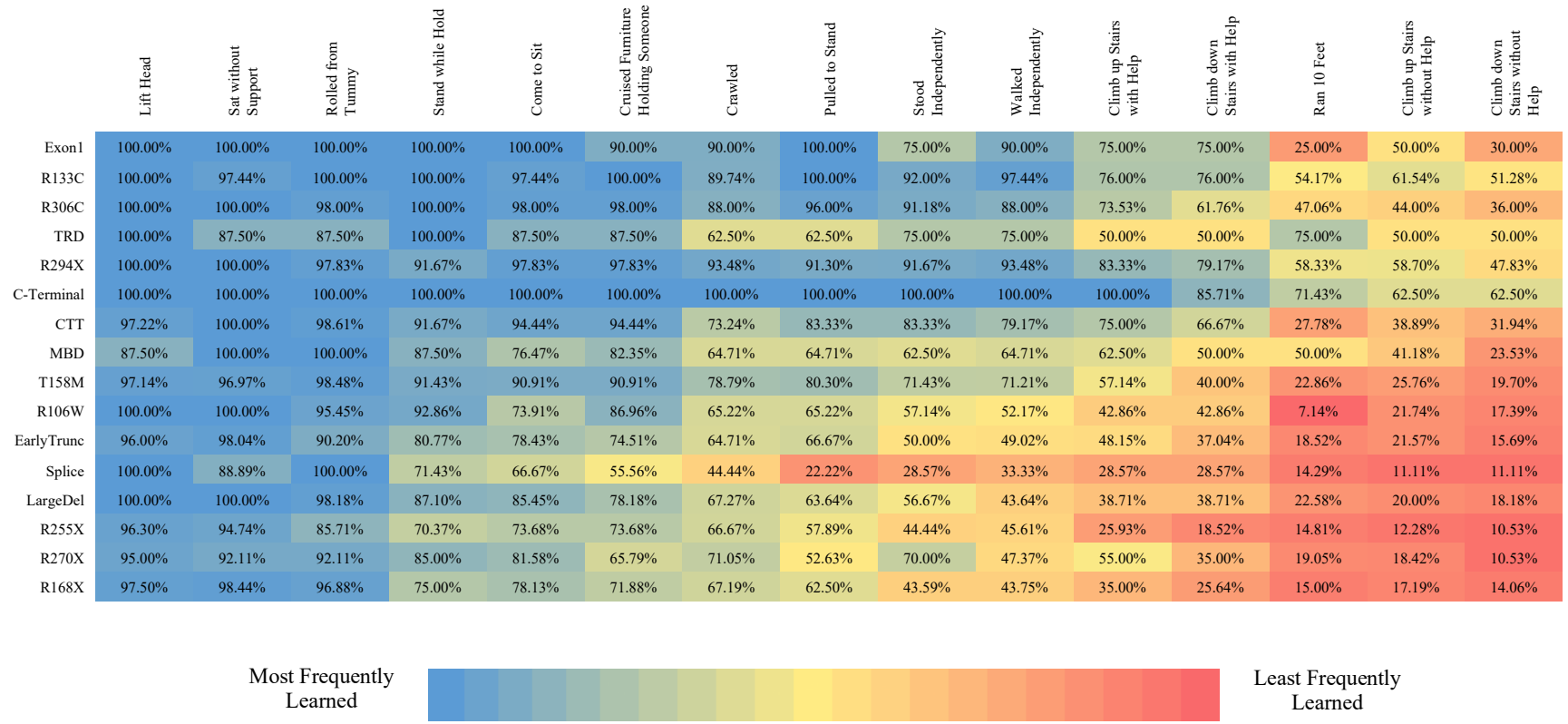



**Figure B.2. Frequencies of gross motor skills learned by *MECP2* variant.**

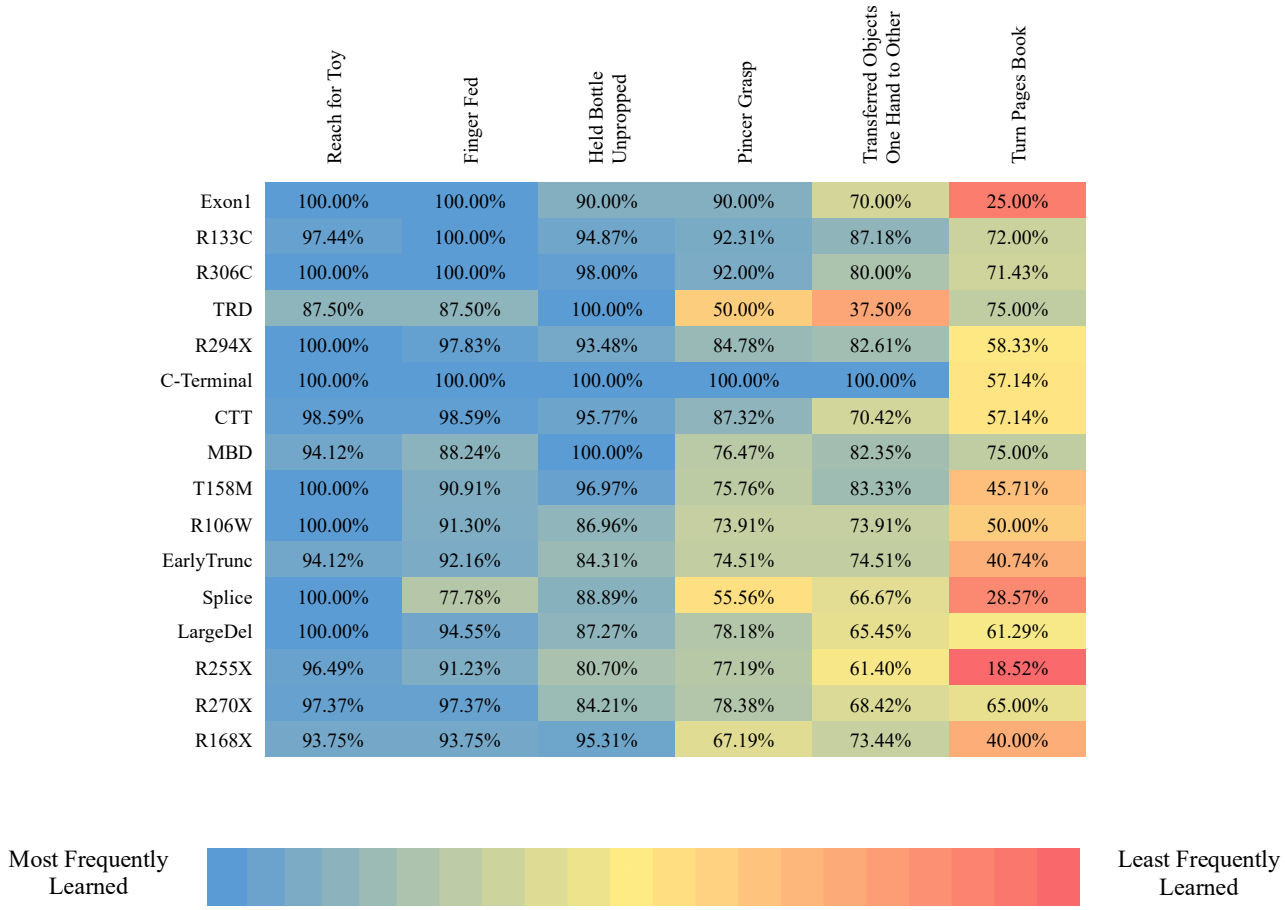



**Figure B.3. Frequencies of fine motor skills learned by *MECP2* variant.**

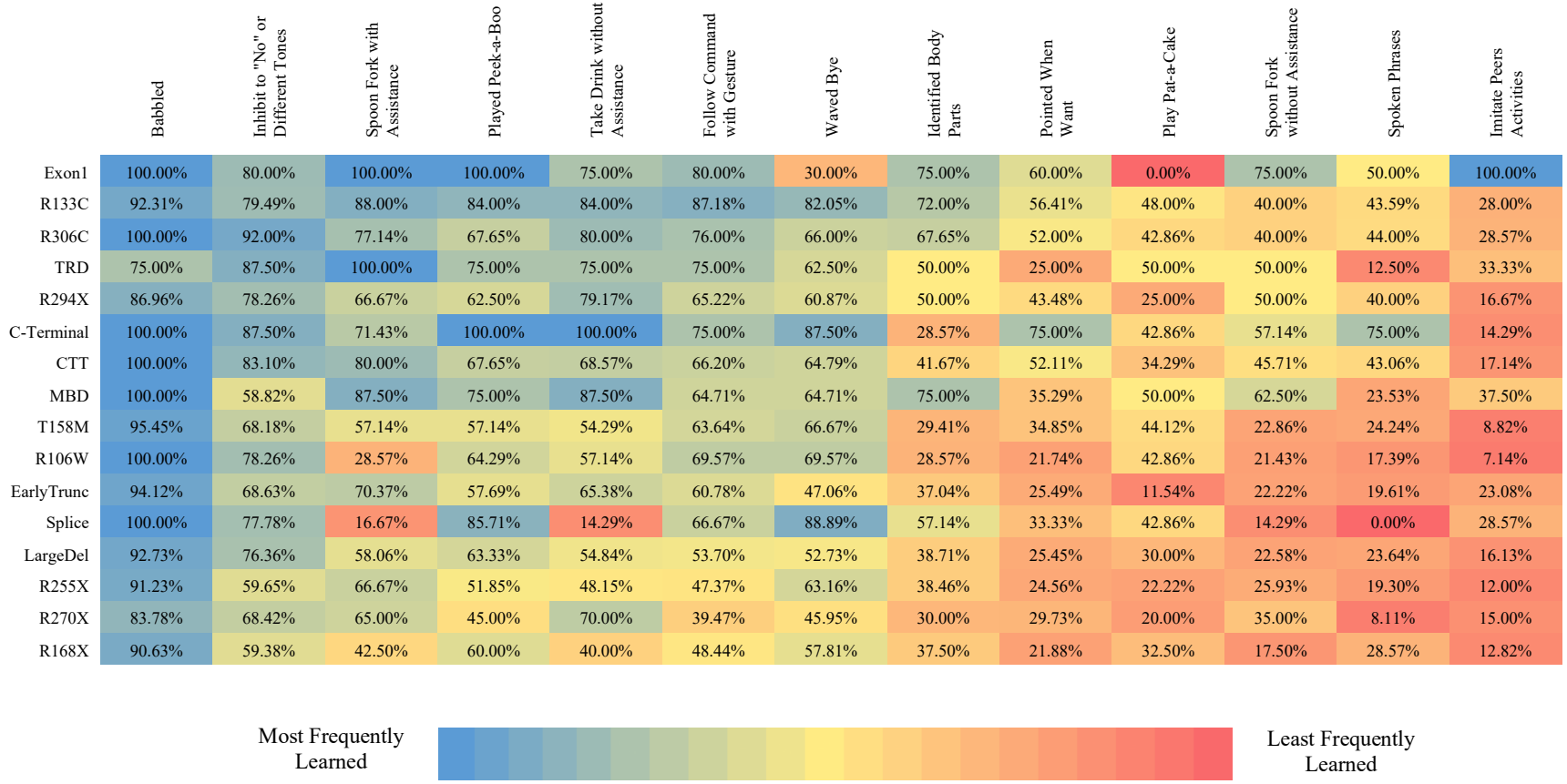
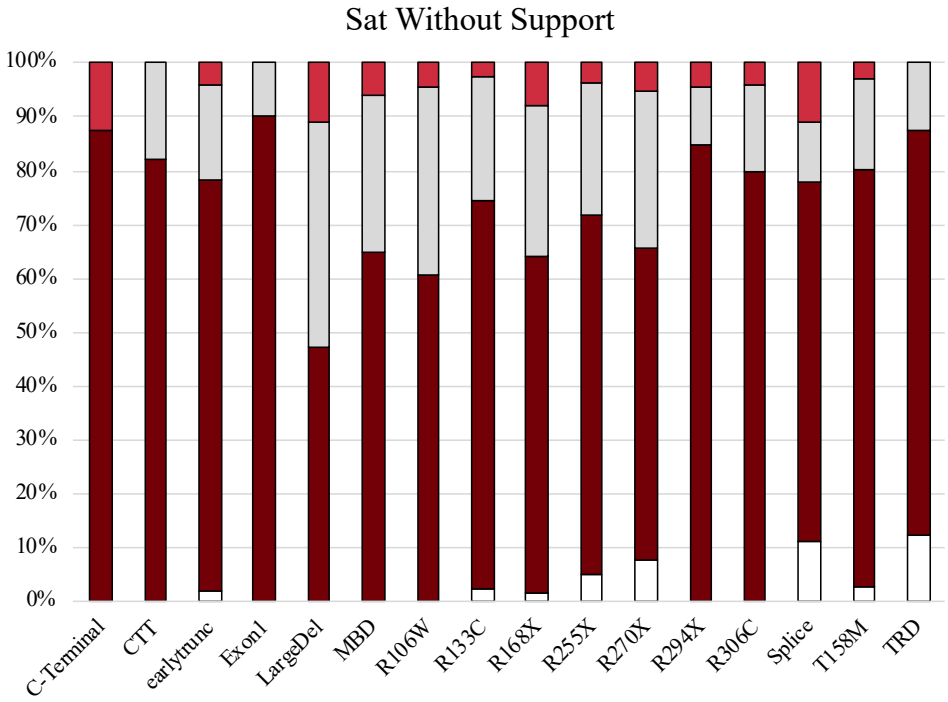
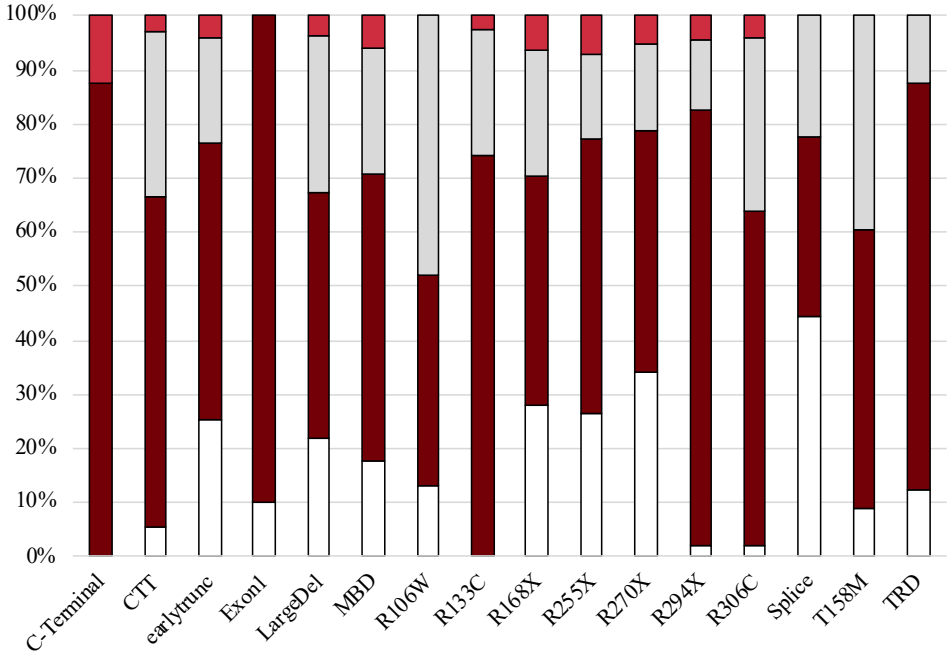


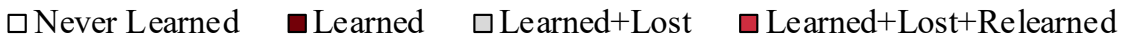

**Figure B.4. Frequencies of language and social skills learned by *MECP2* variant.**

**A.**

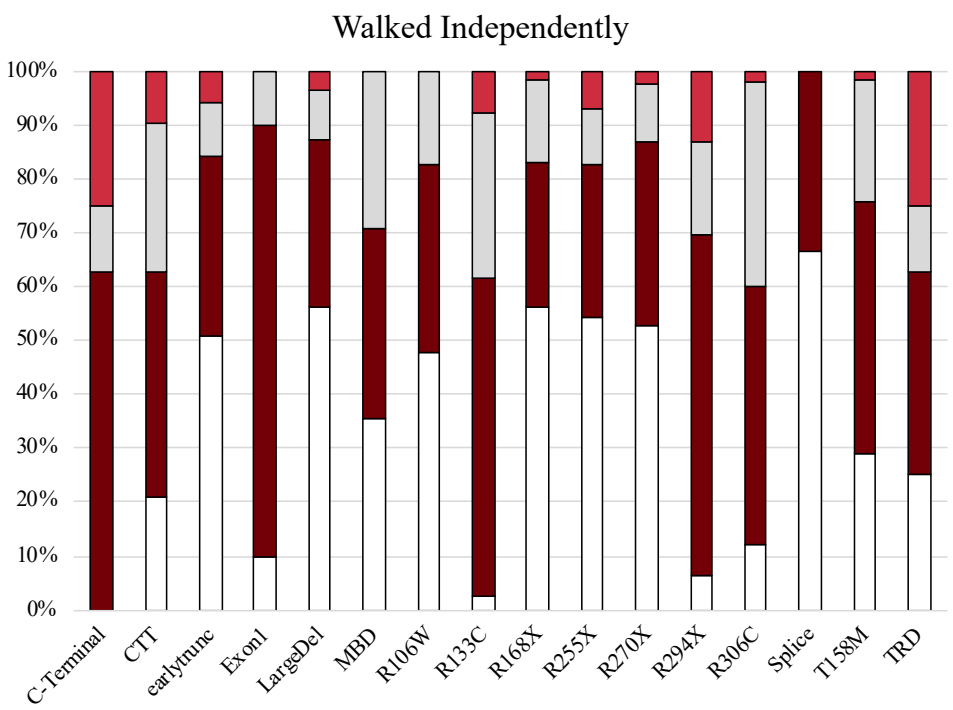

Sat Without Support
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
C-Terminal
CTT
earlytrunc
Exon1
LargeDel
MBD
R106W
R133C
R168X
R255X
R270X
R294X
R306C
Splice
T158M
TRD

**B.**

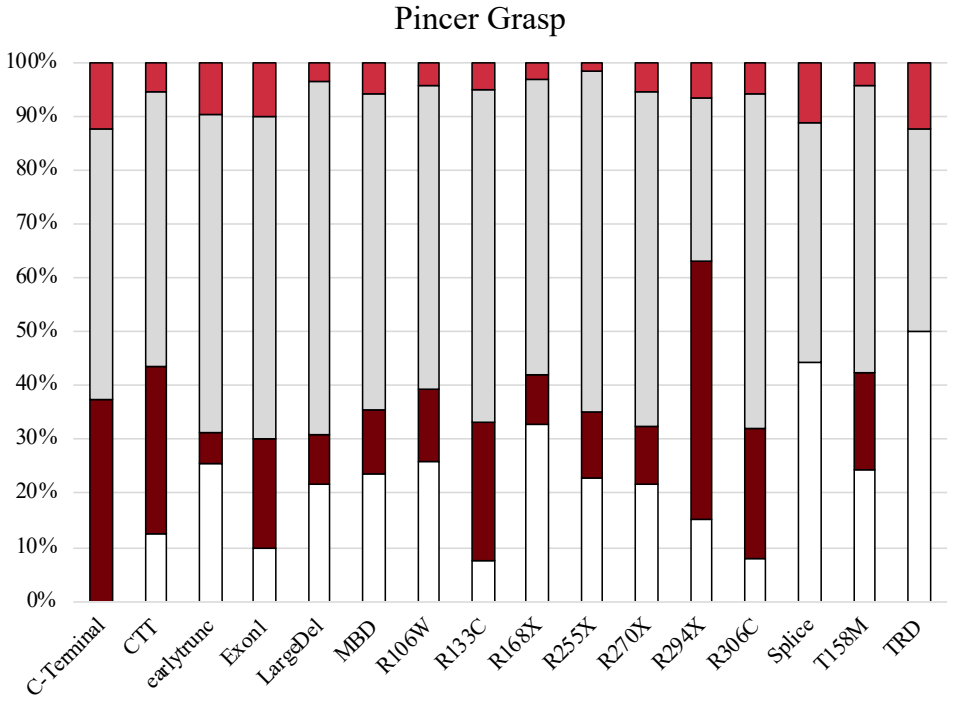

Cruised Furniture Holding Someone
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
C-Terminal
CTT
earlytrunc
Exon1
LargeDel
MBD
R106W
R133C
R168X
R255X
R270X
R294X
R306C
Splice
T158M
TRD


Never Learned
Learned
Learned+Lost
Learned+Lost+Relearned

**C.**


Walked Independently
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
C-Terminal
CTT
earlytrunc
Exon1
LargeDel
MBD
R106W
R133C
R168X
R255X
R270X
R294X
R306C
Splice
T158M
TRD

**D.**


Pincer Grasp
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70%
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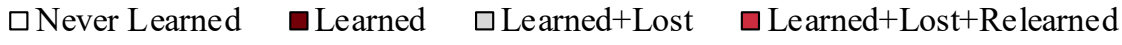
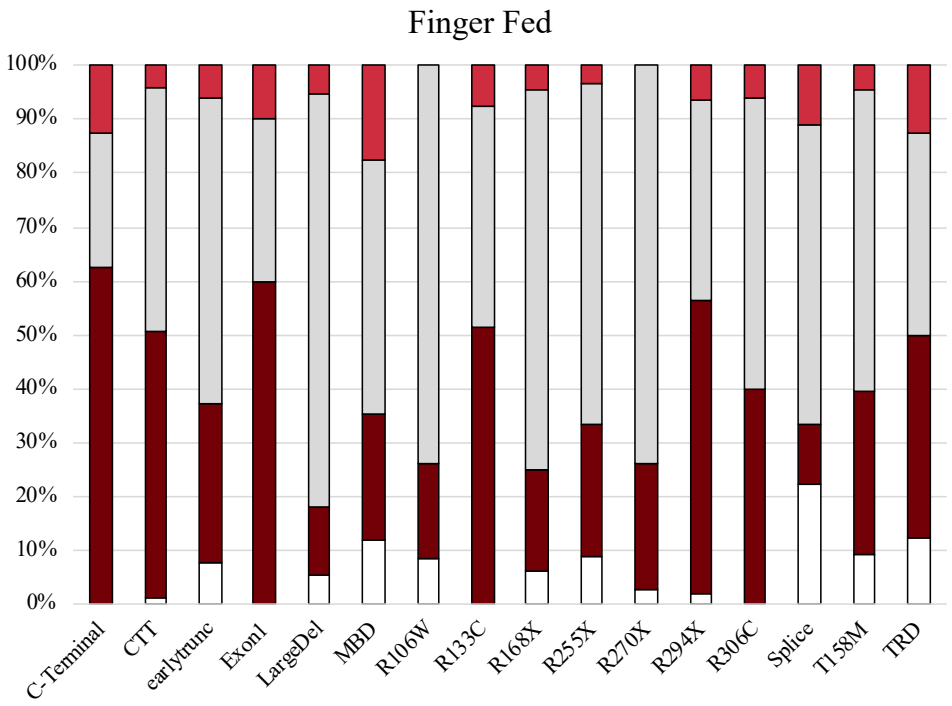
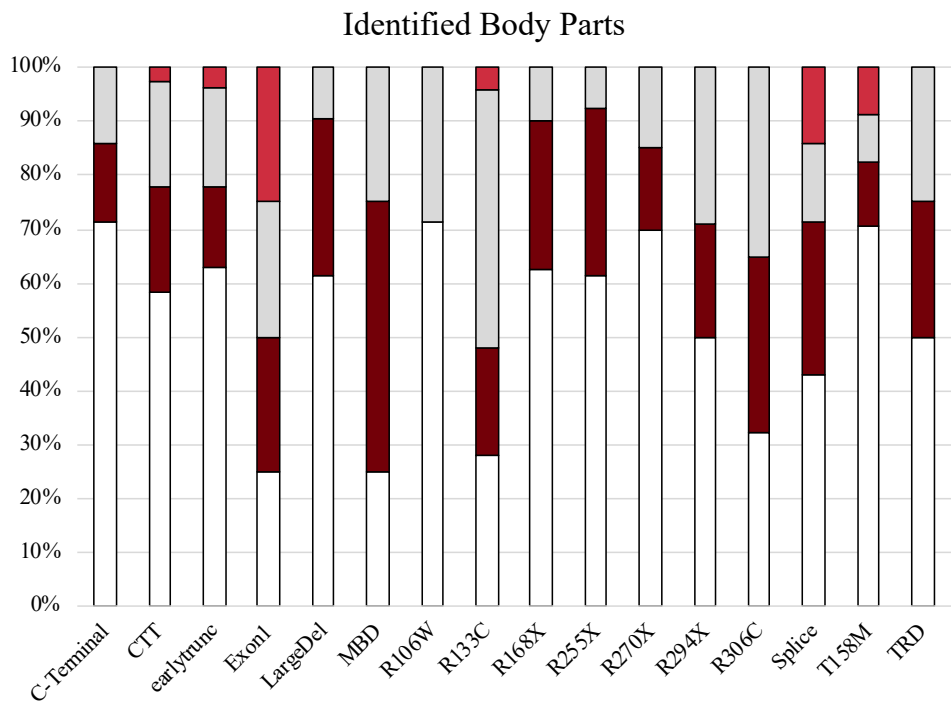
40%
30%
20%
10%
0%
C-Terminal
CTT
earlytrunc
Exon1
LargeDel
MBD
R106W
R133C
R168X
R255X
R270X
R294X
R306C
Splice
T158M
TRD

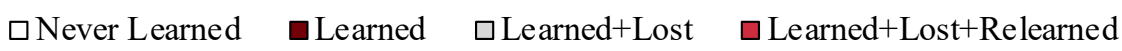

Never Learned
Learned
Learned+Lost
Learned+Lost+Relearned

**E.**


Finger Fed
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
C-Terminal
CTT
earlytrunc
Exon1
LargeDel
MBD
R106W
R133C
R168X
R255X
R270X
R294X
R306C
Splice
T158M
TRD

**F.**


Identified Body Parts
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
C-Terminal
CTT
earlytrunc
Exon1
LargeDel
MBD
R106W
R133C
R168X
R255X
R270X
R294X
R306C
Splice
T158M
TRD


Never Learned
Learned
Learned+Lost
Learned+Lost+Relearned

**G.**

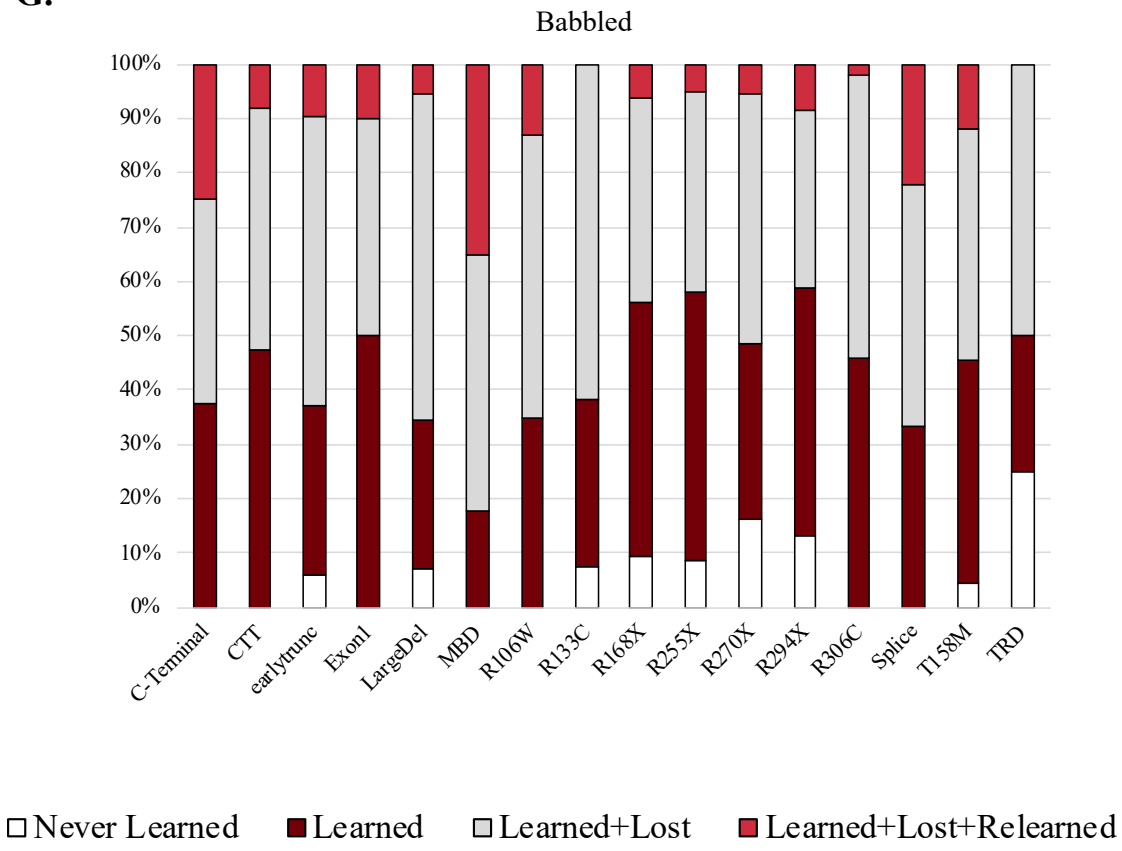



**Figure B.5. Developmental history by *MECP2* variant.**

**Table B.2. Motor skills – frequencies learned, lost, and relearned.**

| Skill | Never Learned | Learned | Learned+Lost | Learned+Lost +Relearned |
|---|---|---|---|---|
| Sat w/ Support | 1.46% | 85.37% | 8.94% | 4.23% |
| Lift Head | 1.79% | 91.84% | 4.08% | 2.28% |
| Reach For Toy | 2.43% | 42.45% | 43.46% | 11.66% |
| Sat w/o Support | 2.77% | 78.12% | 14.25% | 4.86% |
| Rolled Fr Tummy | 3.87% | 63.16% | 28.43% | 4.54% |
| Finger Fed | 7.80% | 33.72% | 52.43% | 6.04% |
| Raking Grasp Retrieve | 10.11% | 38.17% | 46.82% | 4.89% |
| Held Bottle Unpropped | 10.91% | 34.98% | 48.99% | 5.12% |
| Stand While Hold | 13.96% | 61.08% | 22.00% | 2.96% |
| Come to Sit | 17.03% | 60.40% | 19.30% | 3.27% |
| Cruised Furn Hold Someone | 20.91% | 58.35% | 17.38% | 3.36% |
| Pincer Grasp | 23.01% | 18.22% | 53.23% | 5.54% |
| Trans Obj Hand to Other | 23.09% | 22.33% | 51.47% | 3.11% |
| Crawled | 29.10% | 52.90% | 17.07% | 0.93% |
| Pulled to Stand | 32.30% | 39.01% | 25.42% | 3.27% |

| | | | | |
|---|---|---|---|---|
| Stood Independently | 34.86% | 46.81% | 15.88% | 2.45% |
| Walked Independently | 40.91% | 42.50% | 12.99% | 3.60% |
| Turn Pages Book | 43.23% | 14.68% | 39.80% | 2.28% |
| Climb up Stairs w/ Help | 45.92% | 37.58% | 14.38% | 2.12% |
| Climb Down Stairs w/ Help | 55.79% | 30.51% | 12.07% | 1.63% |
| Ran 10 Feet | 70.41% | 16.91% | 11.54% | 1.14% |
| Climb up Stairs w/o Help | 74.50% | 14.60% | 9.82% | 1.09% |
| Climbed Down Stairs w/o Help | 80.87% | 10.40% | 8.05% | 0.67% |

**Table B.3. Language skills – frequencies learned, lost, and relearned.**

| Skill | Never Learned | Learned | Learned+Lost | Learned+Lost+ Relearned |
|---|---|---|---|---|
| Social Smile | 0.50% | 81.11% | 3.61% | 14.78% |
| Respond To Sounds | 2.61% | 81.60% | 3.26% | 12.54% |
| Respond Own Name | 3.92% | 78.79% | 4.73% | 12.56% |
| Babbled | 4.71% | 42.52% | 37.73% | 15.04% |
| Cooed | 6.13% | 56.26% | 29.30% | 8.31% |
| Respond Familiar Words | 8.50% | 73.53% | 8.50% | 9.48% |
| Quiet Soothe Adult Voice | 10.26% | 68.29% | 6.90% | 14.55% |
| Words With Meaning | 23.41% | 8.22% | 57.13% | 11.24% |
| Inhibit Diff Tones | 28.97% | 52.48% | 9.82% | 8.73% |
| Played Peekaboo | 36.72% | 26.72% | 33.77% | 2.79% |
| Follow Command With Gesture | 41.06% | 30.19% | 20.07% | 8.68% |
| Waved Bye | 41.68% | 6.89% | 47.82% | 3.61% |
| Command Without Gesture | 51.97% | 25.61% | 15.28% | 7.14% |
| Pointed Color Asked | 53.59% | 37.25% | 6.05% | 3.10% |

| | | | | |
|---|---|---|---|---|
| Identified Body Parts | 57.61% | 22.75% | 16.86% | 2.78% |
| Pointed When Want | 69.89% | 9.25% | 17.91% | 2.94% |
| Spoken Phrases | 76.37% | 2.86% | 18.67% | 2.10% |
| Shared Stories | 97.55% | 0.82% | 1.47% | 0.16% |

**Table B.4. Personal-Social/Adaptive Skills– frequencies learned, lost, and relearned.**

| Skill | Never Learned | Learned | Learned+Lost | Learned+Lost+ Relearned |
|---|---|---|---|---|
| Like Being Held | 4.04% | 77.38% | 5.21% | 13.37% |
| Fixed Follow Object | 4.97% | 66.39% | 7.50% | 21.15% |
| Attention Loud Pr Sound | 11.13% | 71.52% | 5.07% | 12.27% |
| Desire Social Atten | 15.79% | 68.59% | 4.28% | 11.35% |
| Spoon Fork With Assist | 32.57% | 32.25% | 30.78% | 4.40% |
| Take Drink w/o Assist | 37.91% | 23.69% | 35.13% | 3.27% |
| Been Independent | 64.85% | 20.13% | 12.71% | 2.31% |
| Play PatACake | 71.26% | 5.91% | 22.33% | 0.49% |
| Spoon Fork w/o Assist | 73.25% | 7.01% | 17.94% | 1.79% |
| Imitate Peers Activities | 78.51% | 5.95% | 13.88% | 1.65% |

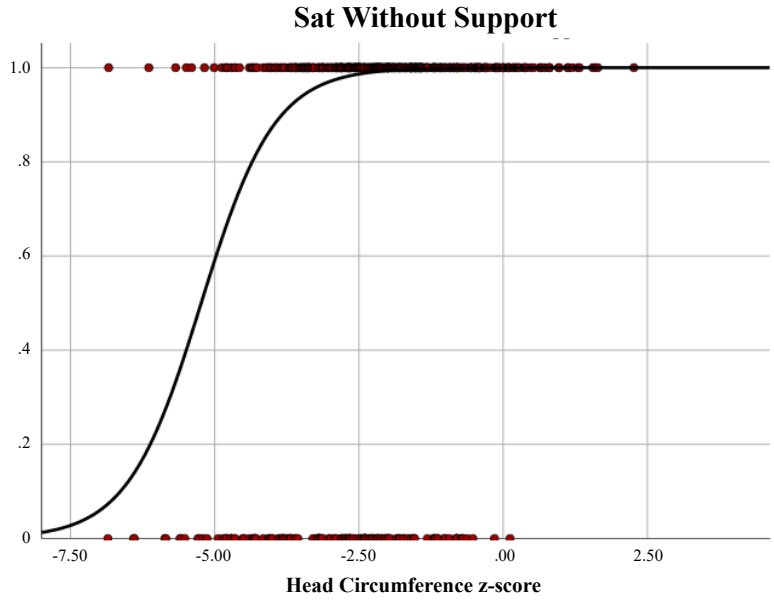
**Table B.5. Age of onset of regression by *MECP2* variant.**

| Variant Grouping | <6mos | 6-<12mos | 12-<18mos | 18-30mos | >30mos | TOTAL |
|---|---|---|---|---|---|---|
| **Exon1** | 1 | 1 | 5 | 5 | 4 | 16 |
| **R133C** | 0 | 2 | 16 | 47 | 10 | 75 |
| **R306C** | 1 | 4 | 22 | 54 | 15 | 96 |
| **TRD** | 0 | 0 | 8 | 6 | 3 | 17 |
| **R294X** | 1 | 2 | 18 | 45 | 9 | 75 |
| **C-Terminal** | 0 | 2 | 5 | 9 | 1 | 17 |
| **CTT** | 2 | 11 | 40 | 58 | 18 | 129 |
| **MBD** | 0 | 3 | 13 | 22 | 5 | 43 |
| **T158M** | 0 | 9 | 53 | 59 | 6 | 127 |
| **R106W** | 1 | 2 | 20 | 20 | 1 | 44 |
| **Early Truncation** | 2 | 14 | 45 | 39 | 7 | 107 |
| **Splice** | 0 | 0 | 6 | 9 | 1 | 16 |
| **Large Deletion** | 2 | 8 | 41 | 52 | 8 | 111 |
| **R255X** | 4 | 9 | 56 | 40 | 7 | 116 |
| **R270X** | 1 | 9 | 28 | 33 | 4 | 75 |
| **R168X** | 1 | 8 | 64 | 53 | 3 | 129 |
| **TOTAL** | 16 | 84 | 440 | 551 | 102 | **1193** |

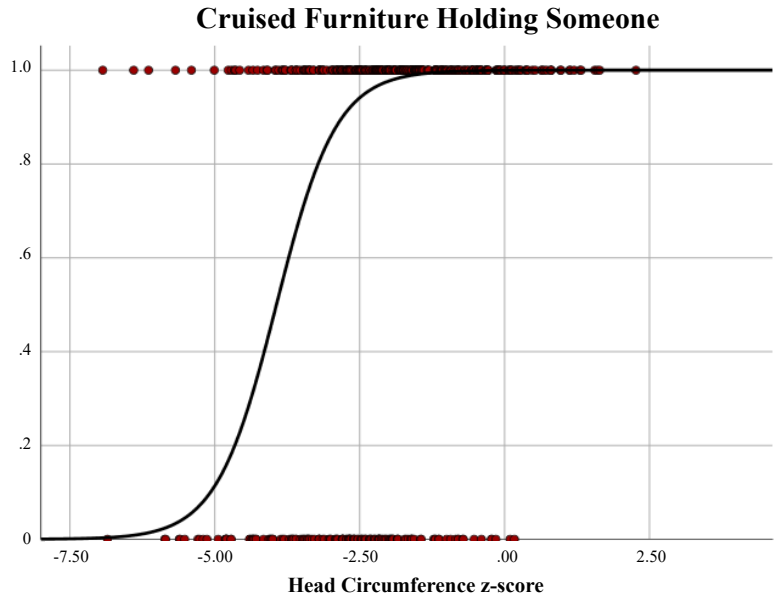
**Table B.6. Developmental skills – average ages learned, lost, and relearned.**
*Ages are reported in months

| Developmental Skill | Average Age Learned* (SEM) | Average Age Lost* (SEM) | Average Age Relearned* (SEM) |
|---|---|---|---|
| Sat Without Support | 7.55 (0.26) | 73.25 (5.73) | 74.47 (13.98) |
| Babbled | 7.98 (0.42) | 27.12 (1.84) | 46.84 (6.15) |
| Finger Fed | 10.67 (0.28) | 36.72 (2.25) | 71.17 (14.02) |
| Respond Familiar Words | 10.80 (2.03) | 23.62 (1.75) | 59.00 (16.48) |
| Raking Grasp | 11.14 (1.25) | 41.57 (5.25) | 84.43 (37.73) |
| Pincer Grasp | 11.64 (0.58) | 31.43 (1.49) | 78.26 (13.14) |
| Words with Meaning | 12.40 (0.36) | 27.99 (1.45) | 49.53 (4.45) |
| Cruised Furniture Holding Someone | 16.17 (0.49) | 67.28 (5.59) | 84.32 (12.00) |
| Walked Independently | 21.02 (0.72) | 110.92 (8.05) | 96.55 (19.98) |
| Identified Body Parts | 33.46 (5.26) | 30.73 (2.73) | 64.00 (17.44) |

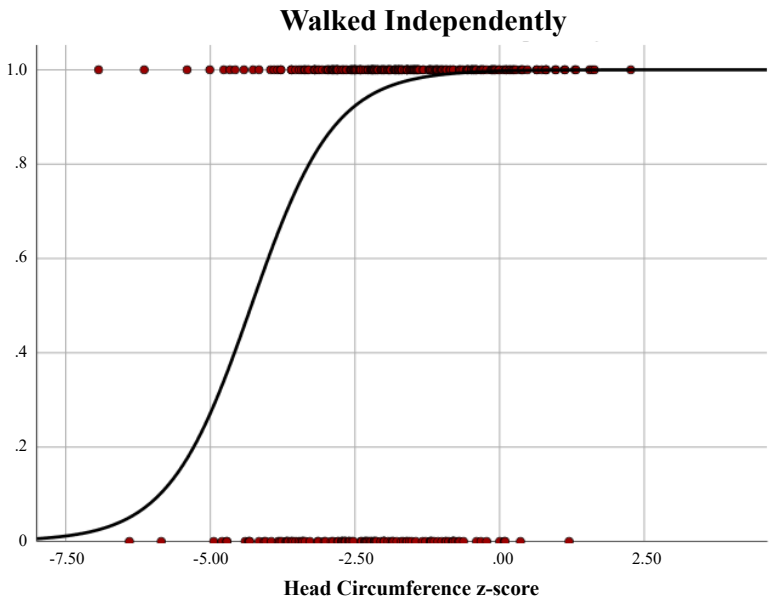
**A.**


Sat Without Support
1.0
.8
.6
.4
.2
0
-7.50
-5.00
-2.50
.00
2.50
Head Circumference z-score

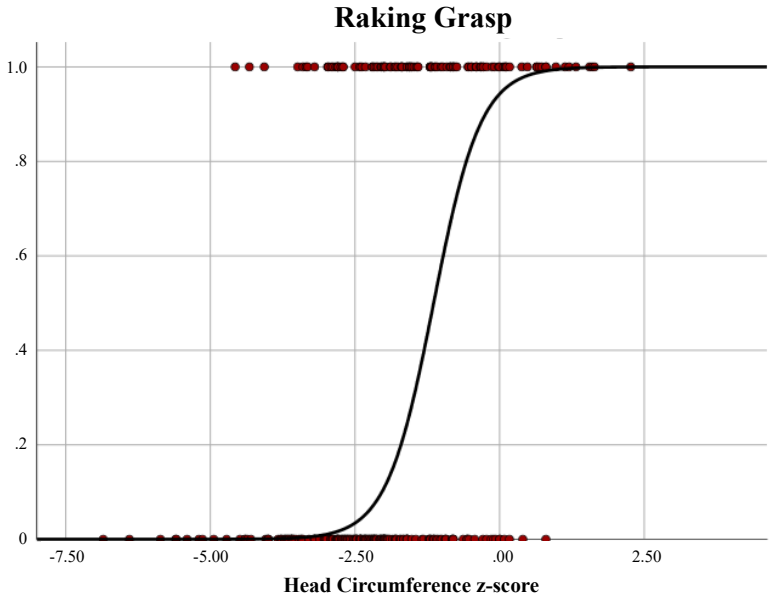
**B.**


Cruised Furniture Holding Someone
1.0
.8
.6
.4
.2
0
-7.50
-5.00
-2.50
.00
2.50
Head Circumference z-score

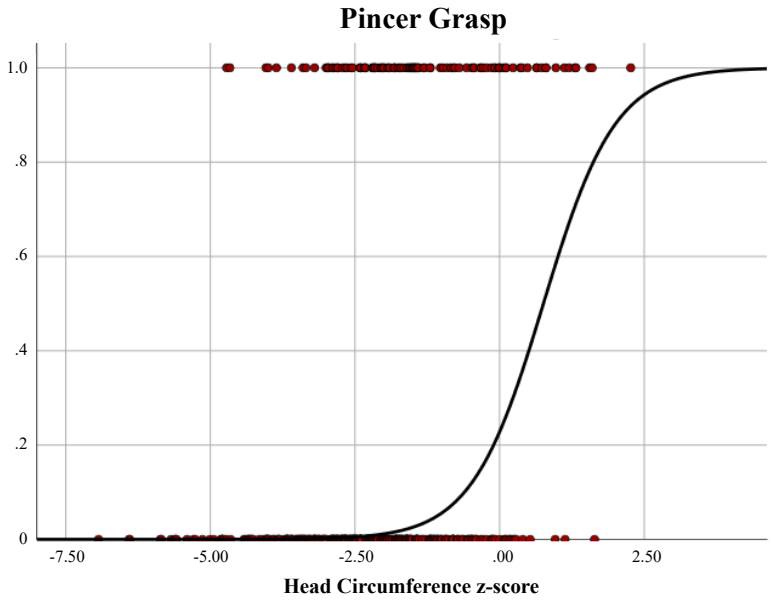
**C.**


Walked Independently
1.0
.8
.6
.4
.2
0
-7.50
-5.00
-2.50
.00
2.50
Head Circumference z-score

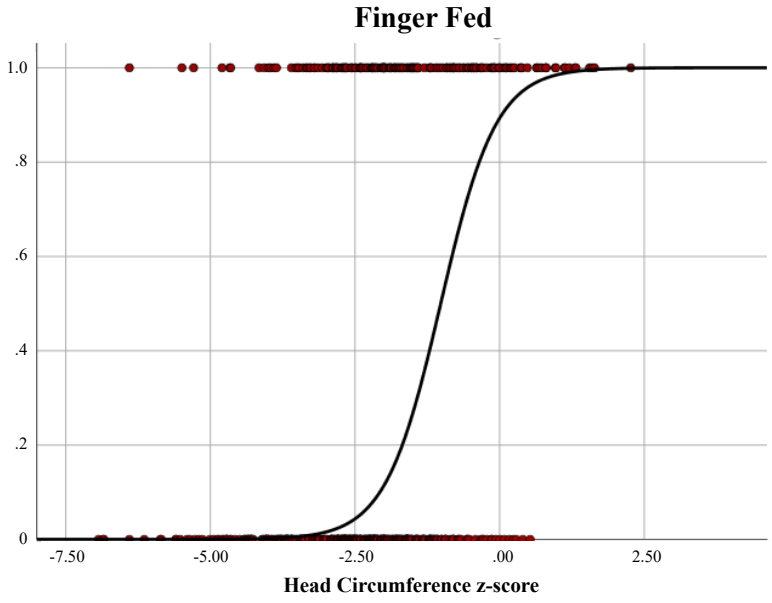
**D.**


Raking Grasp
1.0
.8
.6
.4
.2
0
-7.50
-5.00
-2.50
.00
2.50
Head Circumference z-score

**E.**


Pincer Grasp
1.0
.8
.6
.4
.2
0
-7.50
-5.00
-2.50
.00
2.50
Head Circumference z-score

**F.**


Finger Fed
1.0
.8
.6
.4
.2
0
-7.50
-5.00
-2.50
.00
2.50
Head Circumference z-score

**G.**

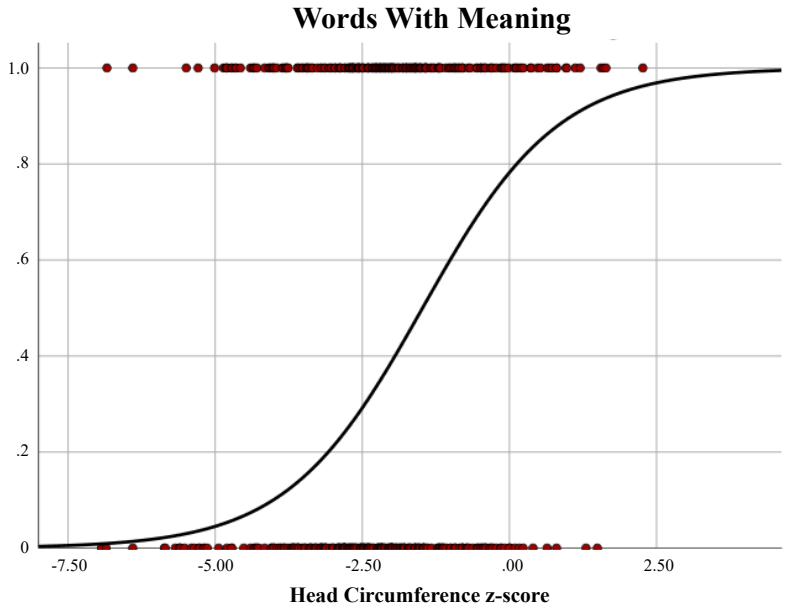



**Figure B.6. Developmental skills by head circumference.** Points represent individual cases. Lines represent logistic curves. A measurement of 0.0 indicates patients who learned and lost a skill, and a measurement of 1.0 indicates patients that learned and retained a skill.