Biomarkers of Familial Speech Sound Disorders: Genes, Perception, and Motor Control

by

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ABSTRACT

Speech sound disorders (SSDs) are the most prevalent type of communication disorder in children. Clinically, speech-language pathologists (SLPs) rely on behavioral methods for assessing and treating SSDs. Though clients typically experience improved speech outcomes as a result of therapy, there is evidence that underlying deficits may persist even in individuals who have completed treatment for surface-level speech behaviors. Advances in the field of genetics have created the opportunity to investigate the contribution of genes to human communication. Due to the heterogeneity of many communication disorders, the manner in which specific genetic changes influence neural mechanisms, and thereby behavioral phenotypes, remains largely unknown. The purpose of this study was to identify genotype-phenotype associations, along with perceptual, and motor-related biomarkers within families displaying SSDs. Five parent-child trios participated in genetic testing, and five families participated in a combination of genetic and behavioral testing to help elucidate biomarkers related to SSDs. All of the affected individuals had a history of childhood apraxia of speech (CAS) except for one family that displayed a phonological disorder. Genetic investigation yielded several genes of interest relevant for an SSD phenotype: *CNTNAP2, CYFIP1, GPR56, HERC1, KIAA0556, LAMA5, LAMB1, MDGA2, MECP2, NBEA, SHANK3, TENM3,* and *ZNF142*. All of these genes showed at least some expression in the developing brain. Gene ontology analysis yielded terms supporting a genetic influence on central nervous system development. Behavioral testing revealed evidence of a sequential processing biomarker for all individuals with CAS, with many showing deficits in sequential motor skills in addition to speech deficits. In some families, participants also showed evidence of a co-

occurring perceptual processing biomarker. The family displaying a phonological phenotype showed milder sequential processing deficits compared to CAS families. Overall, this study supports the presence of a sequential processing biomarker for CAS and shows that relevant genes of interest may be influencing a CAS phenotype via sequential processing. Knowledge of these biomarkers can help strengthen precision of clinical assessment and motivate development of novel interventions for individuals with SSDs.

DEDICATION

This dissertation is dedicated to Dr. Tiffany Mealman, whose life as a professor was an inspiration.

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INTRODUCTION

Speech Sound Disorders

It is not uncommon for speech sound errors to occur in children as they learn to talk, yet some children will present with significant difficulty acquiring speech sounds, impacting their ability to be understood by communication partners. During development, the speech system of a child grows more refined with age and linguistic experience through an interplay of speech perception and production, moving toward adult-like perception around age 10 (Byun, 2012; Kent & Rountrey, 2020) and adult-like production between ages 5-8 (McLeod & Crowe, 2018; Stein et al., 2011). Speech sound disorders (SSDs) "is an umbrella term referring to any difficulty or combination of difficulties with perception, motor production, or phonological representation of speech sounds and speech segments…" (ASHA, 2017). SSDs are the most prevalent type of communication disorder among children (Dodd, 2014), with rates reported to range from 2.3%-24.6% depending on age (Wren, Miller, Peters, Emond, & Roulstone, 2016), with a mean of 8.2% (Pennington & Bishop, 2009). This high variability suggests that establishing a clear categorical boundary for SSDs is not a simple task. Males are 1.5-2.4 times more likely to exhibit SSDs than females (Pennington & Bishop, 2009). In fact, three risk factors that have been associated with speech delay specifically, include being male, lower maternal education, and family history (Campbell et al., 2003; Shriberg, 2009). While some children are affected by SSDs as an isolated disorder, SSDs can co-occur with language impairment (Lewis et al., 2006), literacy difficulties (Farquharson, 2019; Peterson, Pennington, Shriberg, & Boada, 2009), and fine/gross motor deficits

(Farquharson, 2015b; Redle et al., 2015). Such comorbidities suggest the potential for shared genetic etiologies. Within the United States, many children with SSDs receive treatment in the public-school system. The ASHA 2018 Schools Survey indicates that 93.7% of elementary SLPs and 78.7% of secondary SLPs "regularly serve clients" with SSDs (ASHA, 2018, p. 27), making accurate diagnosis and precise treatment of SSDs a priority for pediatric speech-language pathologists (SLPs).

Classification of SSDs

Individuals affected by SSDs make up a heterogeneous group, and for many cases the etiology is unknown (Brosseau-Lapré, Schumaker, & Kluender, 2020; Farquharson, 2019). Such heterogeneity poses a challenge in reaching a universal system of classification; and indeed, there has yet to be consensus on any one classification approach for SSDs (Waring & Knight, 2013). The ultimate purpose of any classification system is to move from heterogeneity toward homogeneity for the purposes of research clarity, accurate diagnosis, and precise treatment. For SSDs in particular, there has been a shift in terminology away from the more dichotomous categorization of *articulation disorders* and *phonological disorders* toward the more general term *speech sound disorders*, thus facilitating inclusion of other disorders that fall beneath the SSD "umbrella" such as childhood apraxia of speech (CAS) (Shriberg, 2009). As such, SSDs have been conceptualized as multifaceted rather than binary in nature (Farquharson, 2019). The literature is filled with a variety of approaches to categorization for SSDs. Broad approaches tend to be influenced by a medical model, as exemplified by the Diagnostic and Statistical Manual (DSM), the International Classification of Diseases and Related Health Problems (ICD-10), and the International Classification of Functioning,

Disability and Health (ICF-CY) (Waring & Knight, 2013). Specific approaches to classification within the area of SSDs have included the Speech Disorders Classification System (SDCS) developed by Shriberg, the Differential Diagnosis system developed by Dodd, and the Psycholinguistic Framework system developed by Stackhouse and Wells (Tyler, 2011; Waring & Knight, 2013).

The Speech Disorders Classification System (SDCS), developed by Shriberg, uses both typological and etiological approaches to SSD classification (Shriberg, 2009). The SDCS describes SSDs based on presentation typology, with *speech delay* describing children with phoneme deletions and/or substitutions, whereas the term *speech errors* describes the group of children with phoneme distortions of sibilants and rhotics (Shriberg, 2009). The SDCS also classifies SSDs based on etiology, with a distal influence of genetic, environmental, and protective factors that impact underlying neurodevelopmental factors, and finally lead to five proximal factors downstream that are reflected in five SSD subtypes. Shriberg proposes that these five subtypes are caused by disruption to one or more of the five proximal factors, including cognitive-linguistic, auditory-perceptual, psycho-social, speech motor control, and phonological attunement (Shriberg, 2009). While there is great value in examining classification from an etiological approach, this system would suggest that an individual with SSD could belong to more than one subtype (Dodd, 2014; Waring & Knight, 2013), hindering clarity and usefulness in clinical application. Shriberg does stress the need for increased focus on research of diagnostic markers that correspond with underlying SSD etiological subtypes, as well as deeper investigation of the distal genetic, environmental, and neurologic factors.

Dodd's Differential Diagnosis system is undergirded by the concept that surface level speech characteristics are reflective of underlying processing deficits unique to specific subgroups. Additionally, this system also incorporates therapeutic response patterns in developing five SSD subgroups: articulation disorder, phonological delay, consistent atypical phonological disorder, inconsistent phonological disorder, and childhood apraxia of speech (CAS) (Dodd, 2014; Waring & Knight, 2013). The Differential Diagnosis system is rooted in delineating between the constructs of *delayed* and *disordered* speech development, yet it lacks a clear articulation of the genetic and neurological factors underlying differences in subgroup processing deficits (Waring & Knight, 2013). An advantage to Dodd's system is its ability to differentiate children affected by SSDs without the use of overlapping subtypes. In addition, Dodd's system demonstrates high clinical utility in relating SSD subtypes directly to clinical treatment approaches.

The Psycholinguistic Framework developed by Stackhouse and Wells examines underlying deficits of speech processing but was originally designed as a developmental model rather than a classification system (Waring & Knight, 2013). Applying this framework to SSDs, a child would experience breakdown at one or more of the five developmental phases: pre-lexical, whole-word, systematic simplification, assembly, and metaphonological (Waring & Knight, 2013). The framework is structured by a series of clinical questions related to speech input, mental representations, and speech output (Stackhouse & Wells, 1993). Thus, the Psycholinguistic Framework can guide SLPs in diagnostic interpretation and therapeutic planning on the level of each induvial child (Stackhouse & Wells, 1993). While this framework has high clinical utility, it does not

provide explanations for the deficits in speech processing but rather sees these deficits as causal in the presentation of SSDs (Waring & Knight, 2013).

Each of these approaches to SSD classification has strengths, and yet none has become universally accepted within the field of communication disorders. As such, more research is needed to provide comprehensive evidence about the nature of SSDs from both biological and behavioral perspectives. To enhance diagnostic accuracy and treatment efficacy, an investigation of genetic and behavioral biomarkers is needed.

Childhood Apraxia of Speech

On the spectrum of speech sound disorders, childhood apraxia of speech (CAS) is a particularly severe disorder that can significantly impact communication abilities. ASHA defines CAS as "a neurological childhood (pediatric) speech sound disorder in which the precision and consistency of movements underlying speech are impaired in the absence of neuromuscular deficits" (ASHA, 2007). For children with CAS, "the ability to plan and sequence speech movements is impaired, thereby decreasing the precision, consistency, and intelligibility of speech" (Chenausky et al., 2020, p. 2; Eising et al., 2018; Peter, Button, Stoel-Gammon, Chapman, & Raskind, 2013; Peter, Lancaster, Vose, Middleton, & Stoel-Gammon, 2018; Peter et al., 2016). Diagnostic criteria for CAS have been debated, with ASHA outlining three consensus criteria: inconsistency with repeated word productions, disruption to articulatory transitions, and inappropriate prosody (Chenausky et al., 2020; Terband, Namasivayam, et al., 2019). A recent factor analysis of 57 children with CAS yielded a three-factor model that aligns with ASHA's three criteria (Chenausky et al., 2020). Because some of these criteria can overlap with other SSDs, determining the incidence of CAS within the population has been challenging,

though estimates suggest 0.1-0.2% (Shriberg, Aram, & Kwiatkowski, 1997). Comorbidities specific to CAS include language and reading impairments (Lewis & Ekelman, 2007), as well as fine and gross motor deficits, with half of children with CAS reportedly needing physical and/or occupational therapy (Iuzzini-Seigel, 2019).

Speech Motor Control

As infants and children are exposed to speech in daily life, there is an interplay of feedforward and feedback mechanisms at work to facilitate communicative development. Auditory and sensorimotor input from their own speech productions, in combination with auditory input from the speech of others around them, helps develop and solidify phonemic representations and are used to refine the planning and programming needed for speech motor output. As children gain increasing confidence in their speech motor productions, reliance on sensorimotor feedback diminishes (Iuzzini-Seigel, Hogan, Guarino, & Green, 2015). The interplay of feedforward and feedback control is captured by the Directions Into Velocities of Articulators (DIVA) model and its extension, the Gradient Order DIVA (GODIVA) model.

Through computational simulations, both DIVA and GODIVA have helped elucidate complex learning processes related to speech motor control as well as corresponding neural substrates (Guenther, 2016). In feedforward control, the brain recruits the required neural circuitry to select a previously learned speech sound map, initiates the corresponding motor program, and generates the appropriate motor commands to produce the desired speech output. Areas of the brain that facilitate this feedforward mechanism can include the premotor and primary motor cortices, basal ganglia, cerebellum, and thalamus (Tourville & Guenther, 2011). The speech output

generated can then be used to train the system via auditory and somatosensory feedback, refining the desired target production through experience. Feedback control involves the auditory cortex, medial geniculate nucleus of the thalamus, the ventral premotor and motor cortices, and the cortico-cerebellar loop (Guenther, 2016). Given that the goal of the DIVA model has been to deepen understanding of the neural underpinnings of speech motor control (Tourville & Guenther, 2011), a natural extension of this research would be to examine possible genetic influences on those neural underpinnings.

In light of the mechanisms at work in the DIVA model, apraxia of speech (AOS) has been presented as an impairment in the feedforward control mechanism (Terband, Rodd, & Maas, 2019). Research has supported this same idea for CAS as well, finding that children with CAS rely more heavily on auditory feedback, likely due to underdeveloped speech motor programs (Iuzzini-Seigel et al., 2015). In doing so, their speech mechanism may be overburdened and slowed by the continuous monitoring of their own productions (Iuzzini-Seigel et al., 2015). In the field of speech-language pathology, research often focuses on consonants, given the high number of children with SSDs who make consonant errors; thus, vowels may be overlooked. For CAS, however, vowel errors present as a hallmark phenotypic trait, necessitating investigation into vowel perception and production.

For SSDs as a whole, it has been hypothesized that there may be neurocognitive deficits in phonological representation and organization, with possible difficulties in auditory perception (Anthony, 2011; Pennington & Bishop, 2009) and auditory-motor integration (Terband, van Brenk, & van Doornik-van der Zee, 2014). When examining speech perception, research indicates that both children and adults with a history of SSDs

can display differences in perceptual speech discrimination tasks compared to typical participants (Cabbage, 2015; Strömbergsson, Wengelin, & House, 2014), though some subsets of children with speech production difficulties do not display co-occurring perceptual difficulties (Byun, 2012), leaving the role of perception in SSDs underspecified.

Genetics of SSDs

Comorbidities

As knowledge of the neural aspects of speech motor control continues to deepen, a natural extension of this research would be to investigate possible genetic influences on involved neural mechanisms. In addition, the comorbidities observed in SSDs suggest a "shared genetic etiology expressed in the brain" (Peter et al., 2013, p. 317) that could be elucidated by expanding knowledge of genotype-phenotype associations. There is overlap between SSDs and language impairment (Macrae & Tyler, 2014; Shriberg, Tomblin, & McSweeny, 1999), and children with early SSDs are often at risk for later literacy deficits (Bishop & Adams, 1990; Tambyraja, Farquharson, & Justice, 2020), even without co-occurring language impairment (Anthony, 2011; Lewis, Avrich, Freebairn, Hansen, et al., 2011). Such comorbidities can leave children vulnerable to poor academic achievement (Eadie et al., 2015; Morgan et al., 2017). Shared biomarkers that have been associated with SSDs and literacy include oral motor skills, phonological awareness, phonological memory, vocabulary, and speeded naming (Lewis, Avrich, Freebairn, Taylor, et al., 2011). Research points to sequential processing as an overarching endophenotype for severe SSDs with clinical manifestations in motor, cognitive, and linguistic domains (Button, Peter, Stoel-Gammon, & Raskind, 2013; Peter

et al., 2013). These findings necessitate research of common underlying biomarkers that are influenced genetically.

Both research and clinical experience suggest a genetic contribution to SSDs. Higher concordance rates of SSDs have been found in monozygotic twins when compared with dizygotic twins (Lewis et al., 2006). In addition, children from families with a history of SSDs are more likely to be affected when compared to those without familial risk (Felsenfeld & Plomin, 1997; Peter, Matsushita, & Raskind, 2012). The investigation of genes of interest that impact communication abilities is a challenging task given the complexities underlying both DNA and human communication (Deriziotis & Fisher, 2017). One way to begin unraveling these complexities is through the study of families. Undertaking a study of the KE Family yielded the breakthrough discovery of the *FOXP2* gene—the first gene found to have an influence on speech and language in humans (C. S. L. Lai, Fisher, Hurst, Vargha-Khadem, & Monaco, 2001). This gene encodes for a transcription factor involved in regulation of neural development, specifically neurite outgrowth (Nudel & Newbury, 2013). Though *FOXP2* illustrates the inheritance of a monogenic trait, it highlights the value of family-based genetic research for the study of communication disorders. Because speech and language are complex traits that are often polygenic in nature, studies of nuclear families can be advantageous to search for rare variants (Fardo et al., 2014). The *FOXP2* story not only raises the question of which genes may influence speech, but how. Both questions are important to answer as the field moves forward in its knowledge of the gene-brain-behavior relationships pertaining to communication. Other genes of interest relevant to SSDs demonstrate roles in neural development, including *BCL11A* (Dias et al., 2016; Peter,

Matsushita, Oda, & Raskind, 2014) and *CNTNAP2* (Centanni et al., 2015). The genetic investigation in this study will help to expand and/or replicate genes of interest, contributing to the catalog of genes associated with phenotypic expressions of individuals with familial SSDs. This catalog could eventually be used to identify young children at genetic risk for SSDs, supporting the use of preventative interventions, as our lab has recently shown (Peter et al., 2020).

Genetic Methodologies

Within the field of genetic epidemiology, methodological approaches have the aim of establishing the relationship between genotype and the phenotypic expression of disease. Two major paradigms underlie these approaches: "Mendel's concept of discrete genes and Galton's biometric approach" that supported the development of statistical genetics (Khoury, 1993, p. 4). Historically, genetic research has used both family-based and population-based studies to examine disease-related variants (Khoury, 1993, p. 16; Laird & Lange, 2006). From the first nuclear family to have their genomes fully sequenced in a study of Miller syndrome (Hartwell, 2015, p. 366), to the family-based experimental approaches used in current research, the investigation of disease transmission within families can provide an important tool in the search for causal disease variants.

Biomarkers

Autism Spectrum Disorder as a Model

Investigating what lies beneath the phenotype of a genetically complex disorder is a challenging task and looking to the research approaches of others studying complex disorders can be helpful. Autism spectrum disorder (ASD) is a heterogeneous

neurodevelopmental disorder that is diagnosed on the basis of phenotypic presentation, and in this way is similar to SSDs, making this disorder an appropriate research parallel to examine. One of the stated goals of ASD research is to investigate genetic and epigenetic factors with the hope that such evidence "might allow individuals with ASD to be characterized into subsets with certain biomarker profiles that would respond more favorably to specific treatments....and lead to earlier diagnosis and more targeted treatments" (Goldani, Downs, Widjaja, Lawton, & Hendren, 2014, p. 1). Within ASD research, genetic, epigenetic, metabolomic, and neurological biomarkers have been researched in an attempt to learn about influential underlying mechanisms (Goldani et al., 2014; D. Li, Karnath, & Xu, 2017; Masi, DeMayo, Glozier, & Guastella, 2017). It has been noted that the aim of such studies is not merely to identify isolated biomarkers, but to ascertain patterns of clinically relevant biomarkers, with recommendations to incorporate aspects of machine learning (Goldani et al., 2014), perform longitudinal research, and use pooling of data samples (D. Li et al., 2017). As ASD research points to a crucial need for identifying biomarkers that can aid in identifying subgroups within a heterogenous disorder, research in SSDs could also follow this paradigm in attempting to learn more about SSD etiology and presentation toward the goal of improving precision and individualization within treatment.

Cerebellar Hypothesis

The traditional view of the cerebellum has focused on its role in the coordination and timing of motor functions, but recent research has expanded the conceptual role of the cerebellum to include linguistic and cognitive processes as well. (Mariën et al., 2014; Peter et al., 2018; Stoodley & Stein, 2011; Vias & Steven Dick, 2017). This

reconceptualizing has yielded three primary cerebellar roles: "timing and coordination; sensorimotor imagery,…and 'as a learning machine that supports the adaptive plasticity needed for the emergence of skilled behavior'" (Mariën et al., 2014, p. 398). Anatomically, the cerebellum contains 50% of the brain's neurons while contributing only 10% to overall brain volume (Stoodley & Stein, 2011), and there is some evidence for a correlation between cerebellar volume and general intelligence in school-age children (Mariën et al., 2014; Pangelinan et al., 2011). Topographical research has identified particular regions associated with differentiated cerebellar functions, with motor control localized within the anterior lobe and a portion of medial lobule VI, and cognitive-linguistic processes localized within the posterior lobe, primarily lobules VI and VII (Stoodley & Schmahmann, 2010). Functional connectivity research confirms topographical findings (Stoodley & Schmahmann, 2018). Functional neuroimaging studies have shown posterior lobe activation during cognitive tasks with no corresponding motor involvement (Stoodley & Schmahmann, 2018).

For communication specifically, it is commonly known that the cerebellum is involved in programming the finely timed movements required for speech, yet it also plays a role in "predicting sensory consequences of ongoing movements" (Guenther, 2016, p. 165). The cerebellar Purkinje cells in particular have been identified as playing an important role in the process of motor learning and error detection (Guenther, 2016). The cerebellum has been implicated in the use of covert speech, suggesting it may be involved in motor planning in the absence of actual motor execution (Callan et al., 2006). Phonological storage and processing involve the cerebellum in the temporal processing of the auditory speech signal, and cerebellar dysfunction has been associated with poor

phonological awareness skills and dyslexia (Mariën et al., 2014; Stoodley & Stein, 2011). Additionally, cases of cerebellar damage have revealed poor verbal working memory in the absence of spatial working memory deficits (Mariën et al., 2014), likely influenced by dysfunction in the phonological loop component of Baddeley and Hitch's model of working memory (Baddeley & Hitch, 1974). Cerebellar damage has resulted in impaired semantics, affecting verb generation, antonym and synonym generation, word associations, and even figurative language (Mariën et al., 2014). The cerebellum has also been implicated in syntactical analysis, with research suggesting that it may play a role in determining occurrences of deviation from expected syntactic rules (Mariën et al., 2014). Thus, the cerebellum appears to play an influential role in a variety of neural processes beyond the motor domain, providing support for the dysmetria of thought theory, that the cerebellum ultimately serves as an "oscillation dampener," smoothing functional performance across motor, linguistic, and cognitive domains (Mariën et al., 2014, p. 403).

The cerebellar deficit hypothesis of dyslexia posits that behavioral difficulties with reading are reflective of impaired neural functions governed by the cerebellum, among which are task automatization and sequential processing, (Nicolson, Fawcett, & Dean, 2001; Peter et al., 2018). Cerebellar implication in dyslexia was suggested by Frank and Levinson, 1973 when they observed "cerebellar-vestibular dysfunction" in 97% of the children with dyslexia in their research sample (p. 699). While a recent functional connectivity study did not find evidence of cerebellar activation during word reading for children with dyslexia or their typical peers, their experiment used an implicit word processing task that required feature identification of letters within real words and false font words rather than a functional reading task (Ashburn et al., 2019). Exploring a

cerebellar deficit hypothesis for SSDs seeks to provide etiological insight into the presence of possible common biomarkers observed in affected individuals across multiple domains. It has been most thoroughly investigated among more severe SSDs, such as CAS (Peter et al., 2018). Research points to sequential processing as an underlying biomarker for CAS, with clinical manifestations in motor, cognitive, and linguistic domains (Button et al., 2013; Peter et al., 2013).

A study involving adults with right cerebellar lesions revealed impairment in antonym generation compared to controls (Gebhart, Petersen, & Thach, 2002). Antonym generation was also found to be difficult for a group of children with CAS (Vose, 2018), providing further evidence for cerebellar involvement in certain linguistic tasks of individuals with impaired speech. Neuroimaging has identified an association between gray and white matter concentrations in the right cerebellum at 7 months with receptive language skills in children 12 months of age (Deniz Can, Richards, & Kuhl, 2013). Functional imaging of the KE family during nonword repetition revealed reduced activation in several brain areas, including the cerebellum (Liégeois, Morgan, Connelly, & Vargha-Khadem, 2011).

Genetic evidence of a cerebellar connection can be found in the variety of candidate genes associated with CAS that have significant expression within the developing cerebellum, including *FOXP2* and *BCL11A* (Estruch et al., 2018; Graham, Deriziotis, & Fisher, 2015; Peter et al., 2014; Sollis et al., 2017; Vernes et al., 2007). In a study of CAS genetics, high expression rates for genes of interest were found within early to mid-fetal brain development, specifically transcription factors and chromatin remodelers (Eising et al., 2018). Thus, extending the cerebellar hypothesis into the

genetic realm would suggest that a subtype of SSDs may exist in which presentation is affected by genetic influences on early neurodevelopment within the cerebellum.

Thalamic Hypothesis

Like the cerebellum, recent research is revisiting older paradigms of thalamic function, recasting its role within the brain as a vital player in cognition in addition to its role as a neural relay station (Saalmann & Kastner, 2015; Wolff & Vann, 2019). Traditionally, the thalamus has been associated with sensory function, but recent findings suggest that it is critical for sensory gating, and in forming, monitoring, and updating mental representations (Wolff & Vann, 2019). Research has also discussed the role of the thalamus and brainstem in tasks requiring distinction of a stimulus from its background noise (Perrachione et al., 2016).

Within the context of dyslexia specifically, fMRI research has identified a general deficit of neural adaptation as a core feature. This reduced capacity for neural adaptation to repetition hinders those with dyslexia from being able "to exploit regularities in stimuli to enhance performance" (Perrachione et al., 2016, p. 2), thereby impeding the formation of mental representations that enable learning. A recent study of 22 adults with dyslexia and 20 controls provides supporting evidence for the role of gating in the formation of stable mental representations (Peter, McCollum, Daliri, & Panagiotides, 2019). When measured with evoked response potentials at N1 to pairs of tones, gating was diminished in adults with dyslexia compared with controls, with the magnitude of the gating corresponding to accuracy of sight word discrimination. This study suggests that individuals with dyslexia display reduced neural adaptation to repetitive stimuli occurring before the deployment of attentional resources.

These research findings in the area of dyslexia may have an analogous application to children with SSDs. Intact sensory gating and mental representation formation are underlying prerequisites for the development and maintenance of stable phonological representations for speech, as these representations form the foundation of speech motor programs incorporated into feedforward and feedback control mechanisms. Thus, it may be that there is a subgroup of individuals with SSDs who present with deficits reflective of a perceptual-thalamic profile due to underspecified phonological representations, whereas others may present with a motor-cerebellar profile as discussed above. Given the role of the thalamus in the creation and regulation of mental representations, this study puts forth a thalamic hypothesis, which posits that a subtype of SSDs may exist in which behavioral presentation is affected by genetic influences on early neurodevelopment within the thalamus and other related subcortical structures.

Rationale

Speech-language pathologists have a long history of using surface-level speech behaviors in therapeutic decision making. Historically, intervention has targeted such behaviors, leading to improved clinical outcomes. Yet, there is evidence that certain underlying deficits may persist even in individuals who have completed treatment for surface-level behaviors. Research has shown that sequential processing deficits can persist into adulthood in those with a history of CAS, suggesting that, though phenotypic behaviors may have improved, residual deficits in the underlying endophenotype remain (Button et al., 2013; Peter et al., 2018). Additionally, children with remediated SSDs were found to display more difficulty with literacy and cognitive skills compared to typical peers. Specifically, these children struggled with vocabulary, word reading,

nonword repetition, and even non-verbal IQ (Farquharson, 2015a). The finding that deficit areas remain despite improved therapy outcomes could suggest that traditional speech-language therapy may not be addressing the mechanisms undergirding SSDs. This suggests a need for additional research to elucidate these mechanisms.

While some children experience difficulty with the acquisition of speech sounds due to a definitive medical cause, more often the etiology of SSDs is unclear, and thus the underlying mechanisms responsible for the manifestation of SSDs also remain unclear. This knowledge gap makes it difficult to establish distinct categories of SSDs, achieve early diagnosis, maximize treatment precision, and may even underlie a more recent trend toward underservicing children with SSDs within the school system (Farquharson, 2015b, 2019). In order to generate knowledge that can address these challenges, a better understanding of genetic and behavioral biomarkers that underlie SSDs is needed. The purpose of this study to identify genetic, perceptual, and motor-related biomarkers within families displaying SSDs, investigating cerebellar and thalamic hypotheses. Results from this study could support or refine current clinical categories with additional biological and behavioral evidence, facilitating earlier, more precise diagnosis and treatment for SSDs. Additionally, this study could generate relevant biomarkers to investigate as potential future therapeutic targets.

Research Questions

The strength of this study lies not in any single part, but rather in its comprehensive approach to investigating SSDs within multiple families. Biomarkers are rooted in genetics and incorporating genetic analysis alongside robust behavioral

phenotyping is essential to address knowledge gaps of SSDs. This study will investigate the following research questions:

1. Do individuals with CAS shows signs of relying on speech feedback control more than feedforward control mechanisms?

   It is hypothesized that individuals who demonstrate CAS have impaired feedforward control for speech and will rely more heavily on feedback control mechanisms.

2. Is there evidence of a motor control biomarker that runs in one or more families?

   It is hypothesized that low scores in measures of sequential processing will be found only in the affected individuals of at least one family.

3. Is there evidence of a perceptual biomarker that runs in one or more families?

   It is hypothesized that low scores in measures of stable mental representations, consistent with perceptual feature extraction, will be found only in the affected individuals of at least one family.

4. Is there evidence that familial genetic variants occur in genes whose early expression in the brain corresponds to the cerebellar or thalamic profiles?

   It is hypothesized that in each family with a familial behavioral biomarker profile, the most likely variant will be in genes that are expressed in the corresponding brain region during early brain development.

5. Are the implicated genes across families in a functional association with each other?

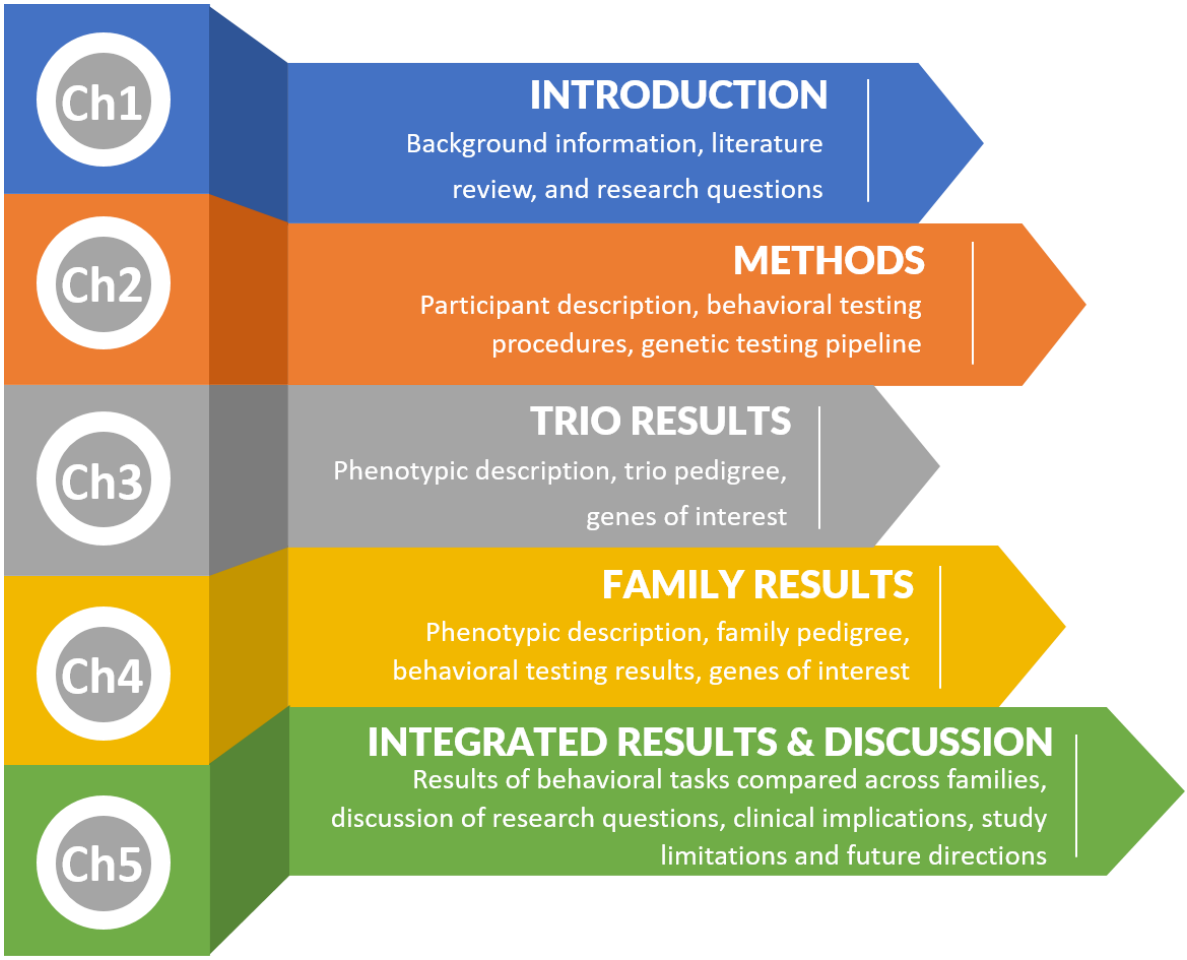
It is hypothesized that genes of interest will share functional properties (e.g., cell-cell adhesion), as has been found with other complex phenotypes such as autism spectrum disorder.

Reader's Roadmap

Chapter 1 has provided introductory background information and reviewed literature relevant for the exploration of these research questions, focusing on speech sound disorders as they relate to genetics, neurology, acoustics, and therapeutic practice. Chapter 2 outlines the participants in this study, along with the rationale and procedures for behavioral testing measures. Additionally, it lays out the genetic testing pipeline and criteria for variant filtering and prioritization. Chapter 3 contains the trio results, organized by presenting each trio's phenotype, pedigree, and the genes of interest that may be associated with their phenotype. Chapter 4 reviews the family results, providing a summary of each family's phenotype and pedigree. Genes of interest and results from their behavioral testing are presented, along with family-level discussion of observed data trends. Chapter 5 presents results of the two acoustic tasks and discussion of these results across the group of participants as a whole. It also contains discussion of each of the five research questions, with questions 2 and 3 discussed together. The chapter concludes with a summary and a discussion of clinical implications, study limitations, and future directions.

Figure 1.1

Reader's Roadmap



CHAPTER 2

METHODS

Participants

The participants in this study included five families and five sets of parent-child trios. Of the genetic samples collected from these individuals, 29 were selected for exome sequencing and passed quality control measures at the University of Washington Center for Mendelian Genomics (UW-CMG). Within each family/trio, all children were biologically related to their parents.

This study was conducted with approval from the Arizona State University Institutional Review Board, with some participants previously consented under Human Subjects Division at the University of Washington. Adults gave written consent for participation, parents gave written permission for their children's participation, children age 14 and older gave written assent, and children age 6-13 gave verbal assent. Funding for whole exome sequencing was generously provided by UW-CMG through their funded grants: NHGRI and NHLBI UM1 HG006493 and U24 HG008956.

Behavioral Testing

Individuals from the five families participated in a variety of behavioral tests to document behavioral phenotype presentation (Table 2.1). Tests given covered areas of speech, language, literacy, cognition, fine motor, and gross motor skills. All testing was conducted according to the guidelines found in the publisher's manual unless otherwise stated. For all standardized tests, resulting scores were converted to z-scores for comparison purposes. When the test did not provide normative data for adults, norms for the highest age range were used. All tasks provided norms up to at least 17;0 except for

the DDK task, which only had available norms up to 13;0. The participants either passed a hearing screening during the testing session or reported hearing functional for testing purposes. All testing sessions were video and audio recorded with participant permission. Two research assistants in the Speech-Language-Genetics Lab performed reliability scoring for 15% of the behavioral data and consensus scoring was used for any discrepancies. There were 8 individuals who participated in behavioral testing who also had genetic samples that were selected for exome sequencing and passed quality control.

Table 2.1

Behavioral Testing Participants

Participant ID	**Sex**	**Age at Testing Years; Months**	**Exome Sequenced?**	**Reported or Suspected Phenotype**
Fam15_SI08	F	8;3	Failed	CAS
Fam15_BR08	M	8;3	Yes	CAS
Fam15_PR10	M	10;7	Yes	CAS
Fam15_FA49	M	49;8	Not selected	Speech
Fam20_PR28	F	28;9	Yes	CAS
Fam20_FA68	M	68	Not selected	CAS
Fam30_BR01	M	1;3	Not selected	Unknown
Fam30_BR04	M	4;6	Yes	CAS
Fam30_PR07	F	7;7	Yes	CAS
Fam30_BR10	M	10;5	Failed	CAS
Fam30_BR12	M	12;1	Failed	CAS
Fam30_BR14	M	14;5	Yes	CAS
Fam37_SI14	F	14;1	Not selected	CAS
Fam37_BR17	M	17;7	Not selected	CAS
Fam37_PR19	F	19;8	Failed	CAS
Fam47_BR03	M	3;11	Not selected	Unknown
Fam47_SI04	F	4;1	Yes	Phonology
Fam47_BR07	M	7;9	Failed	Phonology, Language, Literacy
Fam47_BR10	M	10;0	Failed	Language, Literacy
Fam47_BR12	M	12;5	Not selected	Language, Literacy
Fam47_SI14	F	14;7	Yes	Phonology
Fam47_SI18	F	18;11	Not selected	Academics

Speech Measures

The Goldman-Fristoe Test of Articulation, Third Edition (GFTA-3) is a standardized test designed to assess articulation skills in individuals age 2;0-21;11 (Goldman & Fristoe, 2015). The stimulus pictures provide participants the opportunity to produce all the consonants in Standard American English in various word positions. The Sounds-in-Words subtest was administered using 47 color picture stimuli to elicit production of 60 target words. While the GFTA-3 is helpful for assessing speech sound errors, its usefulness is limited for assessing traits of CAS. Though some of these traits can be assessed informally, there is no standardized method to capture vowel errors, prosody and intonation differences, and inconsistent sound production. This subtest also does not provide the opportunity to assess multisyllabic word production, as only 5% of the 60 target words have more than two syllables. As such, the GFTA-3 was given to provide documentation of current speech sound errors for the participants in this study and not as a means of categorizing SSD subtype. A brief oral mechanism exam was administered to assess structure and function of the speech articulators, incorporating items from the Robbins-Klee Test of Oral and Speech Motor Control (Robbins & Klee, 1987) (Appendix A).

Nonword repetition tasks are particularly helpful in assessing difficulty with sequential processing for speech. These tasks prevent participants from relying on previous experience perceiving the sound sequences and programming motor movements for the novel words they hear (Case & Grigos, 2020; Peter et al., 2013). The Syllable Repetition Task (SRT) was selected because it can assess an individual's ability to repeat nonwords even if they have a limited phonemic inventory or multiple speech sound errors

(Shriberg et al., 2009). The task required participants to use only five early-developing phonemes: the vowel /ɑ/, the voiced nasal consonants /m/ and /n/, and the voiced stop consonants /b/ and /d/. All individuals participating in this study were able to produce these phonemes. Each of the 18 nonwords was presented aloud via a PowerPoint presentation freely available from The Phonology Project at the Waisman Center, University of Wisconsin-Madison: https://phonology.waisman.wisc.edu/administration-scoring-materials/. The task consists of 8 two-syllable nonwords, 6 three-syllable nonwords, and 4 four-syllable nonwords. This task requires sequential processing in order to produce the five phonemes in the correct sequence. SRT scores have been shown to be significantly lower for individuals with speech delay compared to typical peers (Shriberg & Lohmeier, 2008). The SRT allows for the calculation of several types of scores, and analysis for this study yielded a competency score (examining consonant accuracy), an encoding score (examining perceptual encoding of speech sound features), and a transcoding score (examining speech motor planning and programming) (Shriberg, Lohmeier, Strand, & Jakielski, 2012). Encoding errors reflected the percentage of substitution errors that were within-class manner substitutions (e.g., an /m/ for /n/), capturing the partial sound feature knowledge used by the participant. Thus, the encoding score helps to identify individuals who struggle with encoding the phonological information of content they hear, which tend to be those with CAS or who have concomitant speech and language delay (Rvachew & Matthews, 2017). The encoding cutoff score for helping identify individuals with CAS is reported to be 46.9 (Shriberg et al., 2012). Transcoding errors reflected the insertion of additional sounds into the word, capturing difficulty with planning and programming that can be observed in individuals

with CAS. The cutoff score reported for transcoding errors is 80, a score that has shown some utility in identifying individuals with CAS (Rvachew & Matthews, 2017; Shriberg et al., 2012). Preliminary normative data for the SRT were used to calculate z-scores for competency, encoding, and transcoding based on participant age, using the 17-year-old norms for the adult participants (Shriberg & Lohmeier, 2011).

A tongue twister task developed by Haber and Haber (1982) was used to informally explore the ability to plan and execute motor programs when the speech system is challenged with phonemic complexity. This measure has shown to differentiate between adults with a history of SSDs and those without, though both groups performed within the average range (Lewis et al., 2007). Participants listened to a recording of a female voice reading 20 sentences, 10 tongue twisters and 10 control sentences. The control foils were designed to be similar to the tongue twisters in "syntactic complexity, syllable count, and sentential stress pattern" (Haber & Haber, 1982, p. 409), but were filled with sound sequences that were challenging to produce, thus eliciting errors. For example, the control sentence *he finds string beans by the small barn*, was designed to resemble the tongue twister, *she sells seashells by the seashore,* but without the complex planning needed to sequence the similar sibilant phonemes /s/, /z/, and /ʃ/. Participants were given the opportunity to ask for repetitions to minimize working memory load. Participant productions were analyzed for the number of substitutions, deletions, revisions, repetitions, additions, and transpositions, as well as the percentage of phonemes produced correctly for tongue twisters and control sentences.

Diadochokinetic (DDK) rates are a helpful tool in assessing repetitive and alternating speech movements. A slower DDK rate has been found in individuals with

CAS, and is one of the traits in a list of criteria for CAS diagnosis (Chenausky et al., 2020; Peter et al., 2018; Preston et al., 2014; Shriberg, Potter, & Strand, 2011). The DDK disyllables are particularly challenging for those with CAS since they necessitate that motor plans be executed in an alternating pattern. Participants were asked to produce the syllable /pʌ/ in rapid succession, aiming for a minimum of 20 repetitions and taking a breath when needed. This procedure was repeated for the monosyllables /tʌ/ and /kʌ/, the disyllables /pʌtə/ and /tʌkə/ (15 repetitions), and the trisyllable /pʌtəkə/ (10 repetitions). Results were analyzed for consonant accuracy and average syllable rate across each stimulus item using Praat software (Boersma & Weenink, 2002). An average rate for monosyllables and disyllables was also calculated, and z-scores were derived based on normative data from (Fletcher, 1972).

Motor Measures

Keyboard finger tapping tasks were used to assess repetitive and alternating motor movements based on the procedure outlined in Peter and Raskind (2011). It has been shown that finger tapping has been disrupted in individuals with cerebellar damage (D'Angelo & De Zeeuw, 2009). For the first task, participants were asked to repetitively press the spacebar key of a typical laptop computer as quickly as possible. This procedure was repeated 10 times for each hand, alternating hands between trials to reduce fatigue. The second task involved rapidly pressing the left and right arrow keys using the index and middle fingers in an alternating motion. Ten trials with each hand were also performed for this task. The number of key taps and timing were recorded with a program developed by Elias Peter using LabView (Bitter, Mohiuddin, & Nawrocki, 2006). This task has been used in other studies investigating sequential processing (Peter

et al., 2013; Peter & Raskind, 2011). Normative data from Gualtieri and Johnson (2006) and Skogan, Oerbeck, Christiansen, Lande, and Egeland (2018) were used to calculate z-scores for analysis, with the Skogan norms only available through age 16.

Because CAS involves deficits in motor planning and programming, investigating general motor skills is an important step in developing a robust phenotype for these participants. Four subtests of The Bruininks-Oseretsky Test of Motor Proficiency, Second Edition (BOT2) were selected to assess motor skills. The Manual Dexterity subtest involved "reaching, grasping, and bimanual coordination" skills such as sorting cards, stringing blocks, and transferring pennies (Bruininks & Bruininks, 2005, p. 5). The Upper-Limb Coordination subtest examined hand-eye coordination by assessing throwing, catching, and dribbling. The Bilateral Coordination subtest included tasks that required "sequential and simultaneous coordination of the upper and lower limbs," such as jumping jacks, simultaneous finger and foot tapping, and finger pivoting (Bruininks & Bruininks, 2005, p. 6). Finally, the Balance subtest assessed balance and stability during static and movement tasks, such as walking on a balance beam and standing on one foot. The subtest scores did not distinguish between static and dynamic balance.

Language Measures

Given the finding that antonym generation was significantly more difficult for individuals with right cerebellar lesions compared to left cerebellar lesions (Gebhart et al., 2002) and preliminary data showing antonym difficulty in children with CAS (Vose, 2018), participants were given the Antonyms and Synonyms subtests of the Comprehensive Assessment of Spoken Language, Second Edition (CASL-2) (Carrow-Woolfolk, 2017). The Antonyms subtest is an expressive task that required participants

to verbally produce an appropriate antonym for a given stimulus word presented aloud by the examiner, thus requiring both word knowledge and retrieval. In contrast, the Synonyms subtest is a receptive task that required participants to select an appropriate synonym for a stimulus word presented verbally by the examiner from a set of four choices, removing the retrieval aspect of the task. Because the two CASL-2 subtests differed in format, some participants were also given the Antonyms and Synonyms subtests of either The WORD Test 3 Elementary (Bowers, Huisingh, LoGiudice, & Orman, 2014), or The WORD Test 2 Adolescent , depending on age. Both of these subtests involved participants generating an antonym or synonym given a verbal stimulus word, thus providing better data for comparing skill differences across word type. Additionally, the antonyms and synonyms subtests of the CASL-2 are highly correlated with the Oral Language Composite score from the Oral and Written Language Scales, Second Edition (r=0.83 and 0.76, respectively) (Carrow-Woolfolk, 2017, p. 102), and thus could give an indication as to if the participant may have difficulty with overall language skills.

Reading Measures

To examine reading skills, participants were given the Test of Word Reading Efficiency (TOWRE), which assesses accuracy and fluency of reading (Torgesen, Wagner, & Rashotte, 1999). This test contains two timed tasks reflecting skills needed to become a successful reader: a sight word task and a phonemic decoding task. Phonemic decoding is highly sequential in nature, enlisting a sublexical route that taxes sequential processing during visual encoding of the graphemes and motor planning for the verbal output (Peter et al., 2018; Peter et al., 2019). In contrast, sight word reading identifies

words through a lexical route, taxing perceptual processing as the word is stored and retrieved as a single unit (Miles, Rubin, & Gonzalez-Frey, 2018). Participants were asked to read the words on each list as quickly and accurately as possible. Scores were based on the number of words read correctly in 45 seconds. Because the phonemic decoding task involved reading nonwords, participants were unable to rely on their own prior word knowledge to read this list.

Cognitive Measures

The Rapid Automatized Naming and Rapid Alternating Stimulus Tests (RAN/RAS) was given to assess the automatic integration of several overlapping cognitive processes, including sustaining attention, visual tracking, semantic retrieval, phonological assembly, and motor planning and programming for the verbal output. This test is specifically helpful in the early prediction of later reading difficulties (Wolf & Denckla, 2005, pp. 5-6). Participants were given each subtest of the RAN/RAS: objects, colors, numbers, letters, alternating numbers and letters, and finally naming a combination of letters, numbers, and colors.

The Reynolds Intellectual Assessment Scales, Second Edition (RIAS-2) is designed to measure intelligence for individuals age 3-94 (Reynolds & Kamphaus, 2015). The participants in this study were given the Guess What and Verbal Reasoning subtests to estimate verbal intelligence, and Odd-Item Out and What's Missing subtests to estimate nonverbal intelligence. Subtest scores can be combined to yield an estimate of overall intelligence.

Other Measures

For participants who were too young for this study's testing protocol, parent-report measures were used acquire information about overall development for the youngest child in Family 30 (Fam30_BR01) and Family 47 (Fam47_BR03). The Ages & Stages Questionnaires®, Third Edition (ASQ®-3) was used to screen for development in the areas of communication, problem solving, fine motor, gross motor, and personal-social (Squires & Bricker, 2009). Parent responses were scored, and results were plotted on the scoring sheet indicating if the child's development fell below the cutoff score for their age, close to the cutoff score, or above the cutoff score. Speech was rated using the Intelligibility in Context Scale (ICS), a parent questionnaire designed to help provide information about how a child's speech impacts their overall ability to be understood (McLeod, Harrison, & McCormack, 2012). The mothers of each child responded to seven questions about their child's ability to be understood by a variety of communication partners, proving a rating from "never" to "always." The mother of participant Fam30_BR01 also completed questionnaires from the MacArthur-Bates Communicative Development Inventories over time (Fenson et al., 2007). These questionnaires help to capture emerging communication skills by eliciting an inventory of receptive and expressive gestures, words, and sentences that children use to communicate.

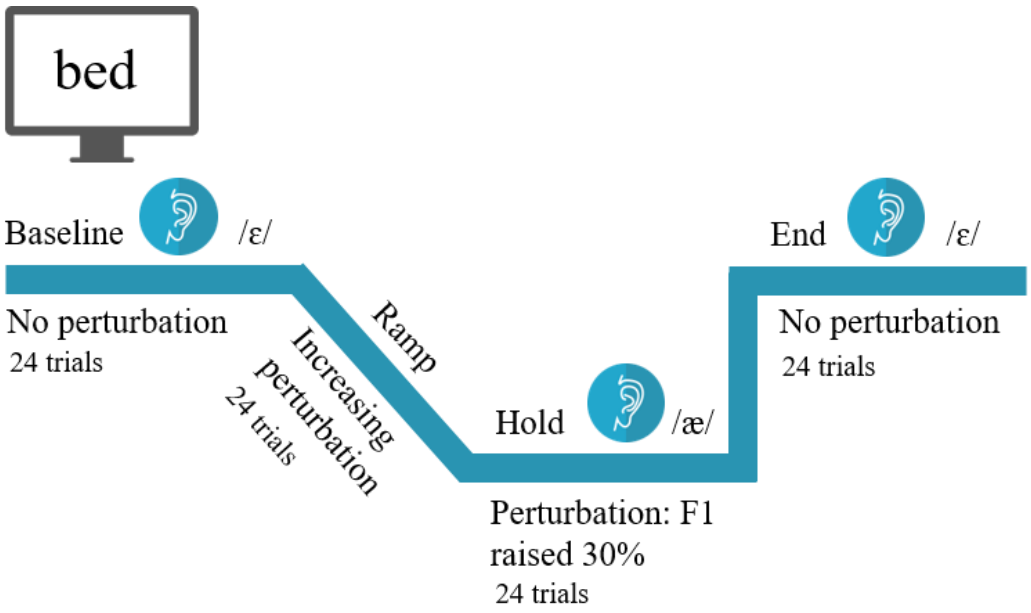
Acoustic Speech Tasks

Two acoustic speech tasks were performed and analyzed as part of this study: a vowel adaptation task to examine degree of adaptation given perturbed auditory feedback and a vowel space task to look at vowel production centralization.

A vowel adaptation task based on Experiment 2 in Ballard et al. (2018) was presented to 12 participants, 8 with a history of CAS and 4 from a single family with phonological and/or language and literacy difficulties. Each participant was visually presented with a CVC word via a laptop computer, either /tɛd/ (ted), /bɛd/ (bed), or /hɛd/ (head). In real time, the participant read the word aloud into the microphone of a Sennheiser® 300 Pro headset, and heard their own speech production through the headphones, as processed by a Motu® MicroBook IIc sound card. The task script was run using MATLAB R2019a software from MathWorks®. Overall, 96 CVC words were presented, with a baseline phase of 24 non-perturbed trials, a ramp phase of 24 trials of increasing F1 perturbation, a hold phase of 24 trials using 30% F1 perturbation, and an end phase with 24 non-perturbed trials (Figure 2.1). Perturbation was introduced by raising F1, shifting the perception of the /ɛ/ vowel towards the /æ/ vowel. Visual presentation time for each trial was 2.5 seconds, and auditory feedback was presented 5db higher than the microphone in order to mask any potential feedback from bone conduction.

Figure 2.1

Adaptation Task



Recorded data from this task were extracted, and the spectrogram of each vowel was visually inspected using ADustics, a custom-written MATLAB script. The steady-state portion of each vowel was manually selected, and average values for F1 and F2 were calculated for the middle 20% of each vowel. Any poor-quality trials were discarded from the dataset. Average formant frequencies for the baseline trials were subtracted from all the trials. The adjusted formant value was then divided by the average of the baseline formants in order to normalize the adaptation responses. Degree of adaptation was then calculated by averaging the normalized formant values for the final 15 trials of the hold phase. Because the perturbation was applied to F1, analysis focused on the adaptation for F1.

A vowel space task was presented to 10 participants with sufficient reading skills, 7 with a history of CAS and 3 from a single family with phonological and/or language

difficulties. Participants were visually presented with one of seven possible /hVd/ words on a laptop computer screen: /hid/ (heed), /hɪd/ (hid), /hɛd/ (head), /hæd/ (had), /hud/ (who'd), /hʊd/ (hood), and /hɑd/ (hod). Each participant read the word aloud into the microphone of a Sennheiser® 300 Pro headset and responses were recorded. The task consisted of 70 trials, 10 of each vowel presented in random order. The spectrogram of each vowel was visually inspected using ADustics and the steady-state portion of each vowel was manually selected for analysis. Any trials with poor production quality or in which a participant read the wrong word (and produced an off-target vowel) were discarded. Average values for F1 and F2 were calculated for the various vowels and were then used to calculate a formant centralization ratio (FCR) for each participant. FCR is used to examine vowel space, but as a measure it shows less variability among speakers of differing sex and age compared with vowel space area calculations (Carl, Kent Raymond, Levy Erika, & Whalen, 2020; Sapir, Ramig, Spielman, & Fox, 2010). The FCR formula used in this study was: (F2u + F2ɑ + F1i + F1u)/(F2i + F2ɑ) (Naderifar, Ghorbani, Moradi, & Ansari, 2019).

Genetic Testing

Genetic samples were obtained from 77 individuals through collection of peripheral blood or saliva. Saliva was collected using either Oragene® OG-510 collection kits or Mawi® iSwab DNA collection kits. Extraction of genetic material was performed by The Translational Genomics Research Institute (TGen) in Phoenix. The genetic material was transported to UW-CMG for exome sequencing. Of these samples, 36 samples failed quality control testing, mostly due to low mass of extracted genetic material. Replacement saliva samples were collected for 21 individuals, which

underwent extraction and resequencing. Eventually, 29 samples were selected for exome sequencing and passed quality control measures (Table 1). Variant calls and CNV calls provided by UW-CMG were analyzed using the procedures discussed below.

Table 2.2

Genetic Testing Participants

Participant ID	Collection Type	Affectation Status
Trios		
Trio13_FA	Blood	Unaffected
Trio13_MO	Blood	Unaffected
Trio13_PR	Blood	Affected
Trio17_FA	Blood	Unaffected
Trio17_MO	Blood	Unaffected
Trio17_PR	Blood	Affected
Trio23_FA	Saliva	Unaffected
Trio23_MO	Saliva	Unaffected
Trio23_PR	Saliva	Affected
Trio31_FA	Saliva	Unaffected
Trio31_MO	Saliva	Unaffected
Trio31_PR	Saliva	Affected
Trio46_FA	Saliva	Unaffected
Trio46_MO	Saliva	Unaffected
Trio46_PR	Saliva	Affected
Families		
Fam15_PR10	Blood	Affected
Fam15_CO22	Saliva	Affected
Fam15_BR08	Blood	Affected
Fam20_PR28	Saliva	Affected
Fam20_NE16	Saliva	Affected
Fam20_NI12	Saliva	Affected
Fam20_NE04	Saliva	Affected
Fam30_PR07	Saliva	Affected
Fam30_BR14	Saliva	Affected
Fam30_BR04	Saliva	Affected
Fam47_PR16	Saliva	Affected
Fam47_SI14	Saliva	Affected
Fam47_SI04	Saliva	Affected

Whole Exome Sequencing

The following is a direct excerpt from the UW-CMG methods paper dated April 24, 2019, outlining exome sequencing procedures, quality control (QC) measures, and details of variant calling and annotation procedures. All analyses used human genome build hg19 unless otherwise indicated.

"All sequencing [was] performed at the University of Washington Northwest Genomics Center (NWGC), an approved recharge center, directed by Dr. Debbie Nickerson. The NWGC has the technical staff to carry out all necessary sample processing steps for second-generation sequencing, including DNA quality control/assurance, library construction, targeted, in-solution capture methods (i.e., exome), DNA sequencer operation and maintenance, variant calling, data analysis, and IT support. The work carried out by the NWGC is done through a per assay rate cost structure that supports the operation, technical staff (i.e. non-key personnel), and reagents needed for this work.

"The UW-CMG centralizes all receipt, tracking and quality control/assurance of DNA samples. Samples [were] assigned unique barcode tracking numbers and have a detailed sample manifest (i.e., identification number/code, sex, DNA concentration, barcode, extraction method) linked to each sample within our laboratory information management system (LIMS). Initial QC entails DNA quantification, gender validation assay, and molecular "fingerprinting" with a 63-SNP OpenArray assay derived from a custom exome SNP set. This 'fingerprint' is used to identify potential sample handling errors prior to sample processing and provides a unique genetic ID for each sample, eliminating the possibility of sample assignment errors. Samples are failed if: (1) the total

amount, concentration, or integrity of DNA is too low; (2) the fingerprint assay produces poor genotype data; or (3) sex-typing is inconsistent with the sample manifest.

Library Production and Exome Capture.

"Library construction and exome capture have been automated (Perkin-Elmer Janus II) in 96-well plate format. 500ng of genomic DNA [was] subjected to a series of shotgun library construction steps, including fragmentation through acoustic sonication (Covaris), end-polishing and A-tailing ligation of sequencing adaptors, and PCR amplification with dual 8bp barcodes for multiplexing. Libraries [underwent] exome capture using the Roche/Nimblegen SeqCap EZ v2.0 (~36.5 MB target). Briefly, 500ng of shotgun library [was] hybridized to biotinylated capture probes for 16 hours. Enriched fragments [were] recovered via streptavidin beads and PCR amplified. Since each library is uniquely barcoded, samples can be captured in multiplex. Prior to sequencing, the library concentration [was] determined by fluorometric assay and molecular weight distributions verified on the Agilent Bioanalyzer (consistently 150 ± 15bp). Processing a sample from genomic DNA into an exome sequencing library requires 6 days (1.5 days for library construction, 3 days for exome capture, and 1.5 days for post-capture processing).

Clustering/Sequencing.

"Barcoded exome libraries [were] pooled using liquid handling robotics prior to clustering (Illumina cBot) and loading. Massively parallel sequencing-by-synthesis with fluorescently labeled, reversibly terminating nucleotides [was] carried out on the NovaSeq sequencer.

Read Processing.

"The processing pipeline consists of the following elements: (1) base calls generated in real-time on the NovaSeq6000 instrument (RTA 3.1.5); (2) demultiplexed, unaligned BAM files produced by Picard ExtractIlluminaBarcodes and IlluminaBasecallsToSam; (3) BAM files aligned to a human reference (hg19hs37d5) using BWA (Burrows-Wheeler Aligner; v0.7.15) (Li and Durbin 2009); and (4) sequence read and base quality are checked using the FASTX-toolkit (v0.0.13). Read data from a flow-cell lane [was] treated independently for alignment and QC purposes in instances where the merging of data from multiple lanes is required (e.g., for sample multiplexing). Read-pairs not mapping within ± 2 standard deviations of the average library size (~150 ± 15 bp for exomes) [were] removed. All aligned read data are subject to the following steps: (1) "duplicate removal" is performed, (i.e., the removal of reads with duplicate start positions; Picard MarkDuplicates; v2.6.0) and (2) base qualities are recalibrated (GATK BaseRecalibrator; v3.7).

Variant Detection.

"Variant detection and genotyping [were] performed using the HaplotypeCaller (HC) tool from GATK (3.7). Variant data for each sample [were] formatted (variant call format [VCF]) as "raw" calls that contain individual genotype data for one or multiple samples and flagged using the filtration walker (GATK) to mark sites that are of lower quality/false positives [e.g., low quality scores (Q50), allelic imbalance (ABHet 0.75), long homopolymer runs (HRun> 3), and/or low quality by depth (QD < 5)].

Data Analysis QC.

"All sequence data [underwent] a QC protocol. For exomes, this include[d] an assessment of: (1) total PE100 reads; (2) library complexity - the ratio of unique reads to total reads mapped to target. DNA libraries exhibiting low complexity are not cost-effective to finish; (3) capture efficiency - the ratio of reads mapped to human versus reads mapped to target; (4) coverage distribution 90% at 8X required for completion; (5) capture uniformity; (6) raw error rates; (7) Transition/Transversion ratio (Ti/Tv) typically ~3 for known sites and ~2.5 for novel sites; (8) distribution of known and novel variants relative to dbSNP (typically < 7% novel using dbSNP build 138 in samples of European ancestry; Ng, Turner et al. 2009); (9) fingerprint concordance > 99%; (10) sample homozygosity and heterozygosity; and (11) sample contamination < 3%. All QC metrics for both single-lane and merged data [were] reviewed by a sequence data analyst to identify data deviations from known or historical norms. Lanes/samples that fail QC [were] flagged in the system and can be re-queued for library prep (< 5% failure) or further sequencing (< 2% failure), depending upon the QC issue. Exome completion is defined as having > 90% of the exome target at > 8X coverage and > 80% of the exome target at > 20X coverage. Typically this requires mean coverage of the target at 50-60X.

Variant Annotation.

"[An] automated pipeline [was used] for annotation of variants derived from exome data, the SeattleSeq Annotation Server (http://gvs.gs.washington.edu/SeattleSeqAnnotation/). This publicly accessible server returns annotations including dbSNP rsID (or whether the coding variant is novel), gene names and accession numbers, predicted functional effect (e.g., splice-site, nonsynonymous, missense, etc.), protein

positions and amino-acid changes, PolyPhen predictions, conservation scores (e.g., PhastCons, GERP), ancestral allele, dbSNP allele frequencies, and known clinical associations. The annotation process has also been automated into our analysis pipeline to produce a standardized, formatted output (VCF-variant call format)."

Though UW-CMG provided annotated files, our lab annotated the VCF files using Ensembl Variant Effect Predictor (VEP) (McLaren et al., 2016). Exome variants were identified and annotated using GEnome MINIng (GEMINI) (Paila, 2013) based upon hypothesized modes of inheritance.

Variant Analysis

Variant calls were filtered and prioritized using the following criteria:

(1) CADD score above 10

A Combined Annotation-Dependent Depletion (CADD) score is a continuous variable designed to capture the deleterious impact a genetic variant can have on a human phenotype (Rentzsch, Witten, Cooper, Shendure, & Kircher, 2019). Higher CADD scores were prioritized as they would be associated with increasingly deleterious effects on a phenotype.

(2) Minor allele frequencies less than 15%

While the analysis was focused on discovering rare variants, a more flexible allele frequency was chosen based on the idea that speech sound disorders are not extremely rare and may present as genetically complex. The minor allele frequencies used in this analysis are from the Genome Aggregation Database (gnomAD) version 2 for Non-Finnish Europeans (Konrad J. Karczewski et al., 2020).

(3) SIFT and PolyPhen-2 Scores

Sorting Intolerant from Tolerant (SIFT) scores are used to identify those amino acid substitutions that are predicted to alter protein function, thus helping to prioritize variants that can impact phenotype. The SIFT score itself is "the normalized probability that amino acid change is tolerated" (Ng & Henikoff, 2003, p. 3813). SIFT scores classified as "deleterious" were prioritized in variant filtering. PolyPhen-2 scores are also used to reflect the probability that a variant negatively affects a phenotype, and each variant is assigned as being "benign," "possibly damaging," or "probably damaging" (Adzhubei et al., 2010, p. 2). The benign variants were filtered out and those classified as "probably damaging" were prioritized.

(4) pLI Scores

A pLI score "reflects the tolerance of a given gene to the loss of function on the basis of the number of protein truncating variants" (Ziegler, Colin, Goudenège, & Bonneau, 2019, p. 839). While pLI scores can be helpful in variant prioritization, caution must be taken when using them to assess autosomal recessive and X-linked recessive variants since these modes of inheritance may not always result in a phenotypic impact within the population. As such, pLI scores were not be used to filter out potential variants, but scores of 0.9 or higher were used to strengthen prioritization (Ziegler et al., 2019).

(5) Verified in IGV

Any potential genes of interest were visually inspected and verified using the Integrated Genomics Viewer (IGV) (Robinson et al., 2011).

Filtered variants were then cross-referenced with a list of genes of interest related to speech and language disorders compiled from relevant literature by Dr. Beate Peter (Appendix B), ensuring that previously identified variants were prioritized. Potential variants of interest were explored using the BrainSpan Atlas of the Developing Human Brain from the Allen Institute, which uses "RNA sequencing and exon microarray" to derive normalized gene expression rates for various brain regions (Allen Institute for Brain Science, 2010). Because the SSDs explored in this study are developmental, expression rates from 8 weeks prenatally through 10 months postnatally were selected for analysis (Eising et al., 2018), focusing on expression within the central nervous system, especially the cerebellum, thalamus, and striatum. Derived expression values are measured in "reads per kilobase transcript per million mapped reads (RPKMs)" (Keil, Qalieh, & Kwan, 2018, p. 2400). Variants were prioritized if values were greater than one (Eising et al., 2018), with values approaching those for known CAS genes of interest given higher priority (Table 2.3).

Table 2.3

BrainSpan Expression Rates for Known CAS Genes of Interest

Known genes of interest for CAS (Eising, 2018)	**Expression in Developing Cerebellum**	**Expression in Developing Thalamus**	**Expression in Developing Striatum**
FOXP2	18.58	20.14	11.46
BCL11A	16.89	8.47	29.58
ERC1	13.6	14.42	15.69

Values given in RPKM

After filtering and analysis, potential genes of interest for each trio or family were aggregated, and gene function was explored using GeneCards to rule-in variants that have potential neurological effects (Stelzer et al., 2016). The DatabasE of genomiC variatIon

and Phenotype in Humans using Ensembl Resources (DECIPHER) was used to investigate reported variants in similar genetic regions and their corresponding clinical presentation, thus aiding in genotype-phenotype interpretation. As such, "This study makes use of data generated by the DECIPHER community. A full list of centers who contributed to the generation of the data is available from https://decipher.sanger.ac.uk and via email from decipher@sanger.ac.uk. Funding for the DECIPHER project was provided by Wellcome" (Firth et al., 2009). ClinVar, a database of the National Center for Biotechnology Information, was used to explore potential variant pathogenicity (Landrum et al., 2018). Based on these analyses, a list of genes of interest were derived for each family or trio.

Gene Ontology

In order to examine gene products and function, gene ontology was explored using the Gene Ontology EnRIchment AnaLysis and VisuaLizAtion Tool (GOrilla) (Eden, Navon, Steinfeld, Lipson, & Yakhini, 2009). This tool searches for gene ontology terms given a list of rank-ordered genes. To assemble gene input lists for analysis, this study used genes from all families ranked by CADD scores. GOrilla outputs provided specific enrichment terms for relevant biological processes, molecular functions, and cellular components for this set of genes, along with p-values for each term.

Copy Number Variation Analysis

The following is a direct excerpt from the UW-CMG methods paper dated October 2013, outlining the procedure for identifying copy number variants (CNVs) using Copy Number Inference from Exome Reads (CoNIFER) (Krumm et al., 2012).

CoNIFER-based CNV discovery from exome read-depth.

"Reads from each exome sample were split into consecutive 36mers, up to two per read, and mapped using the single-end mode of mrsFAST 2, allowing for up to two mismatches per 36mer. We aligned reads to a concatenated hg19 reference genome, which included exome-capture targets based on the Nimblegen EZ Exome v2.0 platform (194,080 targets) as well as 300bp up- and downstream of each target. Using CoNIFER v0.2.2 (http://conifer.sf.net,1), we processed each of the three datasets separately. RPKM values were calculated for 194,080 probes and exons targeted by the Nimblegen EZ Exome v2.0 exome sequence enrichment platform. We set the –svd option to 20, and used default CoNIFER settings for all other options. After CoNIFER analysis, the raw SVD-ZRPKM values were exported (using the export command) for downstream analysis.

Segmentation and filter of CNVs.

"We used DNACopy3 and CGHCall4 to segment and assign deletion or duplication probabilities to SVD-ZRPKM values. In order to prevent excessively strong SVDZRPKM signals from interfering with the models used by CGHCall to assign copy number, we clipped the signal at +/- 3 for each exon. Parameters for DNACopy were as follows: "alpha" was set to 0.02 initially (but increased by 0.01 up to 0.04 if initial segmentation failed to converge), using the "undo.split" to "sdundo" and "undo.SD" set to 2. Default options for CGHcall were used, and we allowed only "deletion" and "duplication" as called states. Raw CNV calls were filtered to exclude those primarily in duplicated or repetitive regions of the genome (using a 50% reciprocal overlap mask for segmental duplications and non-diploid genomic regions), as well as for duplicated

processed pseudogenes. Calls with low signal strength (dependent on the size of the call were filtered to reduce the number of false positives while still retaining high sensitivity (absolute SVD-ZRPKM cutoff values were: ≥ 1.5 for 2 exon calls, ≥ for 3-5 exon calls and ≥ 0.5 for calls with more than 5 exons).

Sample-level quality control steps:

"After filtering raw calls, 75 samples were excluded from further analysis because they had more than 10 raw calls. All calls from filtered samples are listed in the "QC/ *.did_not_pass_filter.csv" files.

Clustering into CNVRs and genotyping:

"Individual CNV calls passing filter were grouped into similar CNV Regions (CNVRs) using pairwise distances between all CNVs based on a modified reciprocal overlap (RO) heuristic. This function calculates the RO between two CNVs based on the minimum fraction of number of overlapping probes, and weights this percentage based on the total number of non-overlapping probes on each end. In this way, the function takes into account the uncertainty in breakpoints and RO for two small CNVs, while allowing two large overlapping CNVs to be count[ed] as distinct entities. In similar fashion, we genotyped CNVs/CNVRs from CMG projects against a set of CNVs discovered in 2,888 control samples (from the NHLBI Exome Sequencing Project). All CNVs from CMG projects were clustered with control CNVs, and the resulting clusters were then split up into CMG and control counts."

CNV Analysis.

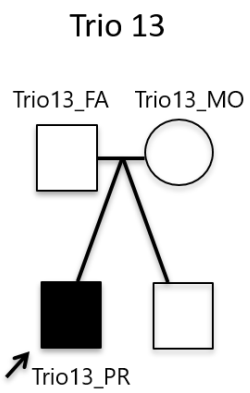
UW-CMG provided data on potential CNVs along with the corresponding CoNIFER plots. For trios, CNV calls were selected for analysis if they were not present

in the unaffected parents. For families, CNV calls were selected for analysis if they were shared with other affected family members. All CNVs of interest were visually inspected using IGV to determine if read depth increased (doubled) or decreased (halved) in the exons at CNV juncture. If a CNV call was real, it was anticipated that duplications would cause an increase in read depth and deletions would show a decrease in read depth. CoNIFER plots were visually inspected to determine their level of trustworthiness in accordance with UW-CMG guidelines. "Very trustworthy" CNV calls encompassed a large set of exons (more than 5 with strong prioritization for more than 20) and consisted of strong signal strength (Krumm et al., 2012). These CNVs were further analyzed using DECIPHER and GeneCards to investigate potential relevance to a speech phenotype.

CHAPTER 3

TRIO RESULTS

Trio 13



Trio 13 consists of two reportedly unaffected parents and one male twin offspring age 27 who is the proband of this trio (Trio13_PR). Per parent report, he and his brother are monozygotic (identical) twins. Prenatal and birth history indicate that the twins were delivered five weeks prematurely via Cesarean section. Developmental milestones were reported to be within normal limits except for speech and language skills. Babbling was minimal to absent and first words were not produced until around 3 years of age, at which time he began receiving intermittent speech-language therapy. Testing at age 6;6 revealed a variety of articulation and phonological errors along with instances of articulatory imprecision and voicing errors. Additionally, vowel distortions during a DDK task and difficulty with the production of multisyllabic words were noted. Prosody was reported to be unusual, characterized by monotone speech and prolongations occurring both within and between word and syllable junctures, significantly impacting his intelligibility. Oral motor skills were delayed and characterized by exaggerated movements. These data indicate difficulty with motor planning and traits associated with CAS. Though initially presenting with an expressive language delay at age 2;6, his receptive and expressive language skills were reported to be within normal limits at 6;6. He received school-based speech therapy from 1st through 6th grade and was diagnosed with high-functioning autism as a college student.

Genetic analysis reveals three instances of autosomal recessive inheritance that yield several potential genes of interest for the proband in Trio 13 (Table 3.1). While there were several CNV calls for Trio13_PR, analysis did not reveal any plausible CNVs. Research has identified an instance of *de novo* translocation in a case of idiopathic autism, involving the q12.1 and q13.2 regions (Castermans et al., 2003). While the case history described in this paper does not mention delayed speech, monotonous speech is noted, a phenotypic similarity to Trio13_PR. The NBEA (Neurobeachin) protein itself affects both synaptic plasticity and efficacy, and within animal models it has been shown to play a role in cognition and social behavior (Gromova et al., 2018). Additionally, *NBEA* had the highest neurodevelopmental expression rates of any of these genes, with its cerebellar expression rate of 17 reaching nearly as high as that of *FOXP2* (18.58) (Allen Institute for Brain Science, 2010).

The autosomal recessive variant found within the *CYFIP1* gene, while rated as benign/tolerated and having a greater occurrence in the population, falls in a region associated with 15q11.2 deletion syndrome. Phenotypic characteristics of this syndrome include delayed speech, autism spectrum disorder, seizures, and attention deficit hyperactivity disorder ("Online Mendelian Inheritance in Man, OMIM®," #615656). Research describes an instance of a single variant at rs4778298 was found to have significant association with changes to the surface area of the left supramarginal gyrus, an area known for its involvement in speech and language (Woo et al., 2016). While not identical in location, Trio13_PR's variant and the one identified in the paper are approximately 160kb apart. Additionally, this same study found a novel relationship between *CYFIP1* and *FOXP2*, a gene known to have a significant impact on speech and

language, including CAS (C. S. Lai et al., 2000; C. S. L. Lai et al., 2001). This was the only gene included that was categorized as benign and tolerant which makes it an unlikely candidate; however, it was included for consideration due to its presence within a known CNV hotspot and its link to *FOXP2*.

One autosomal recessive variant in *LAMB1*was found, a gene that has been implicated in cell differentiation, cell migration, and neurite outgrowth (Stelzer et al., 2016). The *LAMB1* variant had the highest CADD score of all the Trio13_PR genes of interest at 28.5. Within the developing neurological system, *LAMB1* plays a crucial role in axon guidance and there is some evidence linking the gene with an autism spectrum phenotype (Hutcheson et al., 2004).

Given the evidence from variant analysis, it seems plausible that Trio13_PR presents with a phenotype characterized by CAS and high-functioning autism due to multiple, inherited genetic variants. Like the autism spectrum, speech sound disorders exist along a spectrum as well, ranging from mild to severe. In the case of Trio 13, it is possible that the parents carried a few nonpenetrant variants that when inherited by the proband (and his twin), resulted in an aggregated genetic impact on phenotype.

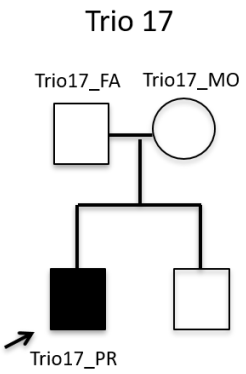
Table 3.1

Variants of Interest for Trio13_PR

Gene	***NBEA***	***CYFIP1***	***LAMB1***
Chr: Locus RS ID	**13**: 36202269 rs11538677	**15**: 22969232 rs7170637	**7**: 107569962 rs35915664
Variant	A>G	G>A	A>G
Type	SNP-missense	SNP-missense	SNP-missense
Allele Freq gnomAD NFE	0.11	0.17	0.02
CADD Scaled	23.5	22.1	28.5
pLI	1	0.97	0
PolyPhen-2	PoD (0.67)	Ben (0)	PrD (0.99)
SIFT	Del (0.03)	Tol (0.58)	Del (0)
MOI	AR	AR	AR
Dev Crblm Exp	17	11.23	11.82
Dev Thal Exp	19.12	7.65	5.33
Dev Strm Exp	12.54	8.09	5.46
Evidence	Gromova, et al., 2018	Woo, et al., 2016	Hutcheson, et al., 2004

SNP=single nucleotide polymorphism; NFE=non-Finnish European; MOI=mode of inheritance; Crblm=cerebellum; Thal=thalamus; Strm=Striatum of Basal Ganglia; AR=autosomal recessive; AD=autosomal dominant; CH=compound heterozygosity; DN=*de novo;* PoD=possibly damaging; PrD=probably damaging; Ben=benign; Del=Deleterious; Tol=tolerated; LoF=loss-of-function; CNV=copy number variation; kb=kilobases

Trio 17



Trio 17 consists of two reportedly unaffected parents and a male offspring age 13, who is the proband of this trio (Trio17_PR). The proband has one unaffected brother. Per medical report, Trio17_PR has a history of developmental delay, hypotonia, macrocephaly, and eczema. Prenatal and birth history provided by parents indicate that the mother had complete placenta previa and low amniotic fluid. Trio17_PR was born prematurely at 34 weeks gestation and was hospitalized for seven days. He had difficulty nursing and had frequent respiratory and ear infections, necessitating the placement of pressure equalization (PE) tubes twice before the age of 18 months. Developmental milestones for speech and language were delayed, with babbling emerging around age 3, first words around age 3;6 and sentences at age 4;6. Parents reported typical gross motor skills, but difficulty with fine motor coordination. He reportedly received a diagnosis of CAS and dysarthria, and at one point a label of mild autism was discussed but was not retained. He has received years of speech-language therapy, physical therapy, and occupational therapy to support his development. He previously participated in genetic testing to evaluate the possibility of Fragile-X, investigating (*FRAXA* and *FRAXE*) and *PTGEN* with no significant findings reported.

Genetic analysis reveals several potential genes of interest for the proband in Trio 17, one potential CNV and one instance of compound heterozygosity (Table 3.2). There is a possible 226 kb copy number variation on chromosome 22 within the q13.33 region

that encompasses multiple genes, including *SHANK3* (Figure 3.1). This gene is well-known in the literature for its association with autism, and deletions in this gene have been found in individuals with Phelan-McDermid syndrome (22q13.3 deletion syndrome), characterized by developmental delay, severe speech impairment, traits of autism, and hypotonia (OMIM, #606232) (Y. Li et al., 2018). This is likely due to the involvement of *SHANK3* in the development of synapse formation and function which, when interrupted in animal models, leads to aberrant behaviors (Uchino & Waga, 2015). While many of the cases involving *SHANK3* reflect genetic deletions (Firth et al., 2009), some evidence for a similar yet milder phenotype has been described for duplications within the 22q13 region (Okamoto et al., 2007; Uchino & Waga, 2015). Thus, it is possible that Trio17_PR is presenting with a milder phenotype that shares some characteristics with 22q13.3 deletion syndrome. This is contingent upon the CNV call being real. While the CNV plot shows a trustworthy call, encompassing areas of strong signal strength and more than 20 exons, the signal strength is lower in the *SHANK3* region and inspection using IGV is more difficult to interpret. The number of reads does show an increase from 24 to 54 near the start region of the CNV, but there is less evidence for a drop in reads at the end of the CNV region. As a result, more investigation would be needed to determine the validity of this CNV call.

In terms of variant calls, *HERC1* presents a possible gene of interest, as it is involved in the development of neural projections (Hashimoto et al., 2016) and has been shown to help regulate cerebellar Purkinje cells within mouse models (Mashimo et al., 2009). A study by Hashimoto et al. (2016) investigating autism risk, found a *de novo*

mutation in *HERC1,* and two instances of compound heterozygosity in *HERC1* within the same family were reported by Ortega-Recalde et al. (2015). Phenotypically, the children in that study presented with developmental delay impacting motor and speech-language skills, hypotonia, macrocephaly, and ataxic gait. In addition, Eising et al. (2018) found a *de novo* variant and a missense variant in *HERC1* for two unrelated individuals with CAS, though the relevance for the variants could not be determined in that study. This preponderance of evidence suggests *HERC1* as a plausible gene of interest for the proband in Trio 17.

Table 3.2

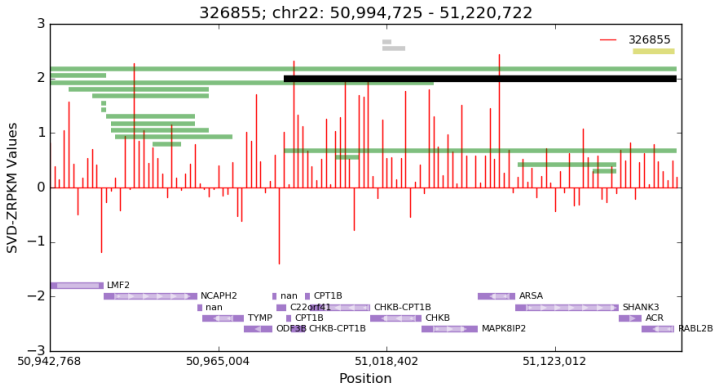
Variants of Interest for Trio17_PR

Gene	***HERC1***		***SHANK3***
Chr: Locus **RS ID**	**15**: 63950887 rs2228513	**15**: 64021853 rs61751109	**22**: 50994725-51220722
Variant	G>A	A>G	226 kb
Type	SNP- missense	SNP- splice region	CNV-duplication
Allele Freq gnomAD NFE	0.05	0.007	
CADD Scaled	28.4	13.56	
pLI	1		
PolyPhen-2	PoD (0.60)	None	
SIFT	Del (0)	None	
MOI	CH	CH	DN
Dev Crblm Exp	10.2		12.43
Dev Thal Exp	11.51		15.55
Dev Strm Exp	11.6		11.47
Evidence	Eising et al., 2018		Okamoto et al., 2007

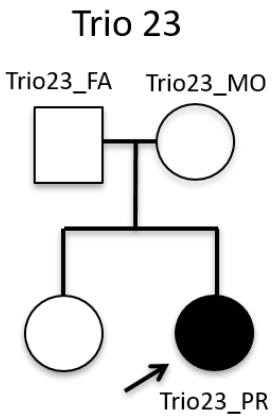
SNP=single nucleotide polymorphism; NFE=non-Finnish European; MOI=mode of inheritance; Crblm=cerebellum; Thal=thalamus; Strm=Striatum of Basal Ganglia; CH=compound heterozygosity; DN=*de novo;* PoD=possibly damaging; Del=Deleterious; CNV=copy number variation; kb=kilobases

Figure 3.1

CNV Involving SHANK3



Trio 23



Trio 23 consists of two reportedly unaffected parents and one female offspring age 15, who is the younger of two daughters and serves as the proband of this trio (Trio23_PR). Her sister is unaffected. Developmental milestones were delayed, as she did not crawl, and walking was delayed until 16 months. Her phenotype is characterized by trunk and hand weakness. Trio23_PR was diagnosed with CAS at 3;6, and she also has a diagnosis of developmental delay. Preliminary genetic testing at age 3 revealed no significant results. She has received a combination of speech-language therapy, occupational therapy, and physical therapy since childhood, but no longer receives these services as a teenager. Her speech is intelligible overall, though she can struggle to maintain sufficient volume, and she continues to show residual speech errors for the /s/ and /r/ phonemes. She experienced two seizures around the age of 12 and currently requires seizure medication. She continues to struggle with fine motor coordination, impacting her handwriting.

Genetic analysis reveals a single gene of interest for the proband in Trio 23 (Table 3.3). While there were several CNV calls for Trio23_PR, analysis did not reveal any plausible CNVs. *MECP2* is a gene known to be causal for Rett syndrome (Fisher & Vernes, 2015; Urbanowicz, Downs, Girdler, Ciccone, & Leonard, 2015). Affecting females, it is a syndrome characterized by microcephaly, seizures, and often a regression of acquired developmental skills between 12-24 months, resulting in cognitive

impairment and speech-language impairment (OMIM, #312750). Annotation data did not provide PolyPhen-2, or SIFT information, and no minor allele frequency was available for this variant from either gnomAD or ExAC (K. J. Karczewski et al., 2017). This *de novo* variant has a CADD score of 50, is classified as loss-of-function (LoF), and the gene has a high pLI score. This indicates that it has a high probability of being intolerant to a loss-of-function variant, thus negatively impacting its function. In addition, this variant was classified as stop-gained, which results in a premature stop codon and termination of protein translation (Chen et al., 2017). The ClinVar archive shows several instances of diagnosed Rett syndrome for this locus (Landrum et al., 2018). When this variant is investigated in the Decipher database, the aggregation of observed cases at this locus shows a phenotypic profile similar to Trio23_PR, with global developmental delay, delayed speech and language, and seizures being the top three traits listed (Figure 3.2). All of this evidence points to *MECP2* being a highly plausible gene for the phenotype exhibited by Trio23_PR, with the possibility of undiagnosed Rett syndrome.

Table 3.3

Variants of Interest for Trio23_PR

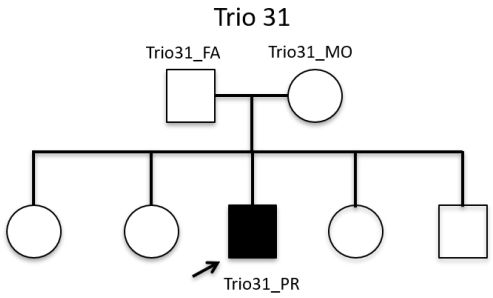
Gene	***MECP2***
Chr: Locus **RS ID**	**X**: 153295922 rs61753979
Variant	G>A
Type	SNP-stop-gained
Allele Freq gnomAD NFE	-1
CADD Scaled	50
pLI	0.89
PolyPhen-2	None
SIFT	None
MOI	DN
Dev Crblm Exp	9.05
Dev Thal Exp	8.31
Dev Strm Exp	9.51
Evidence	Urbanowicz et al., 2015

SNP=single nucleotide polymorphism; NFE=non-Finnish European; MOI=mode of inheritance; Crblm=cerebellum; Thal=thalamus; Strm=Striatum of Basal Ganglia; AR=autosomal recessive; AD=autosomal dominant; CH=compound heterozygosity; DN=*de novo;* PoD=possibly damaging; PrD=probably damaging; Ben=benign; Del=Deleterious; Tol=tolerated; LoF=loss-of-function; CNV=copy number variation; kb=kilobases

Figure 3.2

DECIPHER Phenotypic Traits for MECP2 Locus

Trio 31



Trio 31 consists of two reportedly unaffected parents and a male offspring age 13, who is the proband of this trio (Trio31_PR) and the middle of five children. While family history is negative for speech and language impairment with the exception of the proband, parent report indicates the father and one sister both have a diagnosis of Ehlers Danlos syndrome (EDS) and postural orthostatic tachycardia syndrome (POTS), and the mother has a history of depression, anxiety, attention deficit disorder (ADD), and Raynaud's syndrome. Prenatal and birth history for Trio31_PR were typical, but developmental milestones indicated delayed first words and sentences. While walking developed at a typical age, it was reported that the proband had difficulty with proprioception and would frequently walk into obstacles. He began receiving speech therapy, physical therapy, and occupational therapy at 2 years old and continued until at least third grade. He also received academic support in reading, writing, and math until at least third grade. Trio31_PR has a reported history of CAS, and parents expressed concern with his ability to focus and attend to task.

Genetic analysis revealed two possible genes of interest for the proband in Trio 31, though for both instances of compound heterozygosity, at least one of the variant calls was classified as benign and tolerated (Table 3.4). This would suggest that they are weak candidates at best. While there were several CNV calls for Trio31_PR, analysis did not reveal any plausible CNVs.

The most likely candidate for a relevant gene of interest in Trio 31 is *ZNF142*. In a recent study examining 34 individuals with CAS by Hildebrand et al. (2020), a compound heterozygous missense variant in *ZNF142* was found to be a plausible pathogenic candidate. Their CADD scores were slightly higher for the variant loci, with values of 31 and 26, compared to the scores of 23.6 and 20.8 found in this study. The SIFT and PolyPhen-2 scores they found were also more convincing, with both loci having deleterious and damaging labels. A study by Khan et al. (2019) investigated the genotypes of four unrelated families displaying neurodevelopmental disorders and found the presence of likely pathogenic variants in *ZNF142* in seven of the females within the cohort. All seven individuals presented with cognitive impairment, speech impairment, and motor impairment, with one of the individuals reported to have a CAS diagnosis. Though functioning as a transcription factor, the exact molecular mechanism undergirding the neuronal impact of this gene remains unclear, yet a good candidate for future investigation (Khan et al., 2019).

The compound heterozygosity found for the *LAMA* 5 gene, while having lower CADD scores than *ZNF142*, has possibly damaging and deleterious PolyPhen-2 and SIFT scores for one of the loci. A different *LAMA5* variant was found in a case of CAS investigated by our lab, but with a higher CADD score of 22.7 (Vose, 2018). A *de novo* variant in this gene has also been found in a child with developmental delay, displaying hypotonia, and absent speech at age 2;0 (Han, Jang, Park, & Lee, 2018). While there are limited cases linking *LAMA5* to speech phenotypes, the gene function does support it as a possible gene of interest given its role in cell migration and neurite outgrowth (Stelzer et al.,

2016); however, its evidence for contributing to the phenotype in Trio31 is limited.

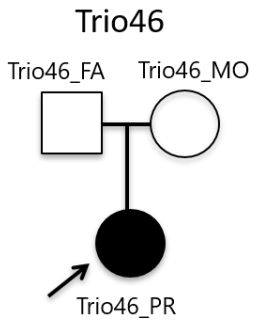
Table 3.4

Variants of Interest for Trio31_PR

Gene	***ZNF142***		***LAMA5***	
Chr: Locus **RS ID**	**2**: 219503277 rs367737510	**2**: 219507166 rs114603798	**20**: 60909316 rs6062223	**20**: 60885362 rs41307203
Variant	C>T	G>A	C>T	G>A
Type	SNP- missense	SNP- missense	SNP- missense	SNP-missense
Allele Freq gnomAD NFE	0.0001	0.02	0.056	0.023
CADD Scaled	23.6	20.8	16.5	11.55
pLI	0		0.01	
PolyPhen-2	PrD (0.99)	Ben (0.27)	PoD (0.70)	Ben (0.001)
SIFT	Tol (1)	Tol (0.12)	Del (0.02)	Tol (0.53)
MOI	CH	CH	CH	CH
Dev Crblm Exp	5.73		5.74	
Dev Thal Exp	4.38		2.1	
Dev Strm Exp	6.18		2.14	
Evidence	Hildebrand et al., 2020; Khan et al., 2019		Vose, 2018	

SNP=single nucleotide polymorphism; NFE=non-Finnish European; MOI=mode of inheritance; Crblm=cerebellum; Thal=thalamus; Strm=Striatum of Basal Ganglia; AR=autosomal recessive; AD=autosomal dominant; CH=compound heterozygosity; DN=*de novo;* PoD=possibly damaging; PrD=probably damaging; Ben=benign; Del=Deleterious; Tol=tolerated; LoF=loss-of-function; CNV=copy number variation; kb=kilobases

Trio 46



Trio 46 consists of two reportedly unaffected parents and one female offspring age 14 who is the proband of this trio (Trio46_PR). She has a diagnosis of Sturge-Weber syndrome with seizures, though that diagnosis has been questioned. A medical report of MRI findings performed at 22 months indicated right cerebral dysplasia with atrophy of both white and grey matter, as well as diffuse atrophy in the left hemisphere. Per parent report, genetic testing was negative for cortical dysplasia. As of 2018, her seizure activity had increased requiring treatment with three medications. Developmental milestones were delayed and, while she did babble, her first words were delayed, and she only used approximately 20 words by the time she reached kindergarten. She presents with cognitive delay, motor difficulties, and has a diagnosis of CAS, for which she receives continuing private and school-based intervention.

Given the complex phenotype of Trio46_PR, genetic analysis proved more difficult. There were no reported CNV calls for Trio46_PR. No instances of variants within known cortical dysplasia genes were found (*DEPDC5, NPRL3, MTOR, TSC1, and TSC2*) (Iffland & Crino, 2017). There are just two genes of interest for this proband, and neither are supported by a robust set of evidence (Table 3.5).

MDGA2 is a gene that has been associated with autism spectrum disorder, schizophrenia, and epileptic encephalopathies such as Landau-Kleffner syndrome (Lesca et al., 2012; Pettem, Yokomaku, Takahashi, Ge, & Craig, 2013). The MDGA2 protein is a contactin protein involved in cell adhesion, and several genes implicated in speech and

language are also contactins, most notably *CNTNAP2* (Lesca et al., 2012). MDGA2 and MDGA1 proteins work together as part of the neurexin-neuroligin pathway, which supports synaptic organization (Pettem et al., 2013). While this gene has a high CADD score and is rated as deleterious, it does not show high expression rates in the developing brain, though it may impact brain expression indirectly through its relationship to *MDGA1*, which does show higher expression rates in the cerebellum (12.19) (Allen Institute for Brain Science, 2010).

Trio46_PR shows an autosomal recessive missense variant of *KIAA0056*, also referred to as *NCAPD3* (Stelzer et al., 2016). Homozygous mutations of *KIAA0056* have been associated with Joubert syndrome (OMIM, #616784), which is best described as a group of disorders characterized by developmental delay, cerebellar hypoplasia, and sometimes hypotonia (Niceta et al., 2020). MRI data for individuals with Joubert syndrome typically shows the molar tooth sign involving hypoplasia of the cerebellar vermis, which Trio46_PR has no evidence of based on the available medical reports. Functionally, *KIAA0556* regulates "microtubule dynamics and ciliary integrity" within the developing brain (Niceta et al., 2020, p. 7). A study by Sanders et al. (2015) found a case of recessive *KIAA0556* mutation associated with a milder form of Joubert syndrome, which provides some evidence for a possible association of this gene with Trio46_PR's phenotype. Overall, there were no clear genes of interest for Trio46_PR, and as such further investigation of this trio is warranted.

Table 3.5

Variants of Interest for Trio46_PR

Gene	***MDGA2***	***KIAA0556***
Chr: Locus **RS ID**	**14**: 47426637 rs12590500	**16**: 27760935 rs16976970
Variant	C>A	G>A
Type	SNP-missense	SNP-missense
Allele Freq gnomAD NFE	0.03	0.02
CADD Scaled	23.3	21.8
pLI	1	0
PolyPhen-2	Ben (0.22)	PoD (0.46)
SIFT	Del (0.04)	Del (0.03)
MOI	AR	AR
Dev Crblm Exp	1.5	2.64
Dev Thal Exp	2.92	1.57
Dev Strm Exp	1.96	2.19
Evidence	Lesca et al., 2012	Sanders et al., 2015

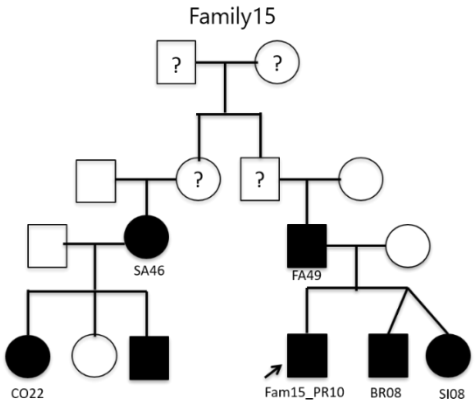
SNP=single nucleotide polymorphism; NFE=non-Finnish European; MOI=mode of inheritance; Crblm=cerebellum; Thal=thalamus; Strm=Striatum of Basal Ganglia; AR=autosomal recessive; AD=autosomal dominant; CH=compound heterozygosity; DN=*de novo;* PoD=possibly damaging; PrD=probably damaging; Ben=benign; Del=Deleterious; Tol=tolerated; LoF=loss-of-function; CNV=copy number variation; kb=kilobases

CHAPTER 4

FAMILY RESULTS

Family 15

Background



Family 15 is four-generation family with known speech affectation for two siblings in the third generation who each have their own families. SA46 is an affected female in the third generation who has three offspring, two daughters (one of whom is affected) and an affected son. This branch of the family did not participate in behavioral testing, but genetic data were obtained for Fam15_CO22. Fam15_FA49 is an affected male in the third generation who has three affected offspring, a son who is the proband for the family and a set of dizygotic twins (male and female). All four of these individuals participated in behavioral testing for this study and will be the focus of analysis.

FA49 was born in Romania and began learning English as a second language in elementary school. Prenatal and birth history were reportedly typical. Developmental milestones for speech and language were delayed, with first words emerging around 2;6 and sentences around 3;0. His mother reported that he displayed speech dysfluencies around age 8;0 and he participated in school-based speech therapy for stuttering. He moved to Canada and began speaking English regularly at the age of 28. His wife reports no history of speech, language, or learning difficulties in her own family history.

Prenatal and developmental history for PR10 was within normal limits, with delivery by C-section at full term. He experienced frequent ear infections when he was young. Developmental milestones were delayed for speech and language, and first words emerged around 3;0, and sentences were delayed until after 4;0. His motor skills were also delayed. He is growing up in a bilingual household that speaks Romanian and English. PR10 began receiving services in the areas of communication, fine motor, and social-emotional skills through the local school district around age 3. Educational testing at age 4 reported that speech skills were significantly below average, along with below average language skills, fine motor skills, and visual-motor coordination skills. A private speech-language evaluation performed at age 4 indicated a diagnosis of CAS. The report also stated that he used 1-2 syllable utterances for communication, and his highly unintelligible speech led to frustration during testing. He was unable to produce CVC words, and his speech was characterized by inconsistent errors, vowel errors, and voicing errors.

SI08 is a female twin born at 32 weeks gestation and was reportedly hospitalized for the first 50 days of life due to respiratory issues. Per parent report, she has a mild form of cerebral palsy. SI08 displayed minimal babbling during development, and she began receiving speech therapy at 21 months old and occupational therapy at 18 months old. A private speech-language evaluation performed at age 2;10 indicated that she produced 15-20 words and was not yet using word combinations. She demonstrated below average receptive and expressive language skills, motor speech skills, and speech production skills, resulting in a CAS diagnosis.

BR08 is a male twin born at 32 weeks gestation. He has a history of CAS diagnosis and began receiving speech therapy at 21 months old. He was reportedly more "talkative" than his twin sister and displayed some babbling as his communication was developing. Per parent report, he experienced difficulties with motor skills. Both twins are also growing up in a bilingual household.

Behavioral Analysis

FA49 took part in behavioral testing along with his three children, PR10, SI08, and BR08 (Table 4.2).

Speech Measures.

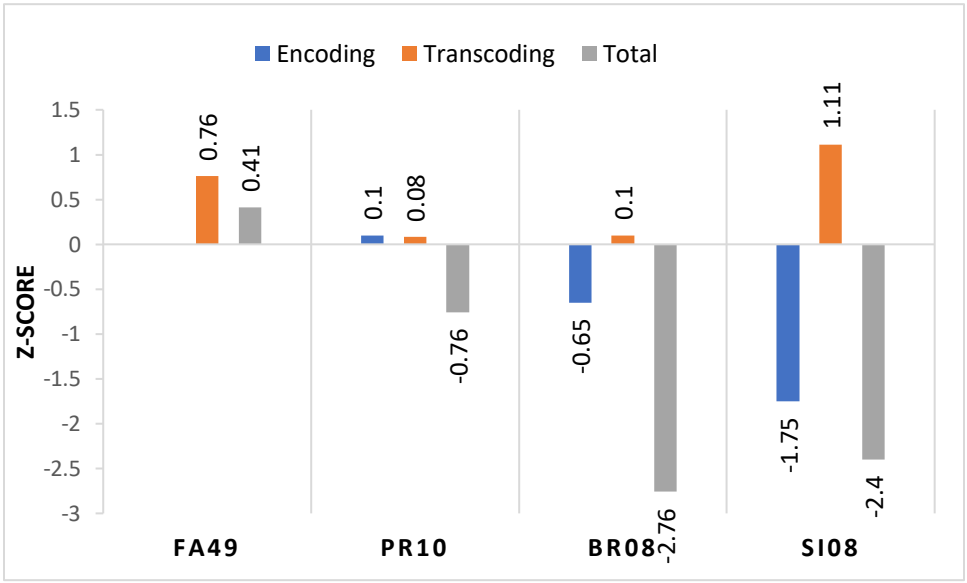
The GFTA-3 was used to capture current speech errors for the three children. PR10 earned a percentile rank of <0.1, displaying errors for some pre- and postvocalic /r/, /ð/, and displaying depalatalization (e.g., / fɪs/ for "fish"). A few vowel errors were noted as well. SI08 earned a percentile rank of 5, displaying errors for /θ/, /ð/, postvocalic /l/, and some slight dentalization of /s/ and /z/. BR08 earned a percentile rank of 21, displaying errors for /θ/ and /ð/. A few vowel errors were also noted informally in his speech. Overall, these data show some residual speech errors among the children, with the proband having the greatest speech impact currently.

Data from speech measures are reflective of the speech difficulties all the children in this family have experienced. SRT results show below average total scores, with the twins showing below average encoding scores as well. This suggests that all the children struggled with nonword repetition, in contrast to their father who scored within the normal range. The twins also struggled with encoding, which reflects the ability to

encode the phonological content of the nonwords. SI08's SRT score actually fell below the identified cutoff score of 46.9, which can be suggestive of CAS.

Figure 4.1

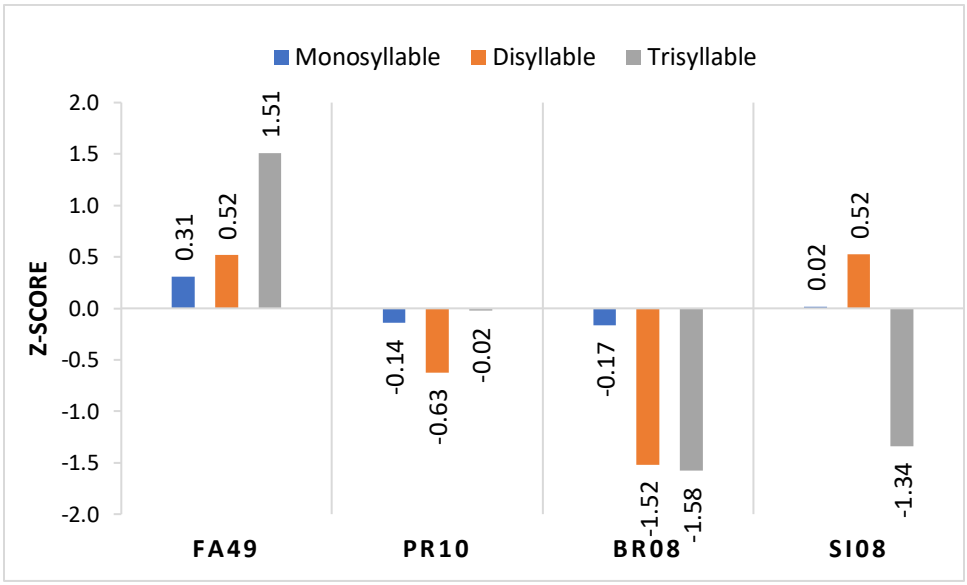
Family 15 Nonword Repetition Task (SRT)



Data from the DDK task indicate that the children struggled more with rapid syllable production compared to their father. PR10 struggled most with disyllables, BR08 struggled with both di- and trisyllables, and SI08 struggled only with trisyllable production. It is noteworthy that the three children had the least difficulty with monosyllables, since the di- and trisyllables require more complex more motor planning.

Figure 4.2

Family 15 DDK Tasks

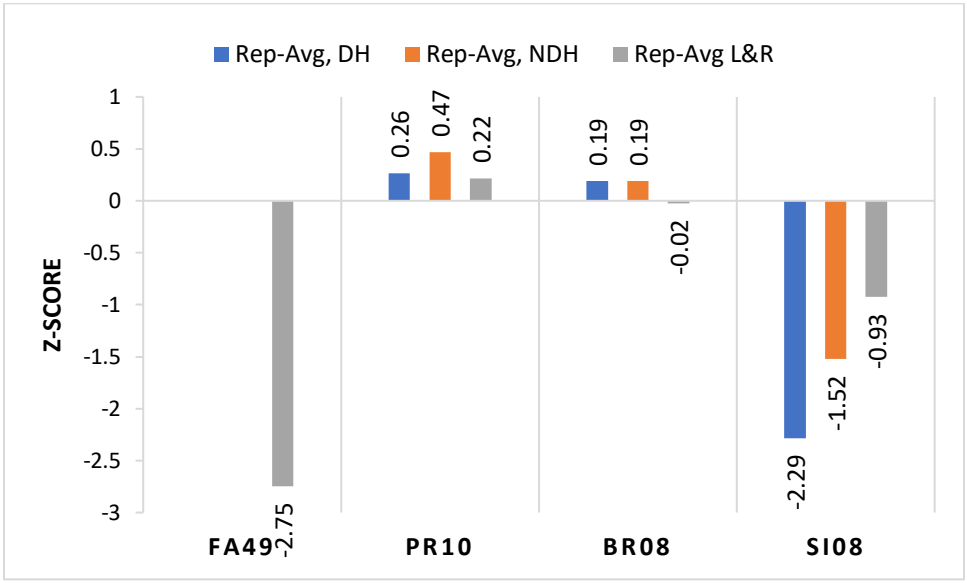


Motor Measures.

The average repetitive finger tapping interval for both hands combined indicates that FA49 and SI08 had significant difficulty with both dominant and nondominant hands, while the brothers were within normal limits.

Figure 4.3

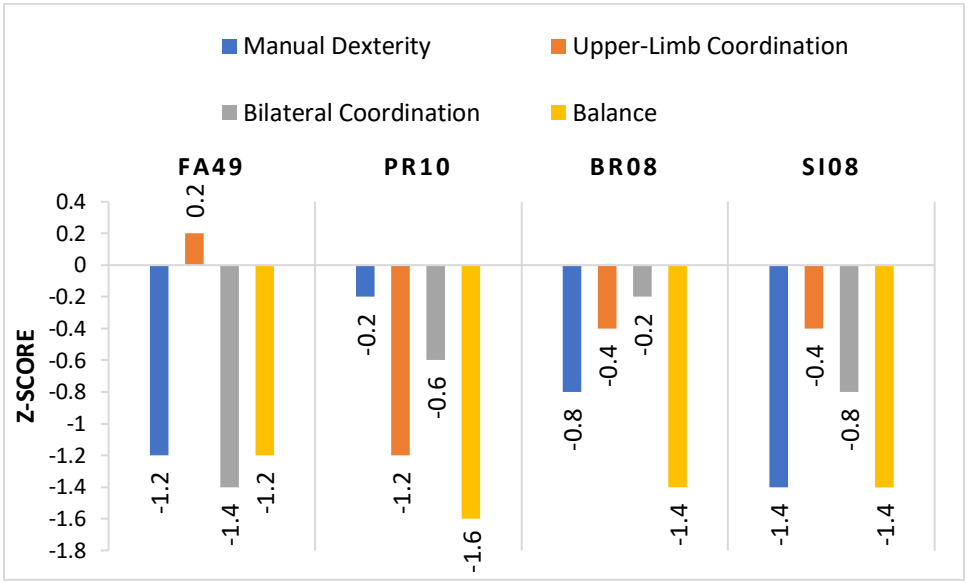
Family 15 Repetitive Finger Tapping



Data from the BOT2 reveal that all four family members struggled with motor skills, particularly balance which is governed by the cerebellum. Manual dexterity was challenging for three of the four family members, as was bilateral coordination.

Figure 4.4

Family 15 Motor Tasks (BOT2)

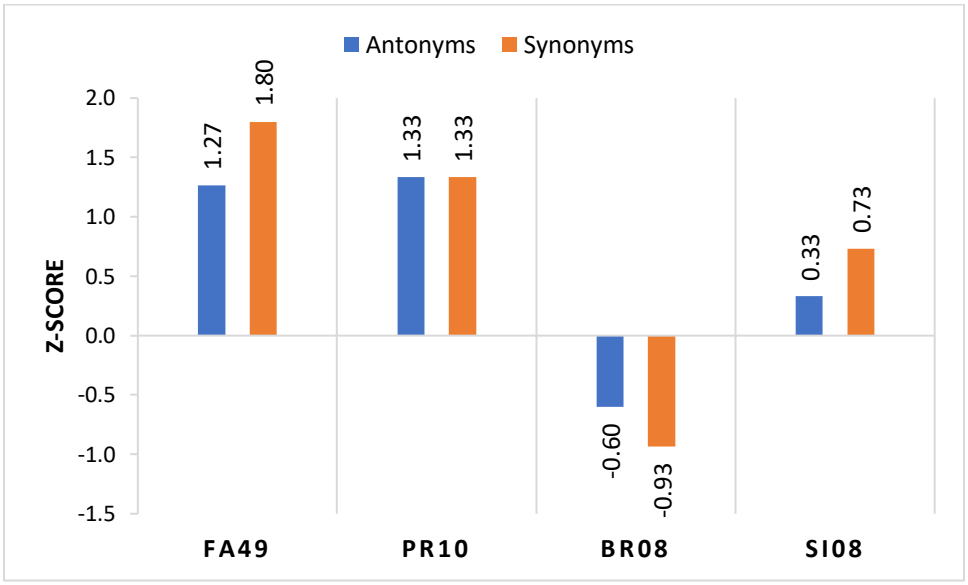


Language, Reading, and Cognitive Measures.

In analyzing antonyms and synonyms, which are reflective of language abilities, all family members had average to above average scores with the exception of BR08, who struggled with both antonyms and synonyms. PR10 had the exact same z-score for both antonyms and synonyms, whereas both FA49 and SI show better performance for synonyms compared to antonyms.

Figure 4.5

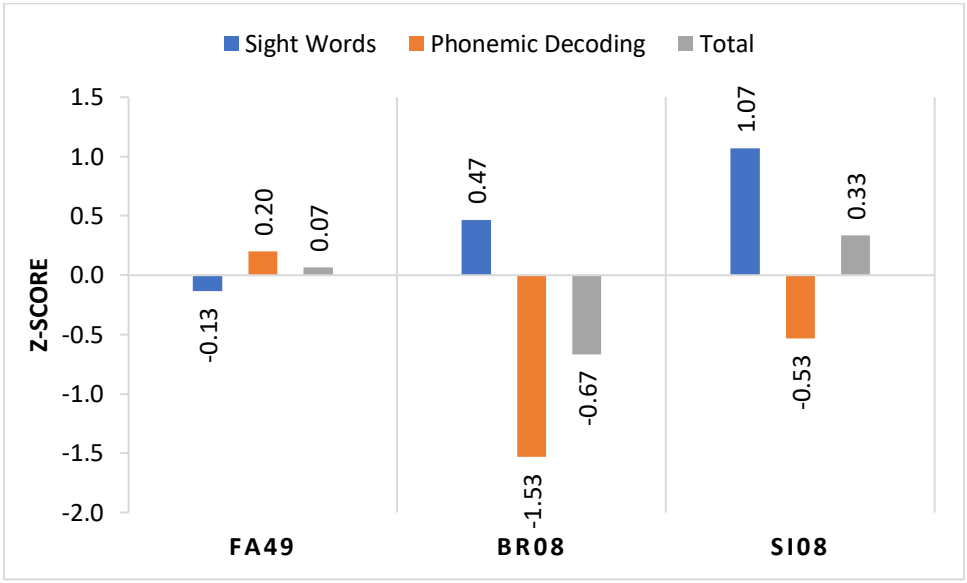
Family 15 Antonyms and Synonyms (CASL-2)



For the reading task, FA49 had average reading ability. Both children had difficulty with reading nonwords as shown by the low phonemic decoding z-scores. Whereas sight words are processed as a whole unit, nonword reading which requires much more sequential processing for word-attack. PR10 had missing data for this task.

Figure 4.6

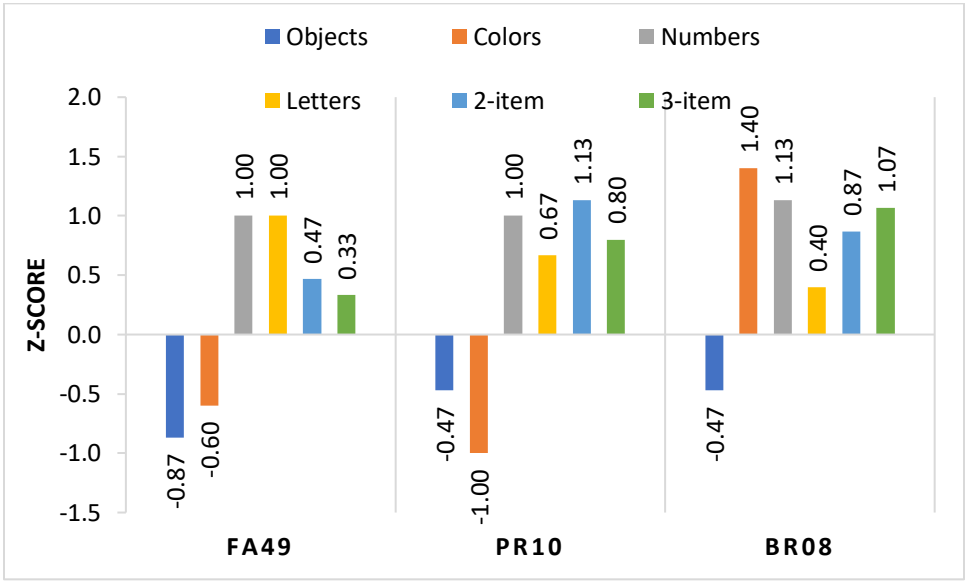
Family 15 Reading Tasks (TOWRE)



For rapid automatic naming, Family 15 did well overall, even when alternating between two items (letters and numbers) and three items (letters, numbers, and colors). FA49 and PR10 had more difficulty with objects and colors than with symbols. SI08 had missing data for this task.

Figure 4.7

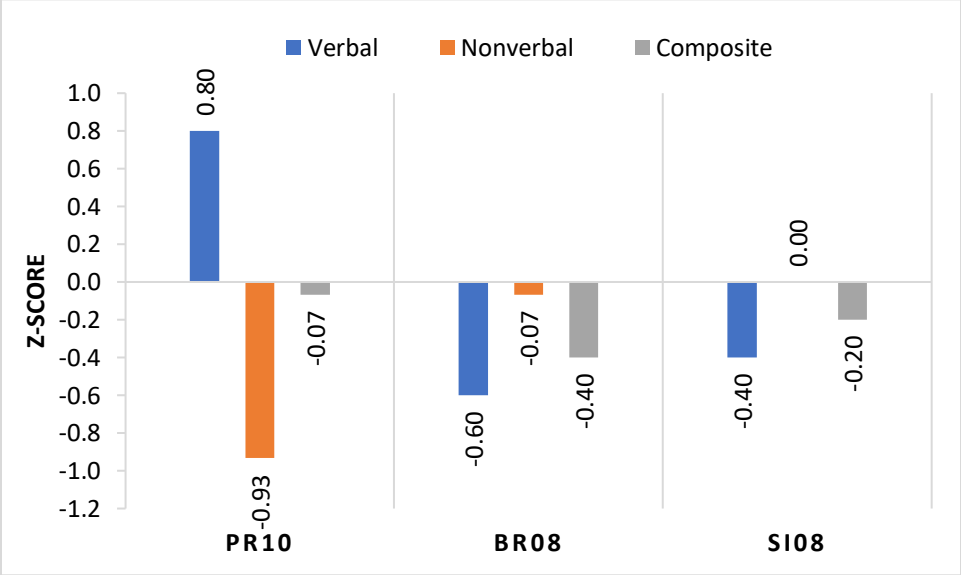
Family 15 Rapid Automatic Naming (RAN/RAS)



Just the children took part in cognitive testing, which showed an overall typical composite measure, yet there were differences between the verbal and nonverbal indices. The proband had a strong verbal index but struggled with the nonverbal subtests, whereas the twins had stronger nonverbal subtests compared to verbal.

Figure 4.8

Family 15 Cognitive Tasks (RIAS-2)



For Family 15, the FA49 shows a mixed phenotype, with poor motor scores that did not affect speech production. He showed strong language skills, especially given that English is his second language. The data from PR10 shows good language skills, but difficulty with sequential speech skills. He displayed poor motor skills overall, but his scores for manual skills were average. BR08 struggled across all the tasks with the exception of rapid automatic naming. He displayed difficulty with sequential speech tasks, but he did show average finger tapping. Additionally, he shows some signs of language difficulties, as he struggled with the synonyms and antonyms. Overall, the

members of Family 15 reflect a phenotype characterized by sequential difficulties of speech, motor, and reading skills, with FA49 not currently affected for the speech modality.

Genetic Analysis

Genetic analysis revealed only one potential gene of interest shared by those members of Family 15 whose genetic data underwent whole exome sequencing: BR08, PR10, and CO22 (Table 4.1). While there were several CNV calls for Family 15, most for PR10, none of these calls were shared among the three family members. All three individuals shared a missense variant in *GPR56*, a gene with limited representation in the neurodevelopmental literature. *GPR56* has been found to regulate cortical patterning and is generally associated with neuronal migration disorders, specifically isolated polymicrogyria (Guerrini & Parrini, 2010). Loss-of-function mutations in this gene can lead to bilateral frontoparietal polymicrogyria, causing epilepsy, cognitive impairment, language impairment, and motor delays (Jin et al., 2007). A 15 bp deletion in *GPR56* was found in several cases of polymicrogyria localized to the Sylvian fissure and Broca's area, with affected individuals displaying cognitive and language impairments as well as seizures (Bae et al., 2014). While Family 15 displays a much milder phenotype than cases of *GPR56* variants described in the literature, it is possible that this particular missense variant, which was not loss-of-function, could yield a milder phenotypic effect. Additionally, it has incredibly high expression rates in the developing brain.

Summary

In general, the phenotype for Family 15 reflects clear deficits in sequential processing, affecting speech and motor domains. The exception to this is FA49 whose speech is typical, but who shows clear motor deficits. SI08 displayed larger motor deficits as evidenced by the finger tapping task, which could be due to her mild cerebral palsy. In addition to sequential processing, BR08 also shows some difficulty with perceptual processing. Genotype analysis suggests *GPR56* as a possible gene of interest for this family, though the cases described in the literature are generally more severe. BrainSpan data indicate that *GPR56* is highly expressed in the cerebellum, thalamus, and striatum of the developing brain, which could be associated with the sequential processing deficits observed in this family. With its high thalamic expression rates, it was hypothesized that there would be additional perceptual processing deficits observed, a pattern only exhibited by BR08. Though there is no concrete evidence, speculation could suggest that the others may have one or more protective genes for a perceptual processing biomarker not shared by BR08.

Table 4.1

Variants of Interest for Fam 15

Gene	***GPR56***
Chr: Locus	**16**: 57693498
RS ID	rs17379472
Variant	T>C
Type	SNP- missense
Allele Freq gnomAD NFE	0.04
CADD Scaled	26.4
pLI	0
PolyPhen-2	PrD (0.97)
SIFT	Del (0)
MOI	AD
Dev Crblm Exp	96.25
Dev Thal Exp	63.21
Dev Strm Exp	69.42
Evidence	Jin et al., 2007

SNP=single nucleotide polymorphism; NFE=non-Finnish European; MOI=mode of inheritance; Crblm=cerebellum; Thal=thalamus; Strm=Striatum of Basal Ganglia; AR=autosomal recessive; AD=autosomal dominant; CH=compound heterozygosity; DN=*de novo;* PoD=possibly damaging; PrD=probably damaging; Ben=benign; Del=Deleterious; Tol=tolerated; LoF=loss-of-function; CNV=copy number variation; kb=kilobases

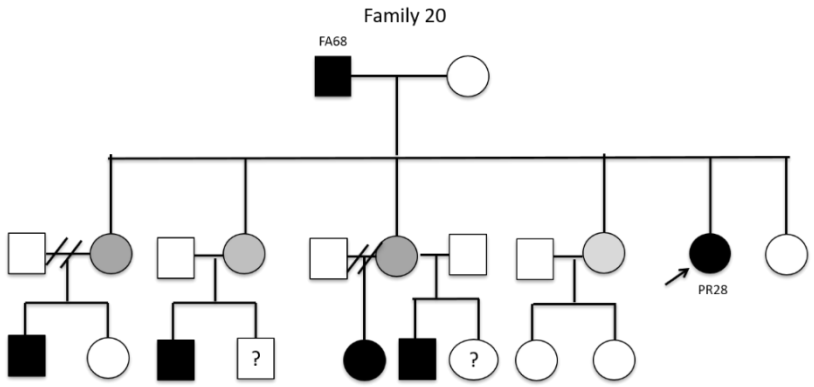
Table 4.2

Family 15 Participants

Participant ID	**Sex**	**Affectation Status**	**Age at Testing**	**Handedness**
Fam15_FA49	M	Affected	49;8	Right
Fam15_PR10	M	Affected	10;7	Right
Fam15_BR08	M	Affected	8;3	Right
Fam15_SI08	F	Affected	8;3	Right

Family 20

Background



Family 20 is a three-generation family consisting of an affected father, an unaffected mother, and six biological children, all female. The fifth daughter serves as the proband of this family and participated in the full behavioral testing battery. Partial data from FA68 that was previously collected were also analyzed. While five of the six daughters show some degree of affectation, the oldest four were not tested for speech disorders during childhood but have reported continuing speech difficulties into adulthood. Several of the grandchildren in the third generation have now displayed speech affectation as well, suggesting a genetic influence on phenotype.

Behavioral Analysis

Table 4.3

Family 20 Participants

Participant ID	Sex	Affectation Status	Age at Testing	Handedness
Fam20_PR28	F	Affected	28;9	right
Fam20_FA68*	M	Affected	68	right

*Tested prior to current study

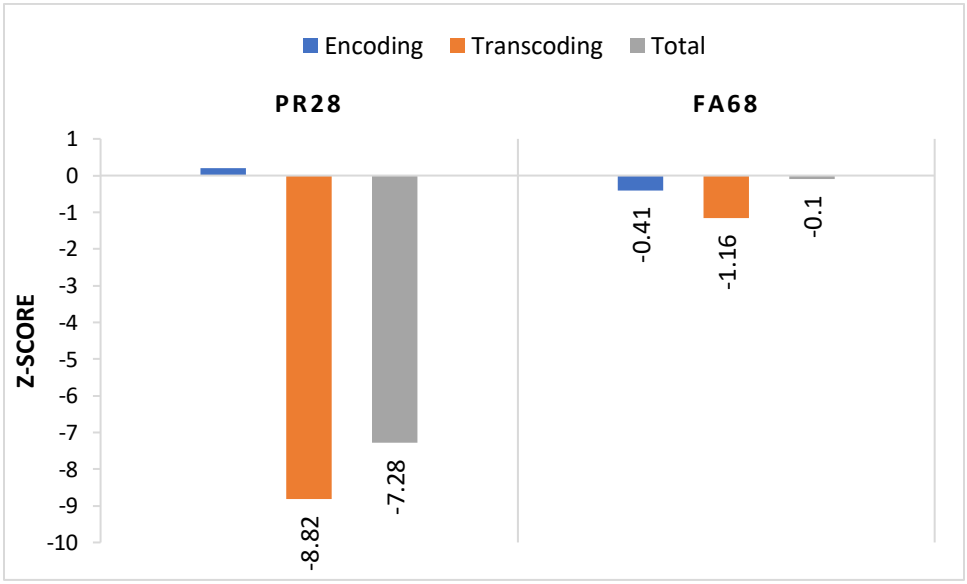
Speech Measures.

The GFTA-3 was used to capture current speech errors for PR28. The oldest GFTA-3 norms available (21 years) were used, and she earned a percentile rank of 50 and made no word-level errors.

PR28 demonstrated significant difficulty with the nonword repetition task compared to her father and compared to all the other participants in this study. She also demonstrated significant difficulty with transcoding, which is the score most suggestive of CAS. This is indicative of adding additional phonemes to the overall CV(CVCVCV) syllable structure. FA68 also had trouble with transcoding, though his overall performance was better.

Figure 4.9

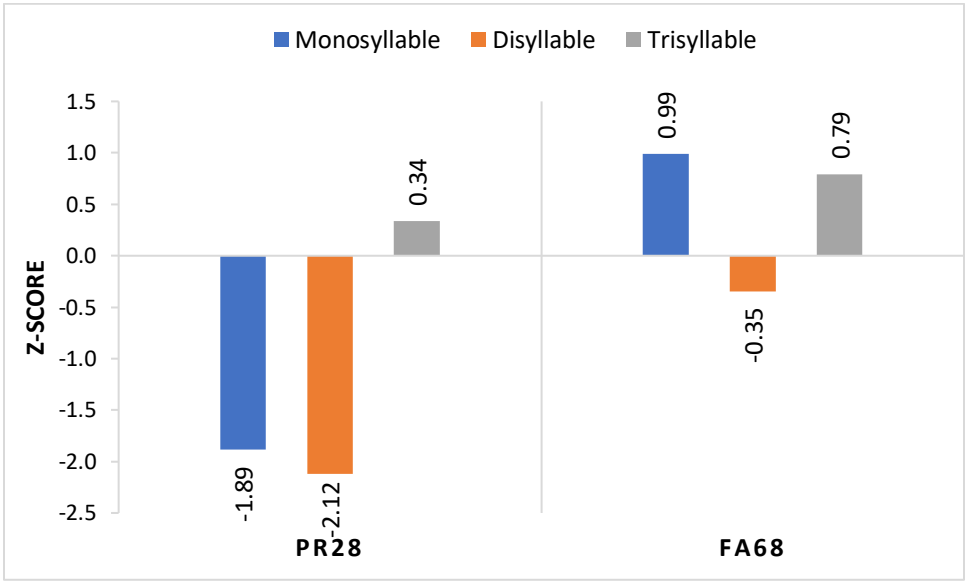
Family 20 Nonword Repetition Task (SRT)



For the DDK task, FA68 showed minimal difficulty with disyllables, but PR28 showed significant difficulty with both monosyllables and disyllables. Her trisyllable score was in the average range.

Figure 4.10

Family 20 DDK Tasks



PR28 had a 14% difference between her phoneme accuracy when producing control sentences compared with tongue twisters. She made a number of alterations to the sentences, with more changes to the tongue twisters than the control sentences overall. For her deletions, she tended to make whole-word deletions rather than single phoneme deletions, which may be indicative of working memory struggles during this task. While participants were given the opportunity to ask for repetitions to minimize working memory load, the tongue twister was presented as a single auditory unit. What is uncertain from this task is if the challenge presented by the tongue twisters is related to

encoding the phonemic complexity, planning and production of the phonemic complexity, or both.

Figure 4.11

Family 20 Phoneme Accuracy in Tongue Twisters Compared to Control Sentences

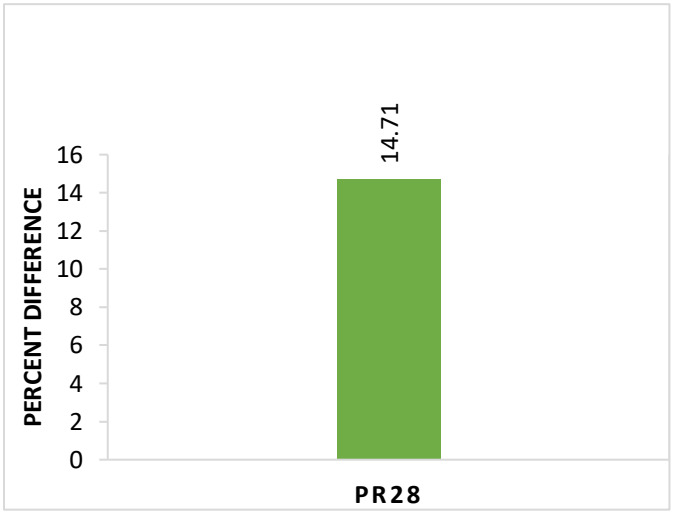
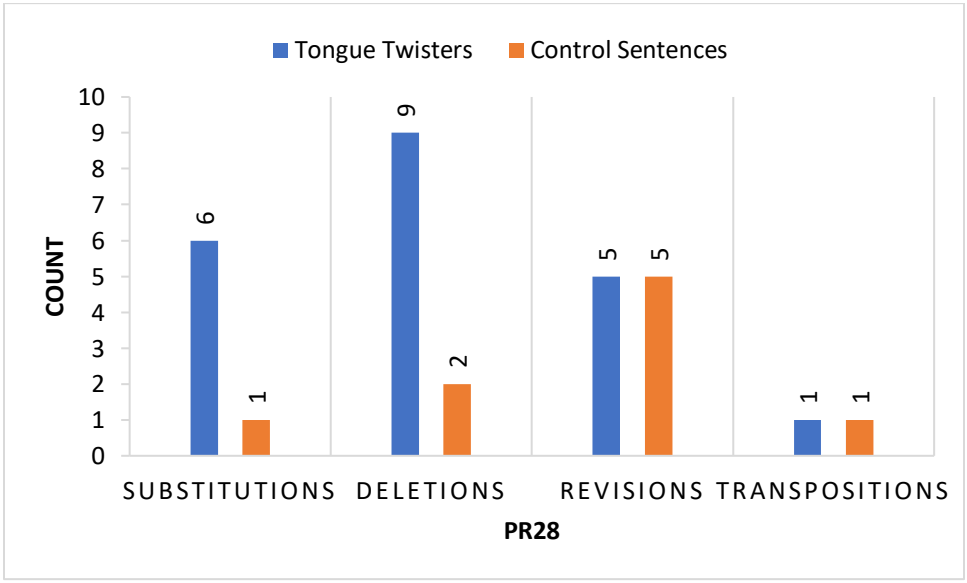


Figure 4.12

Family 20 Sentence Alterations

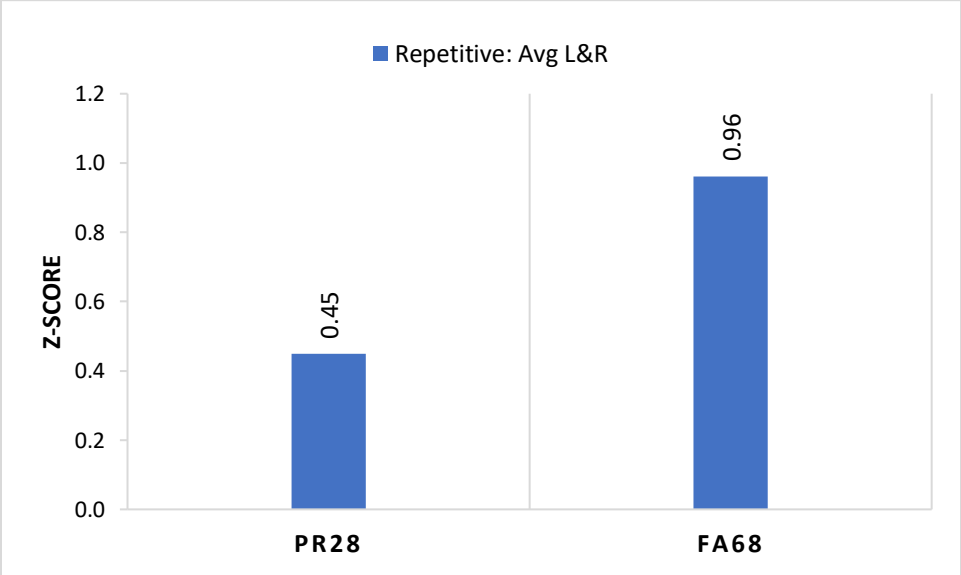


Motor Measures.

PR28 and FA68 both had average finger tapping scores.

Figure 4.13

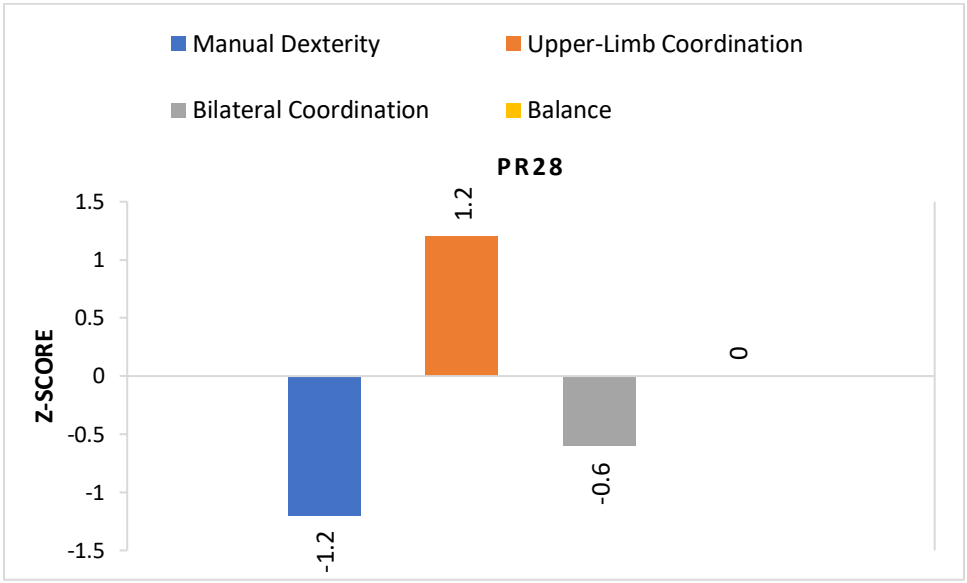
Family 20 Repetitive Finger Tapping



The BOT2 test shows mixed results, with PR28 showing difficulty with manual dexterity and some bilateral coordination, but average scores for upper-limb coordination and balance.

Figure 4.14

Family 20 Motor Tasks (BOT2)

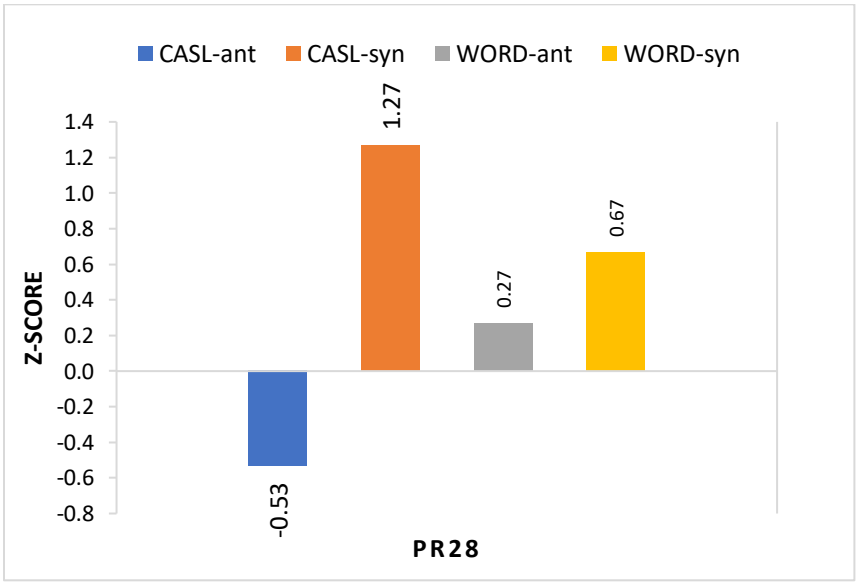


Language, Reading, and Cognitive Measures.

Generally, PR28 performed well on the antonyms and synonyms tasks, except for the CASL-2 antonyms task. She demonstrated a stark difference between the antonyms and synonyms for the CASL-2. These two tasks were structured differently in that the antonym task required the participant to generate a word, whereas the synonym task required the participant to select a synonym from a field of four choices, thus minimizing the need for word retrieval. The WORD-2 tasks both required response generation, and scores show a smaller difference, though the antonym score is still lower than the synonym score.

Figure 4.15

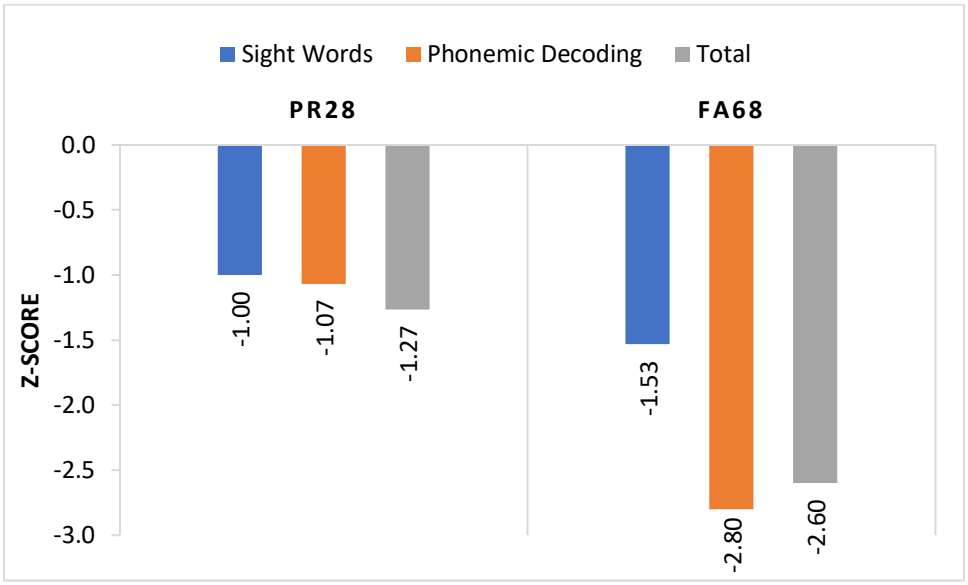
Family 20 Antonyms and Synonyms (CASL-2 and WORD Test 2)



Both PR28 and her father displayed substantial reading deficits across word type, with FA68 showing more difficulty with phonemic decoding compared to sight words, and PR28 showing equal difficulty with both sign words and nonword reading. This indicates that both members of Family 20 struggle with the sequential processing required to read nonwords, and the perceptual processing needed to form, store, and retrieve mental representations need for sight word reading.

Figure 4.16

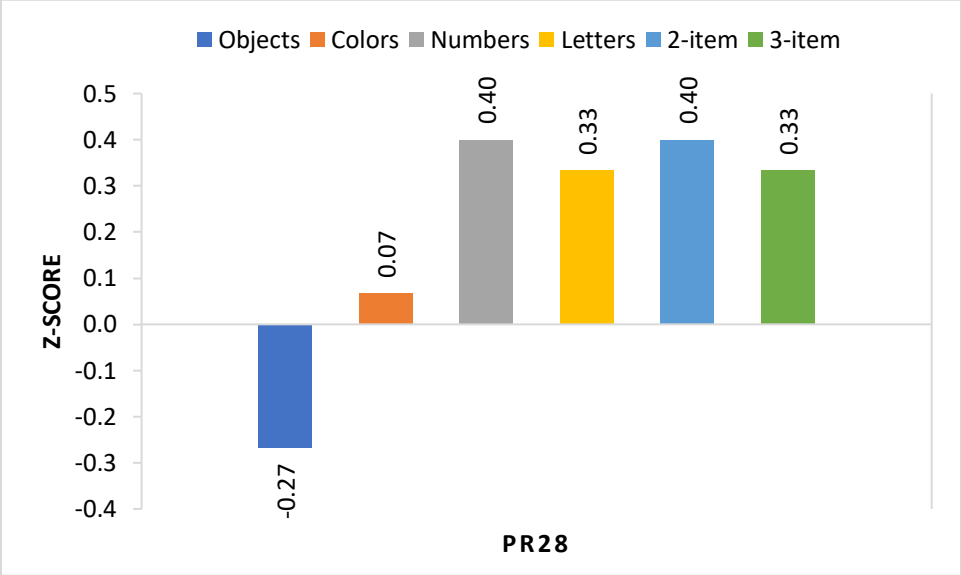
Family 20 Reading Tasks (TOWRE)



In general, PR28 showed average scores for rapid automatic naming, even when asked to alternate between letters and numbers or a combination of letters, numbers, and colors.

Figure 4.17

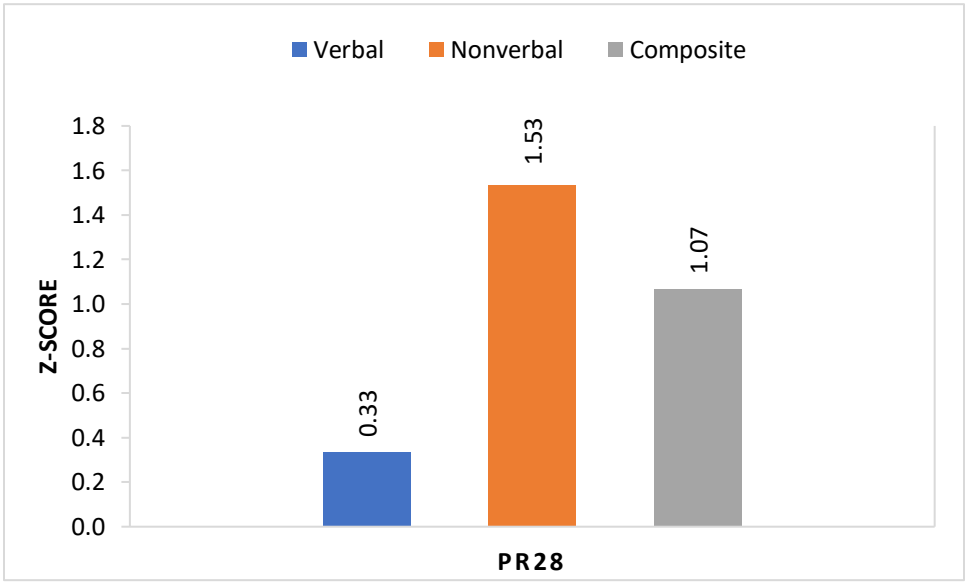
Family 20 Rapid Automatic Naming (RAN/RAS)



The cognitive task scores for PR28 were in the average range, with a higher nonverbal score compared to her verbal score.

Figure 4.18

Family 20 Cognitive Tasks (RIAS-2)



The two individuals from Family 20 that participated in behavioral testing show overall deficits in speech, motor, and reading tasks. PR28 shows a clear pattern of sequential processing deficit, with low scores in nonword repetition, nonword reading, and DDKs. FA68 showed better DDK scores than P28, but still struggled a bit with the alternating sequences required for the DDK disyllable task. Both PR28 and FA68 showed some evidence of perceptual processing difficulty as evidenced by sight word reading.

Genetic Analysis

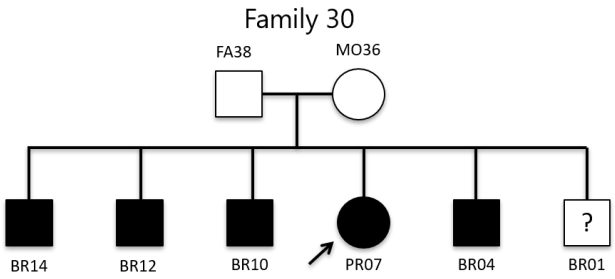
Genetic analysis did not reveal any called variants or CNVs that were relevant for the phenotype in this family.

Summary

Family 20, with a history of CAS, shows a clear sequential processing phenotype, with some evidence of an accompanying perceptual processing deficit affecting reading. Family history suggests genetic contribution to the phenotype, and as such, while this study revealed no genes of interest, further genetic analyses comparing affected and unaffected family members may uncover a potential gene of interest.

Family 30

Background



Family 30 consists of two reportedly unaffected parents and six biological children, five males and one female, who serves as the proband of this family. The oldest five children all struggled with speech development, as evidenced by delayed babbling and emergence of first words. Eventually, all the children have required speech therapy and have a diagnosis of CAS. Given family history, the youngest child began receiving speech-language intervention via parent training starting at 2 months old, with the aim of preventing potential speech deficits. Prenatal and birth history were within normal limits, and all children were born full-term. All children reportedly had multiple ear infections when they were young, though none required pressure equalization (PE) tubes, and all have since passed a hearing screening. Though parents are reportedly unaffected, several extended family members on both maternal and paternal sides of the family report a history of speech, language, reading, and learning disabilities. Both parents are right-handed, but the three oldest boys are left-handed (Table 4.5).

Behavioral Analysis

Speech Measures.

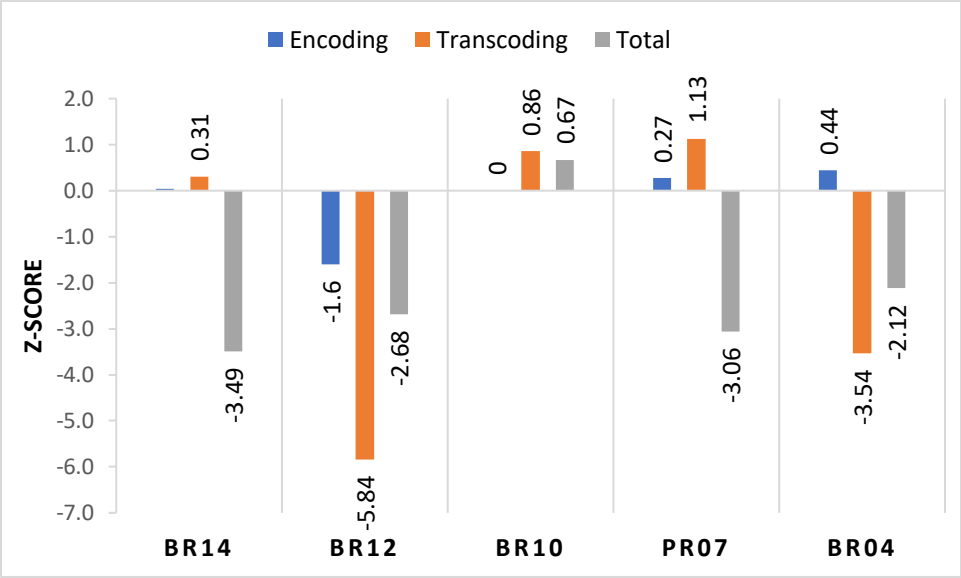
The GFTA-3 was used to capture current speech errors for the children in Family 30. BR14 earned a percentile rank of <0.1 for consistent production of dentalized /s/ and

/z/. BR12 earned a percentile rank of 63. Though he produced no consonant errors on the test, he did display a few intermittent vowel errors. BR10 earned a percentile rank of 68 and made no speech errors. PR07 earned a percentile rank of <0.1, with inconsistent /r/ errors and a few dentalized /s/ and /z/ productions. BR04 earned a percentile rank of 1 and displayed several phonological processes, including cluster reduction (e.g., /wɪŋ/ for "swing"), inconsistent velar fronting (e.g., /d$\overline{\text{oʊ}}$/ for "go"), some use of velar assimilation (e.g., /gʌk/ for "duck"), depalatalization (e.g., /fɪs/ for "fish"), and inconsistent gliding (e.g., /wɪŋ/ for "ring"). He collapsed various fricative phonemes into /f/ (e.g., /f$\overline{\text{oʊ}}$p/ for "soap") and has not yet acquired /r/. His connected speech was sometimes less than 50% intelligible. BR01 was too young to complete this task.

Data from the speech measures are reflective of the speech difficulties the oldest five children have experienced. SRT results show below average total scores for all the children except BR10. Both BR12 and BR04 show below average transcoding scores as well, which is the SRT score most indicative of CAS.

Figure 4.19

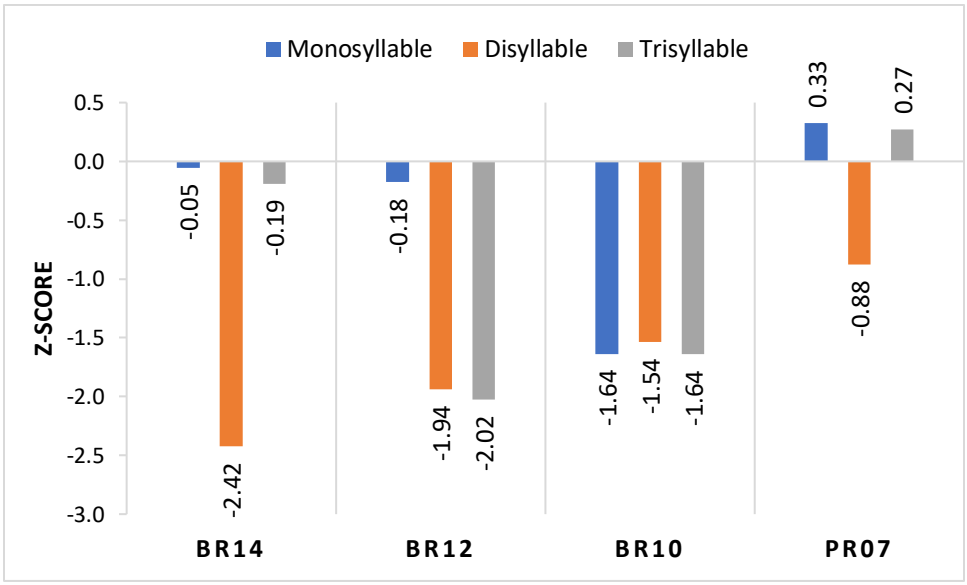
Family 30 Nonword Repetition Task (SRT)



Data from the DDK task indicate that the four children who participated in this task all struggled with disyllables. This suggests difficulty motor planning for the alternating movement of the disyllable task, though BR14 and PR07 moved back into the average range for the trisyllable task. BR10 struggled with all three tasks equally, which contrasts with BR14 who struggled only with disyllables.

Figure 4.20

Family 30 DDK Task



The three oldest children also participated in a tongue twister task. The difference score represents the difference between the percent of phonemes produced correctly while repeating control sentences compared to tongue twisters, and BR12 had a large difference compared to his brothers. BR14 made fewer substitutions overall and used more revisions for his errors. BR12 made the highest number of substitutions. This, in combination with his poor nonword repetition scores suggests that BR12 has significant deficits in sequential processing for speech, despite making no consonant errors on the GFTA-3. This has significant clinical relevance, given that the GFTA-3 is one of the most commonly used tools for assessing speech in pediatric settings, and yet it was unable to capture BR12's underlying sequential deficit.

Figure 4.21

Family 30 Phoneme Accuracy in Tongue Twisters Compared to Control Sentences

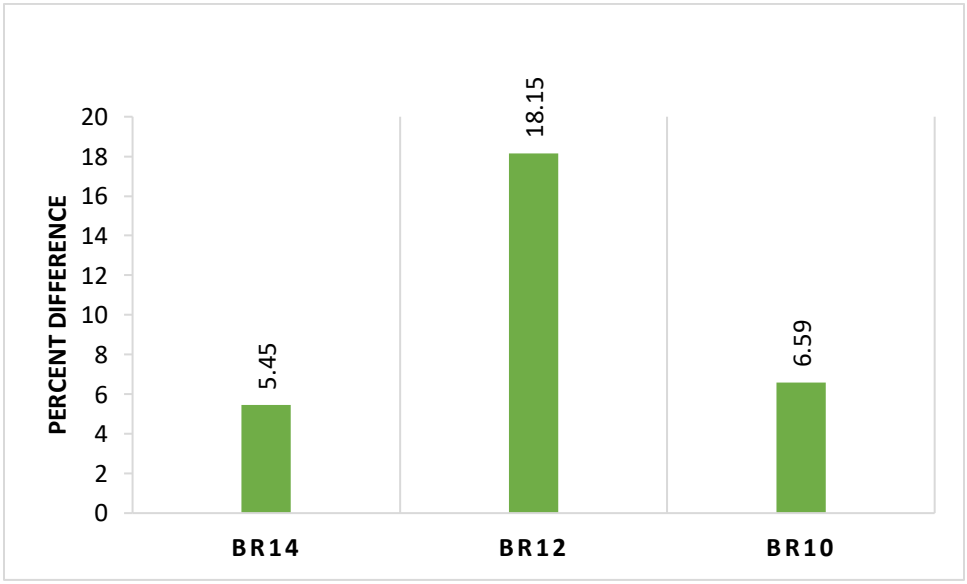
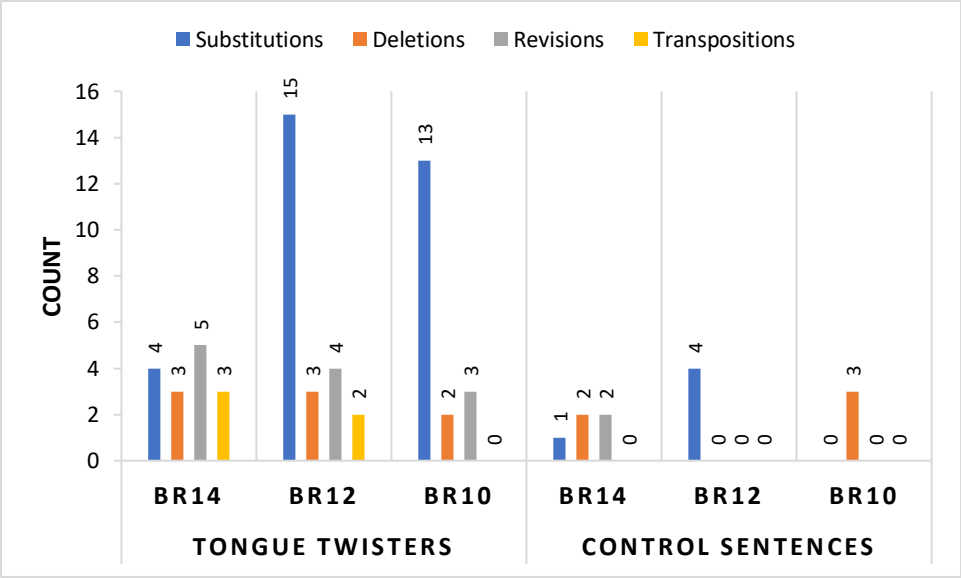


Figure 4.22

Family 30 Sentence Alterations

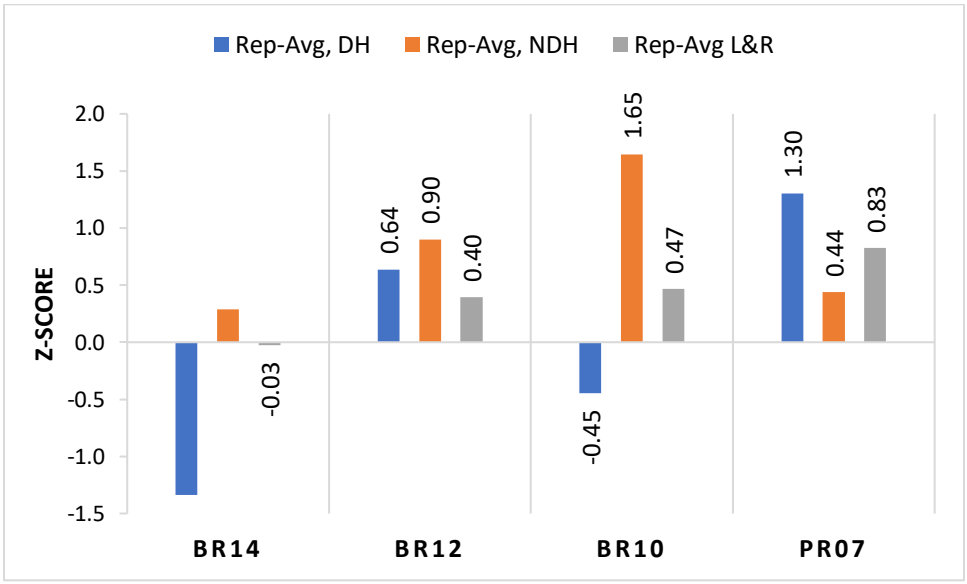


Motor Measures.

The children showed much stronger performance with the finger tapping task. All the conditions were average to above average with the exception of the average tapping interval with the dominant hand for BR14 and BR10. This is interesting to note given that all three of the boys (BR14, BR12, and BR10) are left-handed, but BR12 indicated that his hand dominance does not match his foot dominance. He writes with his left hand, but unlike BR14 and BR10, he kicks with his right foot, and his dominant hand finger tapping was typical. It could be interesting to investigate laterality patterns in participants with sequential processing deficits.

Figure 4.23

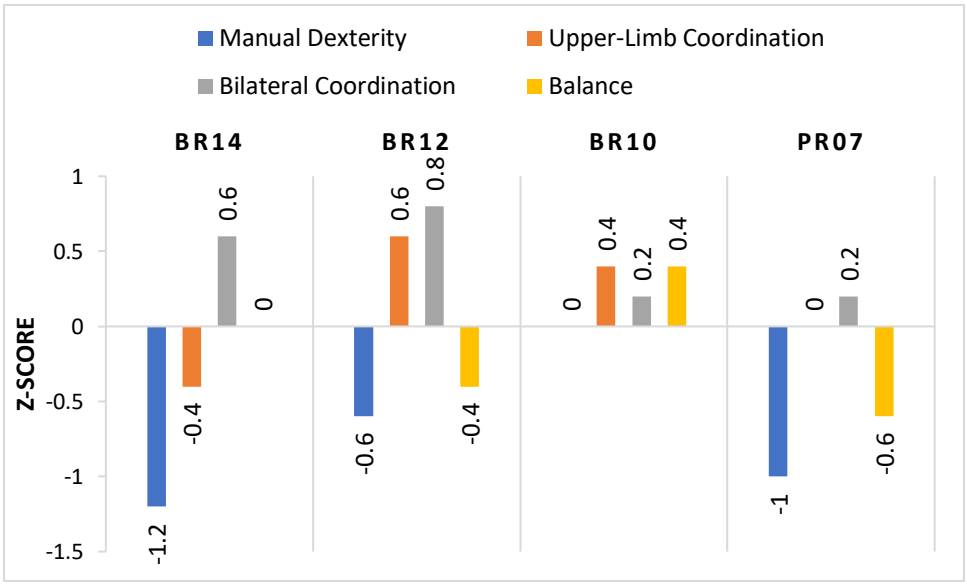
Family 30 Repetitive Finger Tapping



The BOT2 test shows mixed results, with all the children performing well for bilateral coordination, BR12 and PR07 struggling a bit with balance, and all but BR10 struggling with manual dexterity. It is interesting to note that BR14 had the lowest z-score of his family on any BOT2 subtest and he also had the lowest finger tapping z-score, suggesting substantial fine motor difficulties.

Figure 4.24

Family 30 Motor Tasks (BOT2)

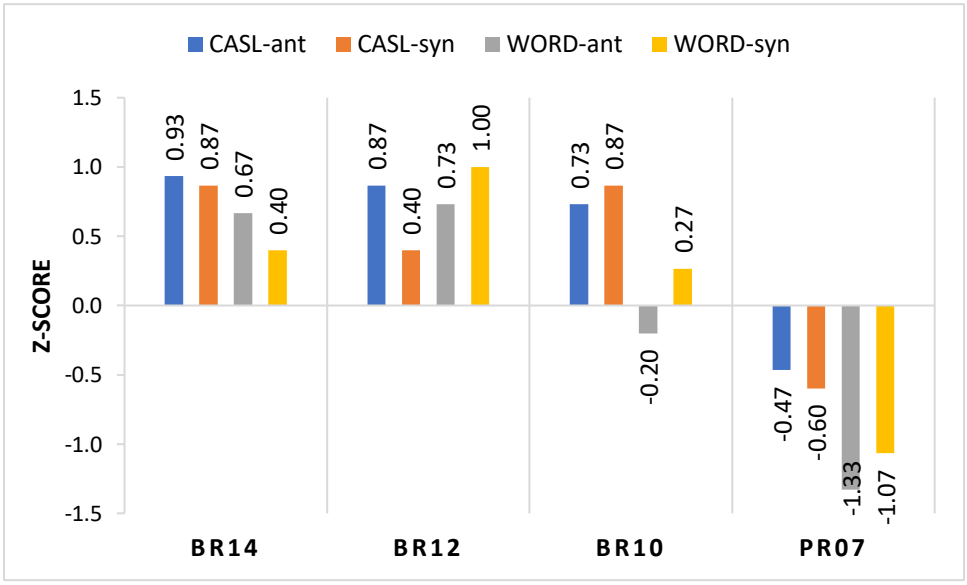


Language, Reading, and Cognitive Measures.

The oldest four children in Family 30 participated in two different tasks to investigate antonym and synonym knowledge. All the children were within or above the average range for these tasks with the clear exception of the proband. She had significant difficulty with all four tasks, particularly those that required her to generate her own response given a stimulus word aloud. There is no clear pattern of difference between the antonym and synonym tasks.

Figure 4.25

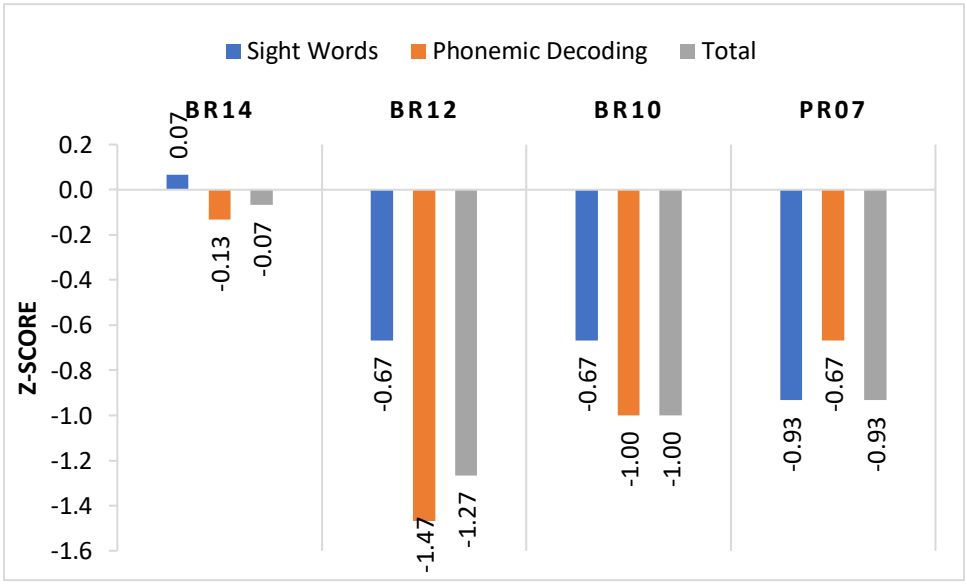
Family 30 Antonyms and Synonyms (CASL-2 and WORD Test 2)



Data from the sight word and nonword reading tasks clearly show a difference between BR14 and his younger siblings, who struggled on all reading tasks. All of the younger siblings show a clear deficit in phonemic decoding as they attempted to read the nonwords, though the proband did worse with sight words. This suggests she has difficulty with both the sequential processing skills needed for decoding and the perceptual skills needed to remember words as a whole unit for sight word reading, whereas the two boys have more difficulty with decoding.

Figure 4.26

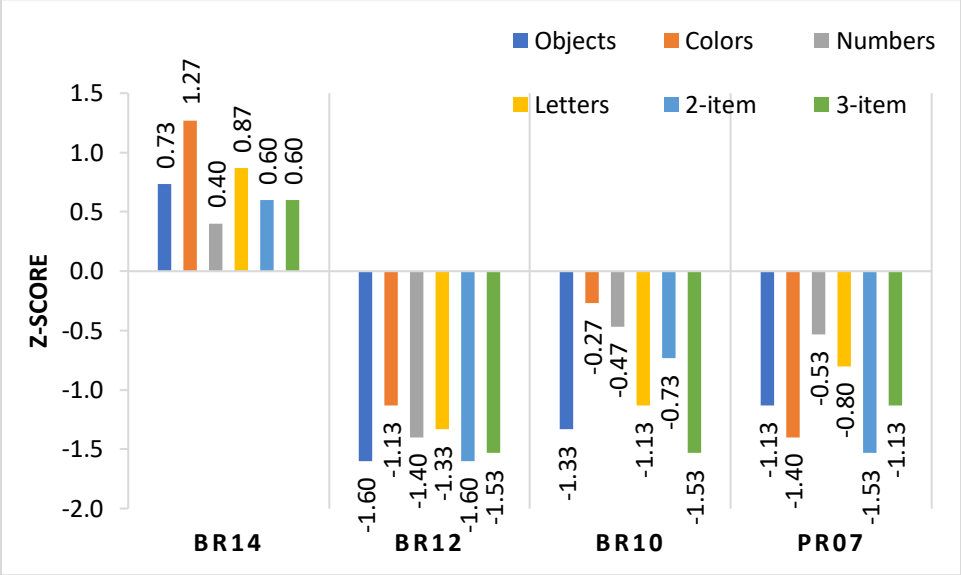
Family 30 Reading Tasks (TOWRE)



It has been said that the rapid automatic naming tasks of the RAN/RAS are a good indicator of reading ability (Wolf & Denckla, 2005), and the results from Family 30 support this trend. Here again it is evident that BR14 shows average skills in contrast to his siblings who show significant deficits in the naming tasks.

Figure 4.27

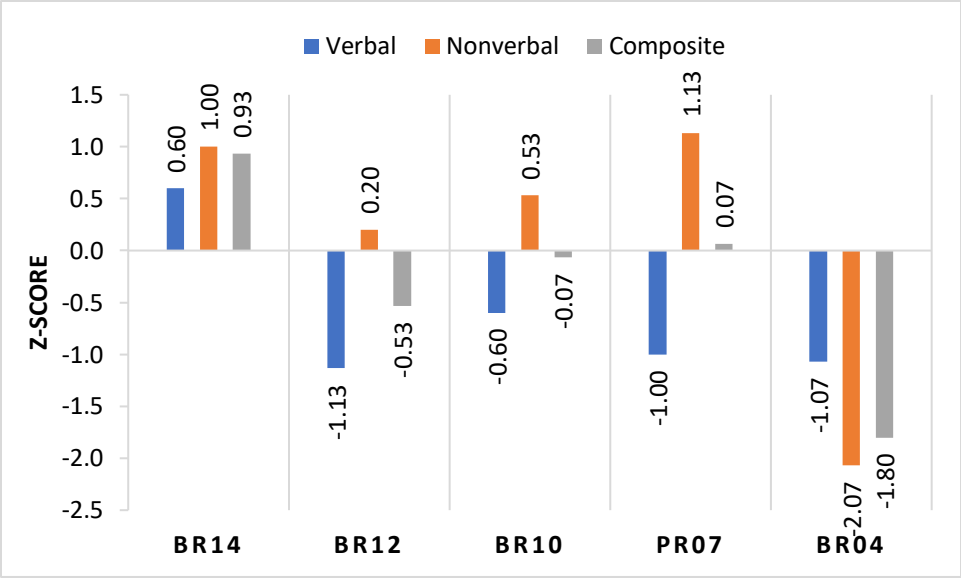
Family 30 Rapid Automatic Naming (RAN/RAS)



Five of the six children were old enough to participate in the cognitive tasks which reveal several interesting trends. Again, BR14 stands out with average to above average scores in all areas in contrast to BR04 who is much lower than average. The middle three children show some mixed results, with all three having more difficulty with verbal tasks compared with nonverbal ones. The proband shows a striking difference between verbal and nonverbal abilities, which could be helpful knowledge for those supporting her education.

Figure 4.28

Family 30 Cognitive Tasks (RIAS-2)



The youngest child, BR01, participated in online speech-language therapy from 2 months old through 24 months old. This intervention was aimed at preventing potential speech-language delays in infants who show genetic risk. His most recent parent questionnaires performed at 24 months indicate that he is performing within normal limits for his age. The ASQ®-3 indicates that BR01's development across all domain areas measured are well above the cutoff scores that would suggest concern. Results from his 24-month MacArthur-Bates CDI questionnaire indicates that he is in the 26th percentile for vocabulary development and the 42nd percentile for combining words. Per parent report, his babbling started at a typical age in contrast to his five older siblings who all had delayed babble. His case provides evidence supporting earliest possible interventions for children who have genetic risk for communication impairments (Peter et al., 2020).

Among the children of Family 30, it is clear that the oldest brother, BR14, is functioning within normal limits across most domains except for a slight lisp and manual dexterity, while the other 4 children show substantial difficulty in a variety of developmental domains. This result could be influenced by multiple factors. It is possible that BR14 has a different phenotypic expression of the genotype he shares with his siblings. It is also possible that BR14's higher scores could reflect the combined effect of education, therapeutic intervention, and his own experiences of learning effective compensatory strategies that the younger children are still acquiring. PR07 presents with the most severe phenotype, showing difficulty across all domains, encompassing sequential processing tasks, reading tasks, and language tasks. The few tasks that BR04 was able to participate in suggest that he may struggle with reading and language abilities in addition to his present speech disorder. BR12 and BR10 show a mixed profile, with both having better speech skills currently according to the GFTA-3. BR12 shows more sequential processing deficits in the speech and motor domains, and both showing deficits with reading and verbal skills.

Table 4.4

Variants of Interest for Family 30

Gene	***CNTNAP2***	***TENM3***
Chr: Locus	**7**: 147600711	**4**: 183714507
RS ID	None	rs184165622
Variant	G>A	C>T
Type	SNP; LoF stop-gained	SNP-missense
Allele Freq gnomAD NFE	-1	0.003
CADD Scaled	47	31
pLI	0	1
PolyPhen-2	None	PrD (0.99)
SIFT	None	Del (0.05)
MOI	AD	AD
Dev Crblm Exp	29.04	NA
Dev Thal Exp	35.07	NA
Dev Strm Exp	64.45	NA
Evidence	Centanni et al., 2015	Leamey et al., 2008

SNP=single nucleotide polymorphism; NFE=non-Finnish European; MOI=mode of inheritance; Crblm=cerebellum; Thal=thalamus; Strm=Striatum of Basal Ganglia; AR=autosomal recessive; AD=autosomal dominant; CH=compound heterozygosity; DN=*de novo;* PoD=possibly damaging; PrD=probably damaging; Ben=benign; Del=Deleterious; Tol=tolerated; LoF=loss-of-function; CNV=copy number variation; kb=kilobases

Table 4.5

Family 30 Participants

Participant ID	**Sex**	**Affectation Status**	**Age at Testing**	**Handedness**
Fam30_BR14	M	Affected	14;5	Left
Fam30_BR12	M	Affected	12;1	Left
Fam30_BR10	M	Affected	10;5	Left
Fam30_PR07	F	Affected	7;7	Right
Fam30_BR04	M	Affected	4;6	Right
Fam30_BR01	M	Unknown	1;3	Unknown

Genetic Analysis

Genetic analysis reveals several potential genes of interest for Family 30, all instances of autosomal dominant inheritance (Table 4.4). While there were several CNV calls for this family, none were shared by all the affected family members. The most significant variant found for this family was in *CNTNAP2*, a gene well-known in the speech-language literature (Centanni et al., 2015; Deriziotis & Fisher, 2017). Because the parents were not selected for exome sequencing, it is unknown from which parent the gene would be inherited. Though the chance of a variant being called in this gene is quite high since it is the largest gene in the entire genome (Rodenas-Cuadrado, Ho, & Vernes, 2014), the variant found in Family 30 is LoF with high pLI and CADD scores. It is classified as a stop-gain variant, providing strong evidence for a phenotypic impact. *CNTNAP2* is a target of the FOXP2 protein, with contact occurring at the first intron site of *CNTNAP2* (Deriziotis & Fisher, 2017). Family 30 shows a heterozygous LoF variant, which has been commonly associated with speech-language phenotypes; whereas homozygous LoF variants can be associated with a more complex phenotype, including autism, seizures, and cognitive impairment, (Deriziotis & Fisher, 2017; Rodenas-Cuadrado et al., 2014). The protein produced by *CNTNAP2*, CASPR2, is part of the neurexin family of proteins and plays a crucial role in neurodevelopment by facilitating neuronal migration and cell-to-cell interactions, and may indirectly contribute to myelination (Centanni et al., 2015; Rodenas-Cuadrado et al., 2014). *CNTNAP2* is highly expressed in the developing brain as the BrainSpan data in Table 4.4 shows. It is thought this "expression pattern recapitulates the cortico-striato-thalamic circuitry known to

modulate higher order cognitive functions, including speech and language, reward, and frontal executive function" (Rodenas-Cuadrado et al., 2014, p. 175). Additionally, there is enriched gene expression found near the Sylvian fissure and surrounding brain areas, including Broca's (Rodenas-Cuadrado et al., 2014). While complex cases do appear in the literature, cases of *CNTAP2* mutation have been found in which CAS appears to be the isolated phenotype, suggesting a wide range of phenotypic impact as well as the possibility that co-occurring language and reading deficits may be a secondary result of the speech phenotype itself (Centanni et al., 2015). Other research proposes sequential processing as an affected, common underlying biomarker that could have impacts across developmental domains (Peter et al., 2018). Thus, the *CNTNAP2* variant found in Family 30 is a clear gene of interest for the phenotype observed in this family.

TENM3 appears as a possible gene of interest for Family 30, with the variant showing a high CADD score, probably damaging PolyPhen-2 score, and a deleterious SIFT score. Its molecular function seems only loosely associated to the phenotype found in Family 30, given its involvement in neurite outgrowth primarily in the developing visual pathway (Leamey et al., 2008). As such, it may influence reading or possibly visual perceptual processing, but it is a much weaker gene of interest for this family.

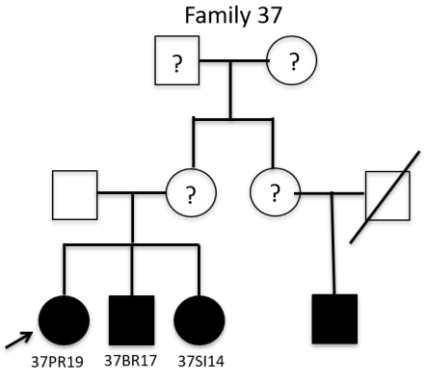
Summary

In general, the phenotype for Family 30 reflects deficits in sequential processing affecting speech and reading domains, as well as some language and motor tasks. In addition to sequential processing, some members of this family also show difficulty with perceptual processing. The exception to this is BR14, the oldest child, who does not

show any language or reading deficits. On literacy tasks, BR12, BR10, and PR07 show difficulty with phonemic decoding and sight word reading, suggesting that they have difficulty with the sequential processing needed to read nonwords, and their difficulty reading sight words is indicative of deficits in creating mental representations of sight words. Genotype analysis suggests that *CNTNAP2* is the strongest gene of interest for this family. Cases found in the literature encompass the phenotypic traits expressed by the individuals of Family30. BrainSpan data indicate that *CNTNAP2* is highly expressed in both the cerebellum and thalamus of the developing brain, and as such, could be related to the combined sequential and perceptual processing deficits observed in this family.

Family 37

Background



Family 37 is a three-generation family consisting of unknown affectation in generations I and II but affectation for all four members of generation III. The three siblings in generation III took part in behavioral testing, and the oldest sister of this family serves as the proband. Per parent report, all three children required speech therapy throughout their childhood due to the presence of CAS. PR19 reportedly had the most severe CAS presentation, and she participated in therapy from the age of 30 months through 8th grade. BR17 participated in therapy from the age of 18 months through 6th grade, and SI14 participated in therapy from the age of 12 months through 2nd grade. The mother indicated that she recognized the need for therapy earlier with each successive child. It is interesting to note that SI14, who started therapy at the youngest age, required the fewest years of treatment. While her phenotype may have been milder, it is reasonable to speculate as to whether the earlier intervention she received may have contributed to a shortened treatment duration.

Behavioral Analysis

Table 4.6

Family 37 Participants

Participant ID	Sex	Affectation Status	Age at Testing	Handedness
PR19	F	Affected	19;8	Right
BR17	M	Affected	17;7	Right
SI14	F	Affected	14;1	Right

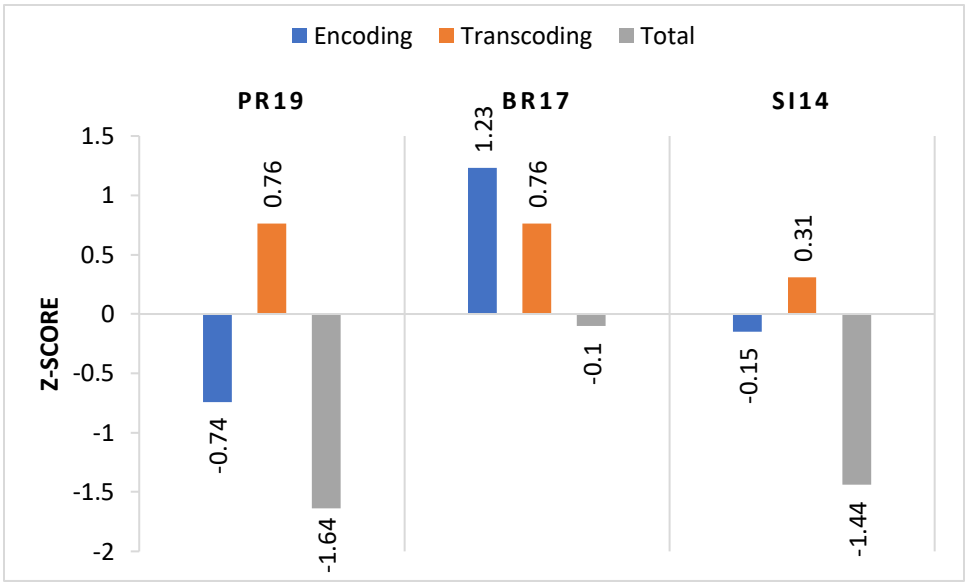
Speech Measures.

The GFTA-3 was used to capture current speech errors for the offspring in Family 37. PR19 earned a percentile rank of 53 and made no word-level errors. BR17 earned a percentile rank of 55 on the GFTA-3 and made no word-level errors. SI14 earned a percentile rank of 58 and made no word-level errors.

PR19 and SI14 demonstrated significant difficulty with the nonword repetition task, contrasting with BR17 who showed average skills. PR19 struggled with encoding, indicating that she made a higher number of across-class manner substitutions (e.g., /b/ for /m/) rather than within-class. This score reflects an individual's ability to encode at least some of the phonetic features of a sound. SI14 had average encoding and transcoding scores but had a lower total score due to instances of deletions, which are not captured in the other scores.

Figure 4.29

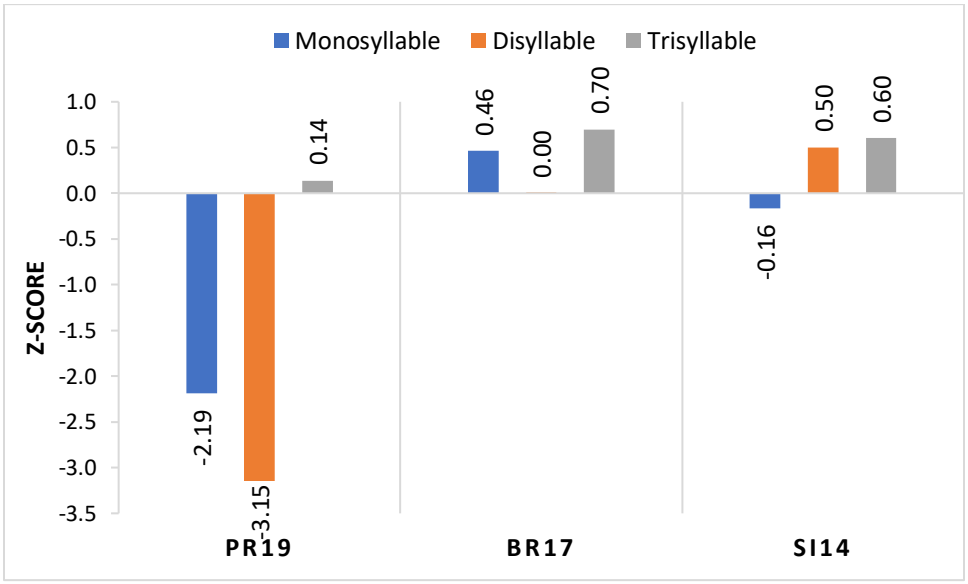
Family 37 Nonword Repetition Task (SRT)



The DDK task clearly showed PR19 had more difficulty with monosyllables and disyllables compared to her siblings, but all of the children produced average trisyllables.

Figure 4.30

Family 37 DDK Tasks



PR19 had the highest difference between her phoneme accuracy when producing control sentences compared with tongue twisters at 8.59%. She also made the highest number of sentence alterations of this family, with more changes to the tongue twisters than the control sentences overall. Her deletions were a combination of word and phoneme deletions, with the phoneme deletions indicating difficulty with motor planning for a phonetically complex task. When he produced errors, BR17 made more revisions, suggesting that he was able to monitor his productions against the target sentence and adjust as needed. SI14 also made a number of tongue twister deletions, with all of her deletions being whole-word rather than phoneme-level deletions, suggesting difficulty with working memory for this task.

Figure 4.31

Family 37 Phoneme Accuracy in Tongue Twisters Compared to Control Sentences

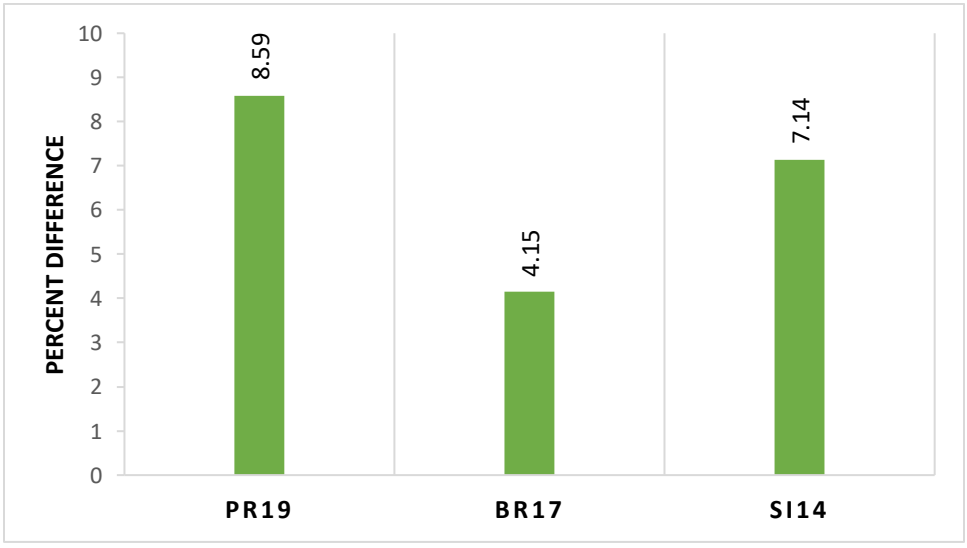
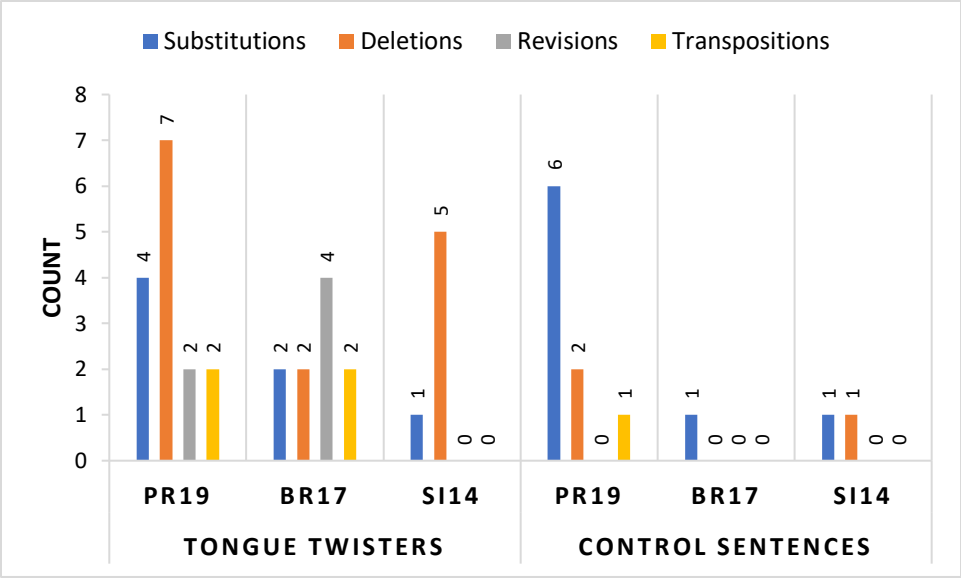


Figure 4.32

Family 37 Sentence Alterations

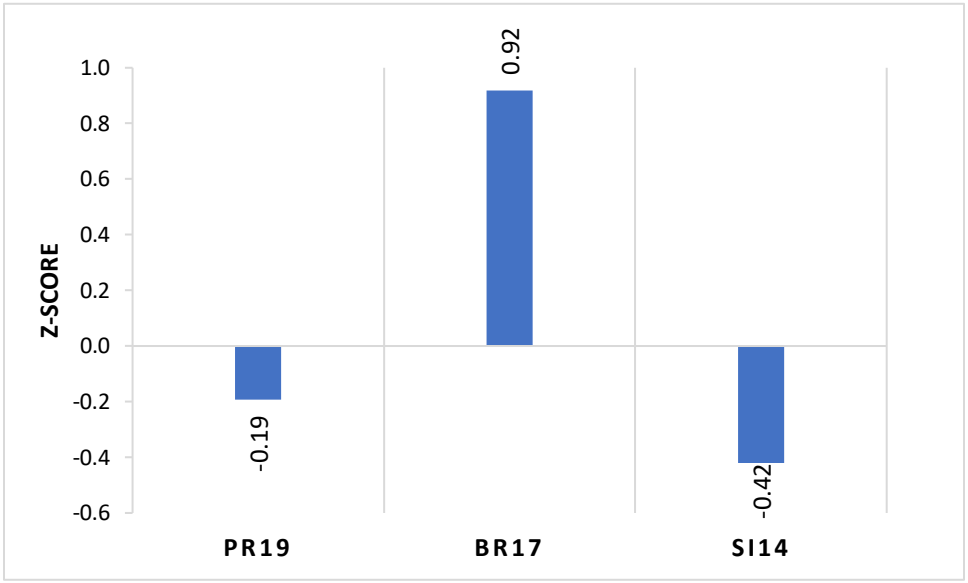


Motor Measures.

For the finger tapping task, BR17 shows above average skills, while his sisters show low-average scores.

Figure 4.33

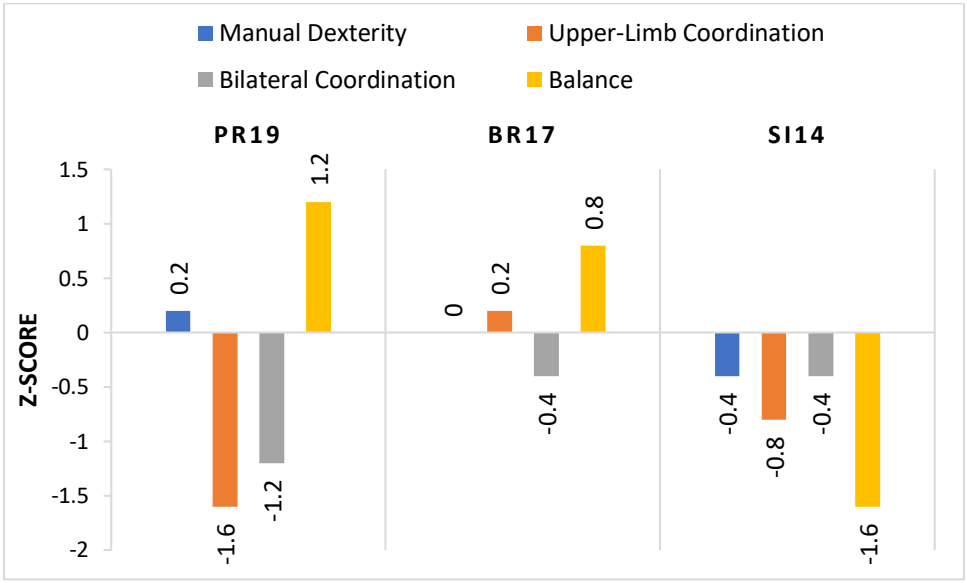
Family 37 Repetitive Finger Tapping



The BOT2 test shows mixed results, with BR17 showing generally average scores, but with a low-average score for bilateral coordination. PR19 shows difficulty with upper-limb coordination and bilateral coordination, whereas SI14 shows some difficulty across all tasks, with a particular deficit in balance.

Figure 4.34

Family 37 Motor Tasks (BOT2)

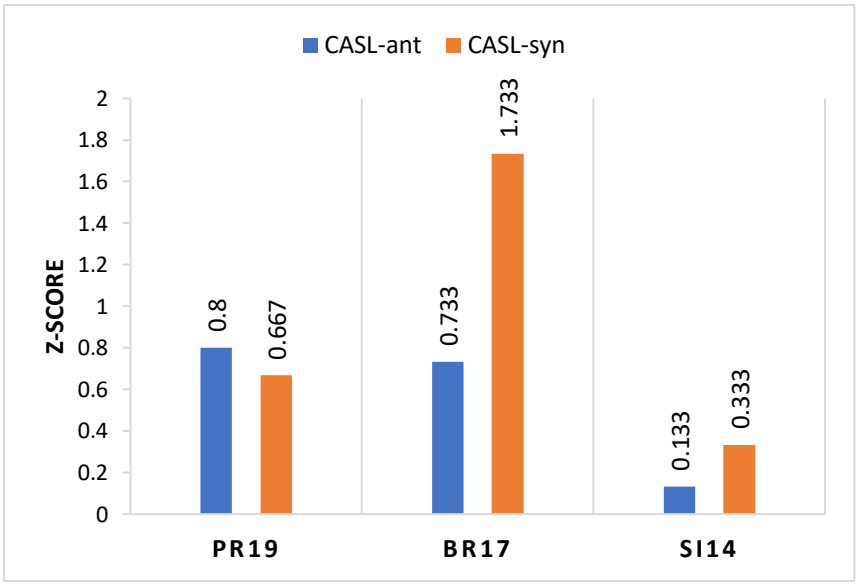


Language, Reading, and Cognitive Measures.

Family 37 participated in the CASL-2 synonym and antonym tasks, demonstrating average to above-average scores. While BR17 had high scores, he did show a full z-score difference between the antonyms and synonyms, in contrast to his sisters who were about even on the two tasks.

Figure 4.35

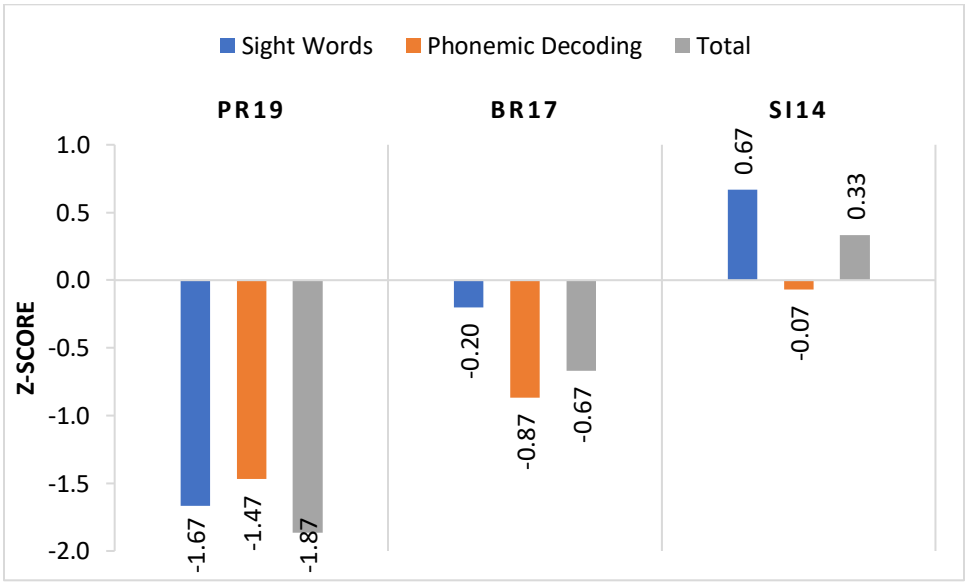
Family 37 Antonyms and Synonyms (CASL-2)



For the sight word and phonemic decoding (nonword reading) tasks, SI14 had the strongest performance with scores in the average range. Both PR19 and BR17 show difficulty, with PR19's scores all more than one standard deviation below the mean. Because these tasks are timed, there is more pressure placed on the speech system to produce a quick response. It is interesting to note that the pattern seen here mirrors the amount of time spent in speech therapy, with PR19 requiring the longest period of intervention. Thus, if time to CAS remediation is reflective of CAS severity, it is possible that the speech production requirement for this task may be influencing the scores.

Figure 4.36

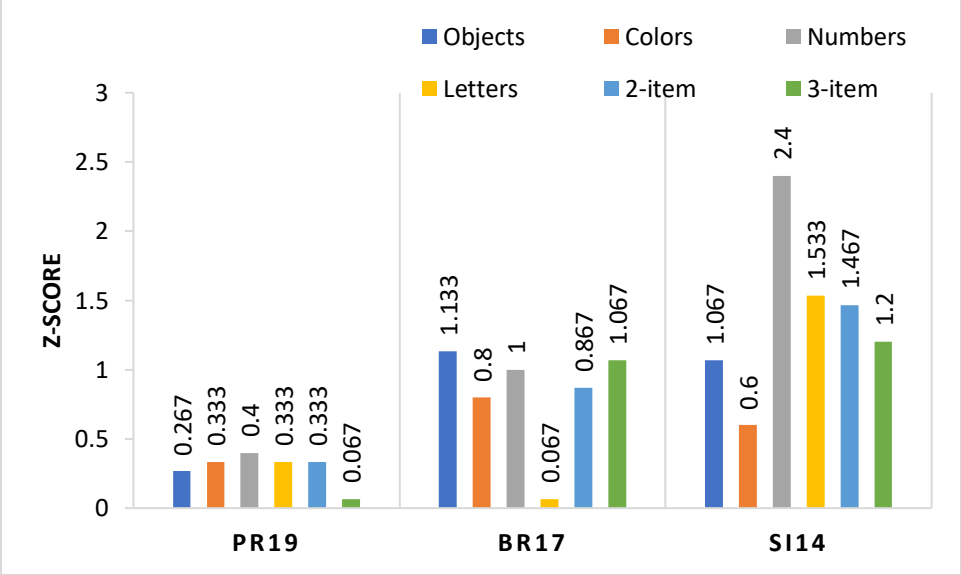
Family 37 Reading Tasks (TOWRE)



With the rapid automatic naming task, we see a pattern similar to the reading task. While all the scores for Family 37 were average to above average, the same pattern is observed with PR19 scoring lowest and SI14 scoring highest. Because this task also includes speed as a component, it is possible that the residual effects of CAS are slowing the speech production for PR19 compared to her siblings.

Figure 4.37

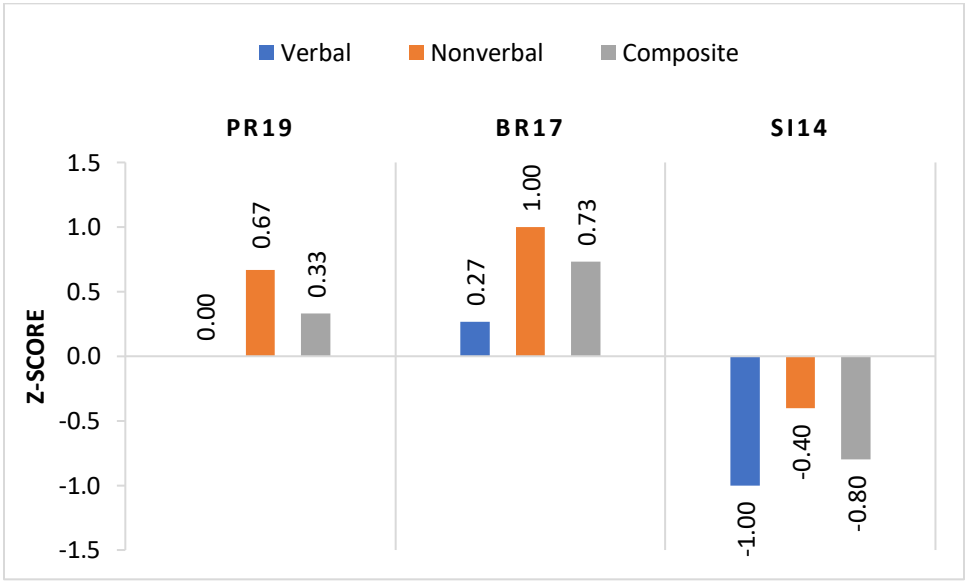
Family 37 Rapid Automatic Naming (RAN/RAS)



The cognitive task scores for Family 37 show the oldest siblings in the average range, but SI14 struggling across tasks, with nonverbal being more difficult. It is noteworthy that for all the siblings, the nonverbal scores are all at least a 0.5 standard deviation higher than the verbal ones.

Figure 4.38

Family 37 Cognitive Tasks (RIAS-2)



Genetic Analysis

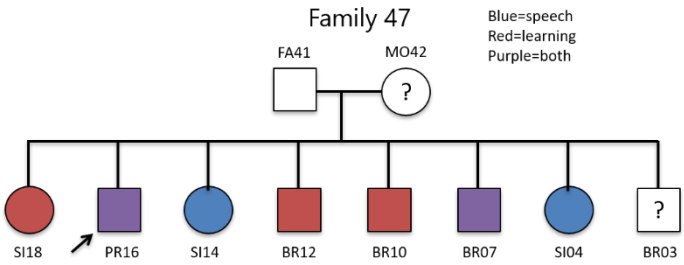
The proband and cousin in Family 37 were selected for exome sequencing; however, both samples had issues with quality control, and sampling for PR19 failed twice. As a result, genetic analysis was not performed for this family.

Summary

Family 37, with a history of CAS, shows evidence of a sequential processing phenotype for both sisters, but less so for BR17 since his speech and motor tasks were within the average range. The only tasks he showed some difficulty with were the reading tasks, particularly the phonemic decoding task which does require sequential processing to read nonwords. SI14 may also be showing signs of mild language difficulties, with lower verbal cognition scores than her siblings.

Family 47

Background



Family 47 consists of an unaffected father, a possibly affected mother, and eight biological children, five males and three females. The oldest male who is the second child serves as the proband of this family. All the children have struggled with either speech, language, academics, or some combination. Prenatal and birth history were within normal limits for all the children with the exception of the oldest, SI18, who was born at 35 weeks gestation. Mother reported that she spent the last month of pregnancy in the hospital and had preeclampsia. Many of the children had multiple ear infections when they were young, especially SI18, PR16, SI14, and BR07. SI18, PR16, and SI14 required multiple sets of pressure equalization (PE) tubes. All the children have consistently passed their school hearing screenings and there are no current concerns with hearing loss. In the extended family, a few cousins have some speech difficulties, and one struggles with reading. The mother reports some challenges with math during her school years. Unlike the other families in this study, the children in Family 47 had typical developmental milestones, but several of the children were very difficult to understand when they were young, especially PR16 and BR07. In terms of intervention, SI18 received academic support, PR16 and BR07 received academic support and speech therapy for phonological disorder, SI14 received speech therapy only for phonological disorder, BR12 and BR10 both receive academic

support. Currently, SI04 is in preschool and is reportedly demonstrating good pre-reading skills and her speech skills are improving and being monitored.

Behavioral Analysis

All of the children in Family 47 participated in some behavioral testing with the exception of PR16. It should be noted that SI18 participated in testing online due to COVID-19 related testing restrictions, and thus her scores may be an underestimate of her actual abilities (Table 4.8).

Speech Measures.

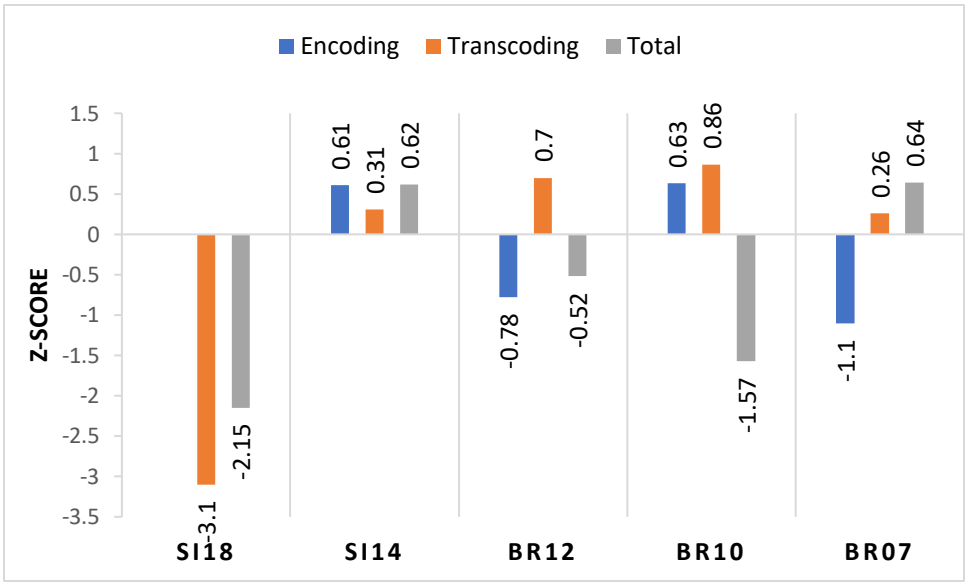
The GFTA-3 was used to capture current speech errors for the children in Family 47. SI18 earned a percentile rank of 53, made no errors on the GFTA-3, and no errors were noted in her conversational speech. SI14 earned a percentile rank of 58, made no errors on the GFTA-3, and no errors were noted in her conversational speech. BR12 earned a percentile rank of 63, made no errors on the GFTA-3, with just a few distortions of dark /l/ noted in his conversational speech. BR10 earned a percentile rank of 53 and made one f/θ substitution on the GFTA-3. BR07 earned a percentile rank of 8 on the GFTA-3. He made consistent f/θ substitutions, produced intermittent dentalized /s/ and /z/, and had a few errors on medial /l/. He made one /n/ for /ŋ/ substitution, and had one instance of alveolar assimilation, substituting /d/ for /g/ in "guitar." SI14 earned a percentile rank of 8 and made 47 errors on the GFTA-3. During the task, she used a dentalized /s/ and /z/, velar fronting (e.g., /d$\overline{\text{oʊ}}$/ for "go"), stopping (e.g., /n$\overline{\text{aɪ}}$p/ for "knife"), and all /s/ blends were substituted with /f/. In addition, she substituted /j/ for /l/ but was able to produce /r/. The errors she made that are not considered developmental

included use of an intermittent glottal stop substitution (e.g., /ʔʌp/ for “cup”), and she made several voicing errors. These errors made her speech difficult to understand. BR03 earned a score of 4.14 out of a possible 5 on the Intelligibility in Context Scale per parent report, indicating that his speech is usually understood by his communication partners. His mother indicated that he is easier to understand than some of his other siblings were at his age.

Data from the nonword repetition task reflect average overall performance for those children who have participated in speech therapy compared to those who have not shown a need for speech services. It is likely that the low scores exhibited by SI18 are due at least in part to testing online, as some sounds can be distorted through telecommunication and using nonwords created a task in which she had no semantic context to support her understanding. She was given repetitions when requested. Encoding the phonemic features of the words they heard was more difficult for BR12 and BR07.

Figure 4.39

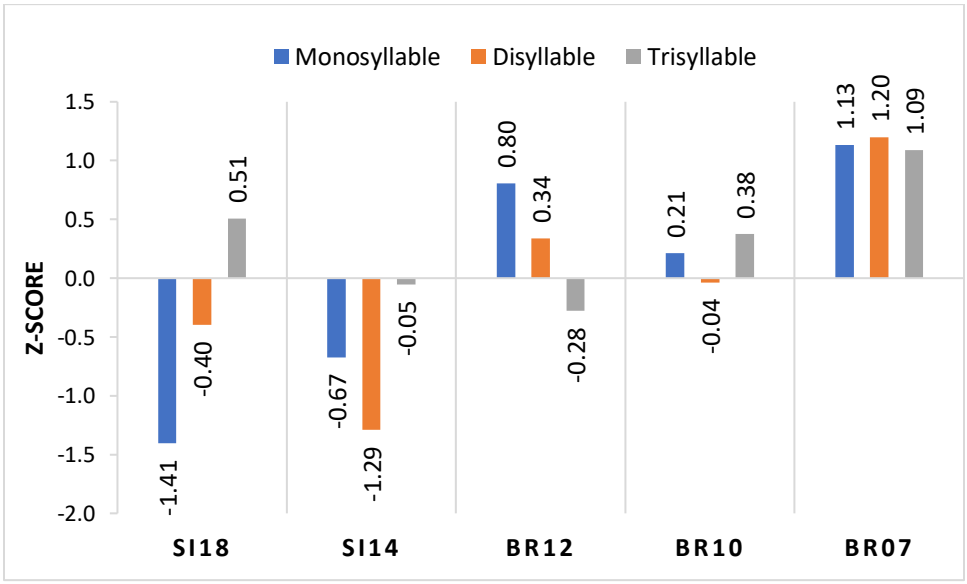
Family 47 Nonword Repetition Task (SRT)



Data from the DDK task indicate that the sisters struggled more overall than their brothers, showing difficulty with monosyllables and disyllables but average trisyllables.

Figure 4.40

Family 47 DDK Tasks



Four of the children participated in a tongue twister task. The difference score represents the difference between the percent of phonemes produced correctly while repeating control sentences compared to tongue twisters. Here, the sisters outperformed the brothers, with BR07 having a 27.03% difference score. BR12 had a large number of substitutions in the tongue twisters but no substitutions for the controls. BR07 had a high number of phoneme substitutions and deletions, with a combination of word and phoneme-level deletions. While other family members have received speech therapy in the past, currently, BR07 is the only member of Family 47 receiving speech therapy, which may be reflected in his performance during this task. He was also the youngest member of any family to attempt this task, and since it is not standardized, it is difficult to know what would be considered a typical score for someone his age.

Figure 4.41

Family 47 Phoneme Accuracy in Tongue Twisters Compared to Control Sentences

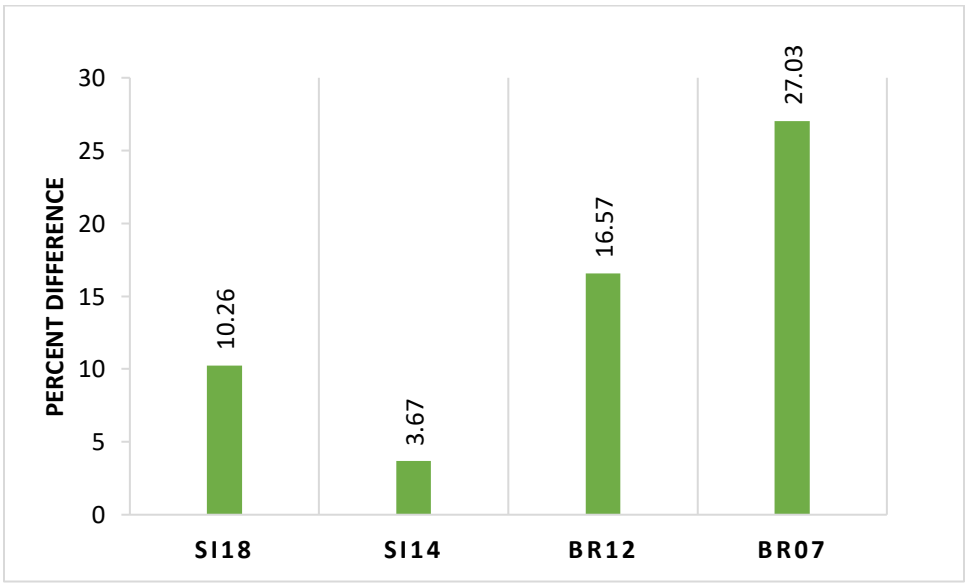
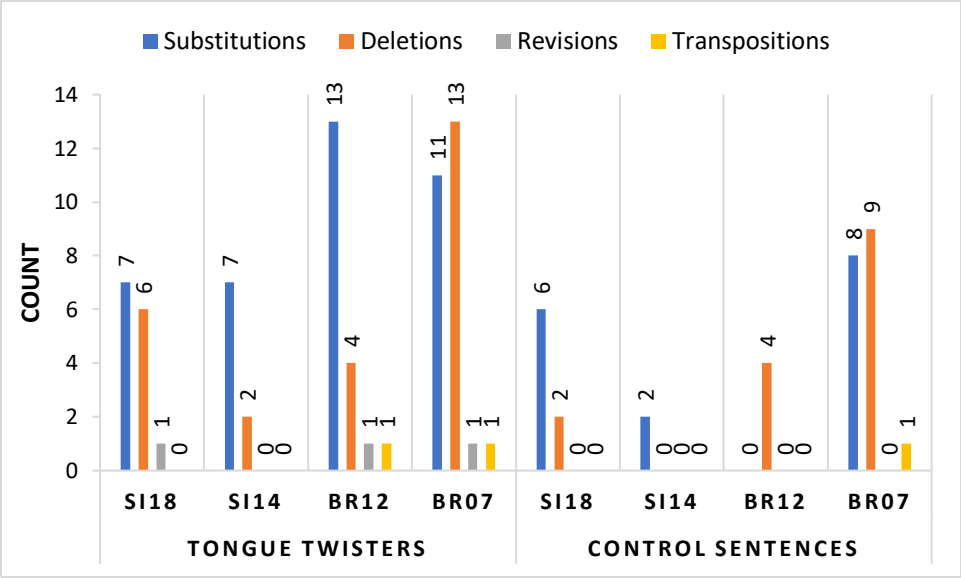


Figure 4.42

Family 47 Sentence Alterations

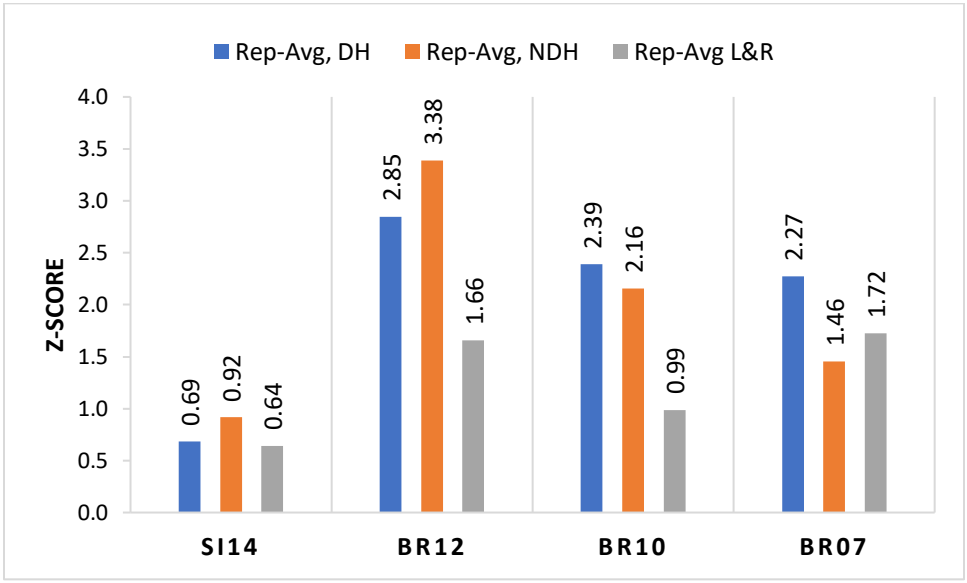


Motor Measures.

All of the children in Family 47 who participated in the finger tapping task showed average or above average scores. This is a stark contrast compared to the other families, most of which had at least some members that struggled with this task.

Figure 4.43

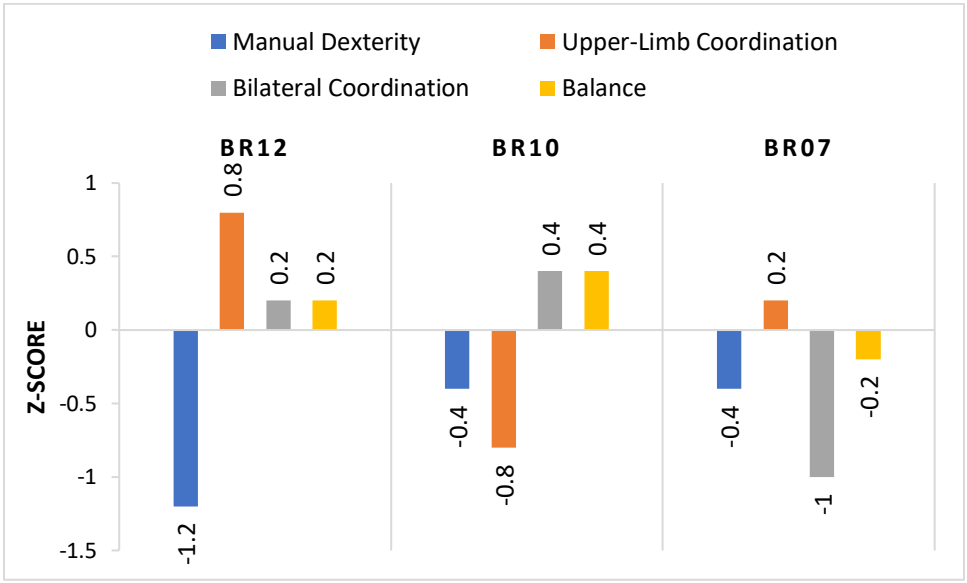
Family 47 Repetitive Finger Tapping



The BOT2 test shows mixed results, with the three brothers each demonstrating a different area of struggle: manual dexterity for BR12, upper-limb coordination for BR10, and bilateral coordination for BR07.

Figure 4.44

Family 47 Motor Tasks (BOT2)

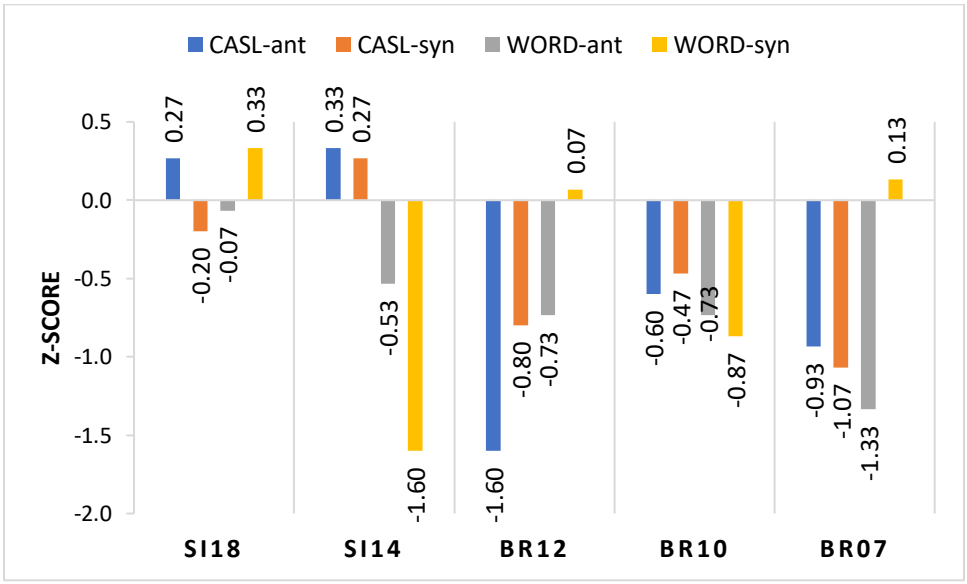


Language, Reading, and Cognitive Measures.

Five of the children in Family 47 participated in two different tasks to investigate antonym and synonym knowledge. SI18 performed in the average range for these tasks, and SI14 performed in the average range for the CASL-2 tasks, while she struggled with the WORD tasks. The WORD-synonym task was more challenging compared with the CASL-2 synonym task because it required word generation rather than selection from four choices. She struggled significantly with the WORD-antonym task but was average on the CASL-2 antonym task, which were both word-generation tasks. All three brothers struggled with the antonym and synonym tasks in general.

Figure 4.45

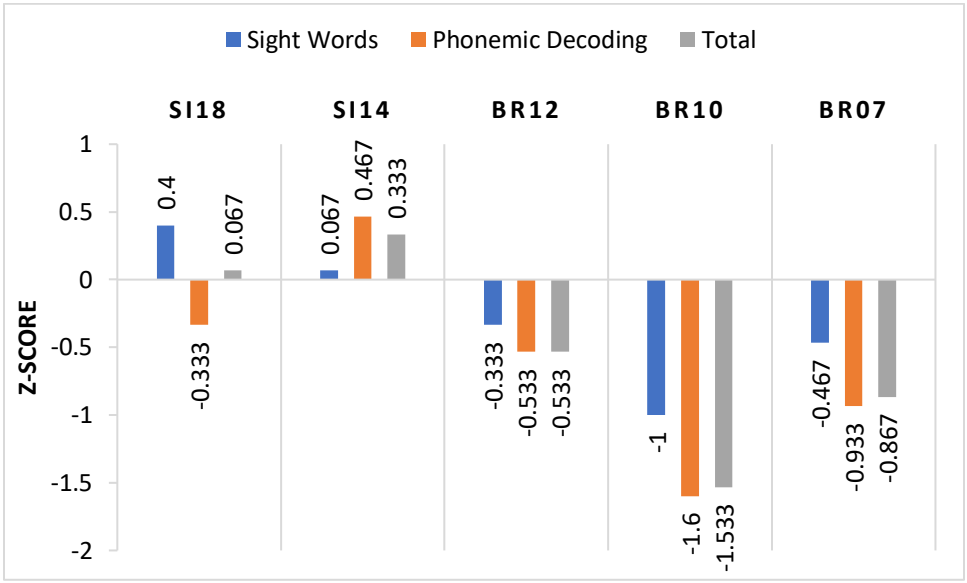
Family 47 Antonyms and Synonyms (CASL-2 and WORD Test 2/3)



Data from the sight word and nonword reading tasks also shows a difference between the brothers and the sisters. The brothers showed difficulty across all reading tasks, and phonemic decoding was harder for all of them compared to sight word reading.

Figure 4.46

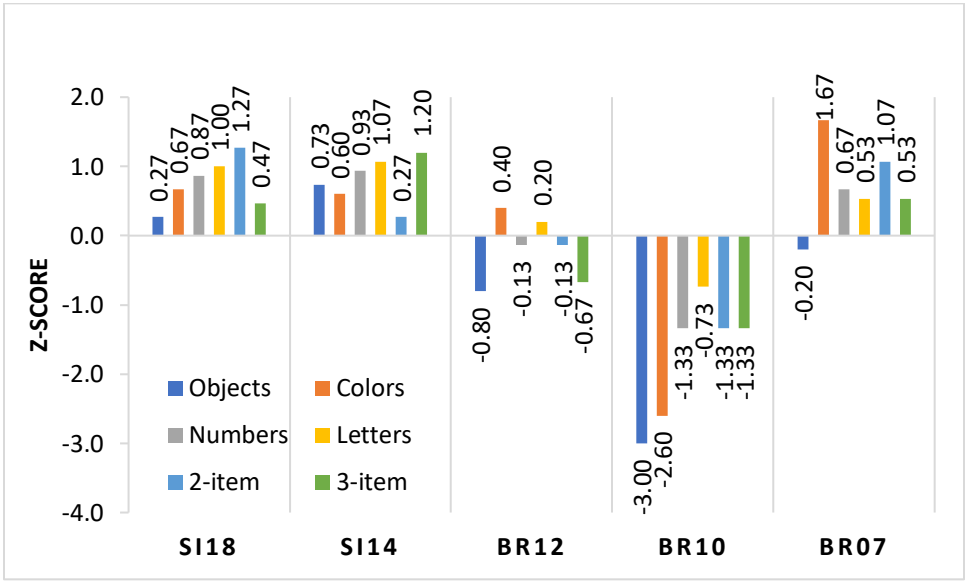
Family 47 Reading Tasks (TOWRE)



Despite the three brothers having difficulty with reading, only one of them showed difficulty with all the rapid automatic naming tasks, BR10. While BR12 did struggle with a few, including the 3-item tasks which requires the participant to name a combination of letters, numbers, and colors, BR07 was average to above average.

Figure 4.47

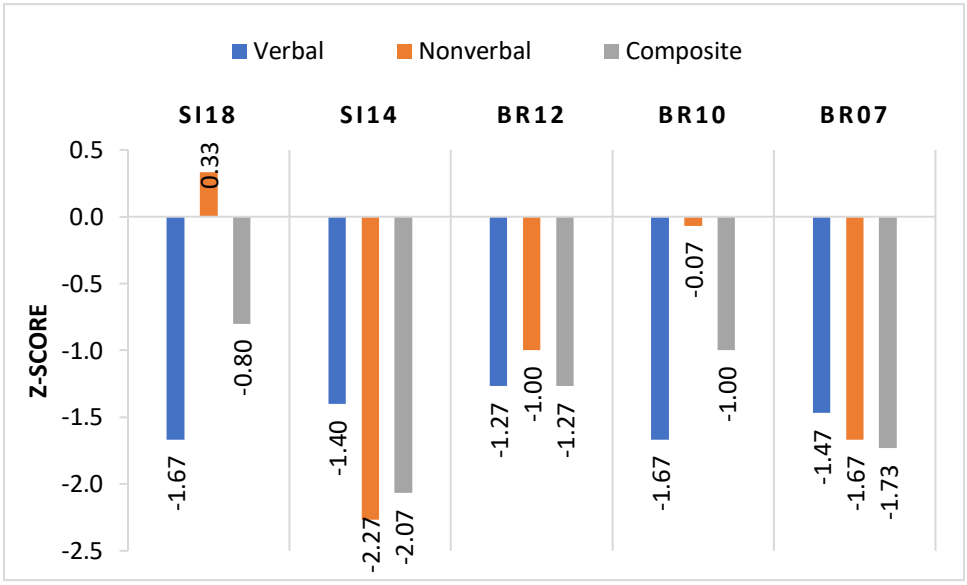
Family 47 Rapid Automatic Naming (RAN/RAS)



The cognitive tasks showed very low scores for this particular family, so much so that it brings into question a possible third variable that may be influencing the outcome. All of these children participate in the general education classroom with some academic support, and SI14 has consistently shown strong academic performance. While some of the members of this study cohort have struggled with aspects of cognition, none showed this pattern for all family members across all tasks. This could be reflective of subtle difficulties with attention.

Figure 4.48

Family 47 Cognitive Tasks (RIAS-2)



The children of Family 47 demonstrate a different speech phenotype compared to the other families, with the affected children having a phonological disorder or learning difficulties rather than CAS. This is most evident when examining the DDK and the nonword repetition tasks for the two speech affected individuals: SI14 and BR07. SI14 does show difficulty with the DDK task and BR07 struggles with the tongue twister task,

yet both of their total nonword repetition scores are average. Each of the brothers seemed to have a unique motor profile, but all five children were average to above average in finger tapping. The brothers show more deficits in reading and language, and the cognitive data does not provide much clarity for this family.

Table 4.7

Variants of Interest for Family 47

Gene	***NBEA***
Chr: Locus	**13**: 36202269
RS ID	rs11538677
Variant	A>G
Type	SNP- missense
Allele Freq gnomAD NFE	0.11
CADD Scaled	23.5
pLI	1
PolyPhen-2	PoD (0.67)
SIFT	Del (0.03)
MOI	AR
Dev Crblm Exp	17
Dev Thal Exp	19.12
Dev Strm Exp	12.54

SNP=single nucleotide polymorphism; NFE=non-Finnish European; MOI=mode of inheritance; Crblm=cerebellum; Thal=thalamus; Strm=Striatum of Basal Ganglia; AR=autosomal recessive; AD=autosomal dominant; CH=compound heterozygosity; DN=*de novo;* PoD=possibly damaging; PrD=probably damaging; Ben=benign; Del=Deleterious; Tol=tolerated; LoF=loss-of-function; CNV=copy number variation; kb=kilobases

Table 4.8

Family 47 Participants

Participant ID	**Sex**	**Affectation Status**	**Age at Testing**	**Handedness**
Fam47_SI18*	F	Unaffected	18;11	Right
Fam47_PR16~	M	Affected	NA	Right
Fam47_SI14	F	Affected	14;7	Right
Fam47_BR12	M	Unaffected	12;5	Right
Fam47_BR10	M	Affected	10;0	Right
Fam47_BR07	M	Affected	7;9	Right
Fam47_SI04	F	Affected	4;0	Right
Fam47_BR03	M	Unknown	3;11	Right

*Tested virtually
~Unavailable for testing

Genetic Analysis

Genetic analysis revealed only one potential gene of interest for Family 47, *NBEA*. While there were several CNV calls for this family, none were shared by all the affected family members. This same *NBEA* variant was also found in Trio 13, which is not unexpected given that its allele frequency is more common at 0.11. The variant in this family shows a heterozygous presentation, whereas the proband in Trio 13 was homozygous (with heterozygous parents). As discussed previously with Trio 13, NBEA (Neurobeachin) is involved in synaptic plasticity and efficacy, with animal models demonstrating its role in cognition and social behavior (Gromova et al., 2018). This gene is only weakly related to the phenotype found for Family 47. None of the members of Family 47 display characteristics of autism; however, their cognitive scores were lower overall compared to the other families. This could suggest the possibility of some neural disruption but with a much milder phenotype.

Summary

Family 47 shows a range of phenotypic impact, with some individuals affected for speech only, others for learning only, and some for a combination. In this family, SI14 and BR07 who have both shown affectation for speech did not struggle with the SRT nonword repetition task. This is in contrast to the other families in which the overall scores on this task were consistently lower. The heterogeneous phenotype in this family suggests that any genetic influence may be originating from multiple genetic hits, with various family members impacted by a different set of genes. While *NBEA* is a possible gene of interest, it is only weakly associated with the family phenotype.

CHAPTER 5

INTEGRATED RESULTS AND DISCUSSION

Analyses of behavioral testing from all five families helped to elucidate the presence of familial biomarkers related to sequential and perceptual processing.

Research Question 1:

Do individuals with CAS show signs of relying on speech feedback control more than feedforward control mechanisms? It was hypothesized that individuals who demonstrate CAS would show evidence of reliance on feedback control mechanisms. To investigate this question, families participated in a vowel adaptation task and a vowel production task.

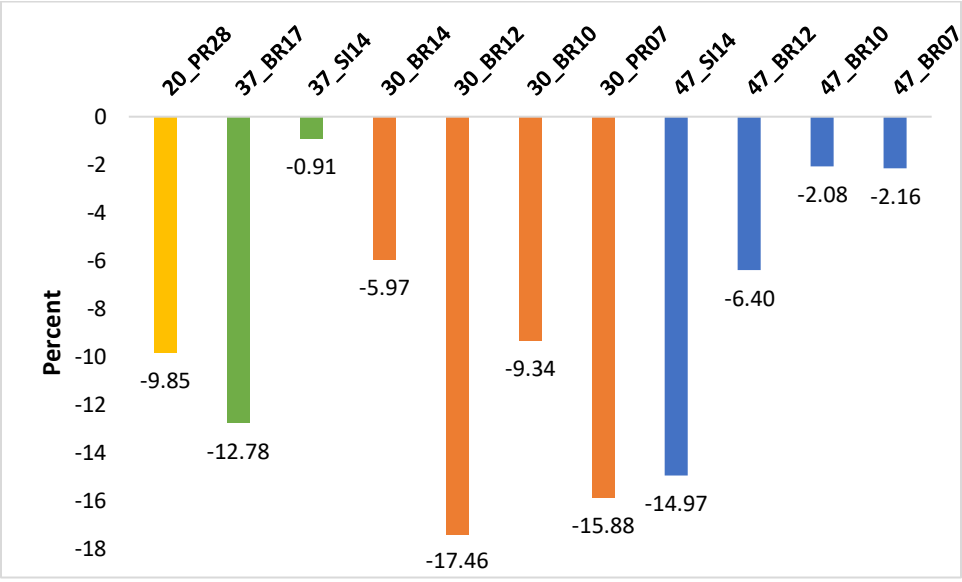
Vowel adaptation in response to perturbed F1 was determined for individuals in four of the families (Figure 5.1). In general, when given perturbed auditory feedback, typical individuals tend to show an adaptation response in the direction opposite the perturbation in an attempt to compensate for their perceived error (Terband et al., 2014). In a group of children with SSDs, some of whom had CAS, the opposite effect was found, with responses occurring in the direction of the perturbation, though there was high variability among the children (Terband et al., 2014). Results displayed in Figure 5.1 indicate that all participants in this study showed an adaptation response in the opposite direction of the perturbation, thus compensating for it, yet the degree of adaptation varied. In general, the individuals with a higher degree of adaptation are those with a history of CAS. 37_SI14 showed very minimal adaptation, suggesting that she may not have actually perceived the perturbation. This is supported by her data from the

tongue twister task, as she did not make any revisions of her errors during this task. 47_BR10 and 47_BR07 also showed minimal adaptation. 47_BR10 has missing data for the tongue twister task, but BR07 also shows only one revision during this task. In averaging percent adaptation across families, Family 47 shows the lowest adaptation overall, and given that they do not have a history of CAS, this could indicate that they are not relying on feedback mechanisms as much as the CAS participants.

The study from Terband et al. (2014) also found a significant correlation between nonword repetition and amount of adaptation. This relationship was investigated using the nonword repetition task (SRT) and no significant correlation was found; however, the SRT only uses five early-developing phonemes, which may have underchallenged the speech motor control system for the participants in this study.

Figure 5.1

Percent F1 Adaptation for All Families



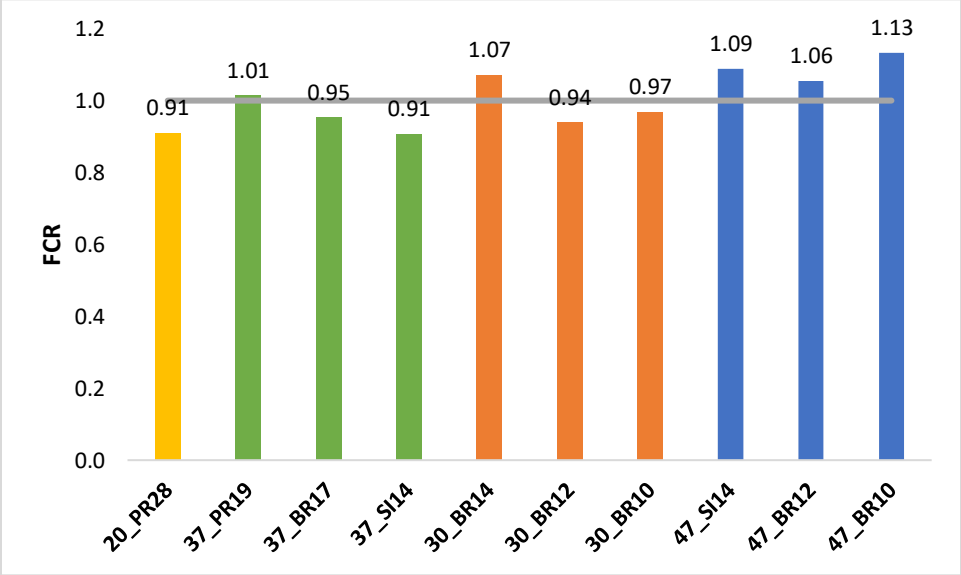
To analyze vowel production, formant centralization ratio (FCR) values were calculated for the individuals who participated in acoustical speech tasks (Figure 5.2). An FCR value of around 1.0 is considered average for typical speakers of Standard American English (Naderifar et al., 2019; Sapir et al., 2010), the dialect used by all of the participants in this study. FCR values greater than 1.0 are indicative of a more centralized vowel system, which is often found in individuals with speech deficits (Sandoval, Berisha, Utianski, Liss, & Spanias, 2013). Research by Sapir et al. (2010) found an average FCR for typical children of 0.97 but a higher FCR value of 1.14 for children with dysarthria due to cerebral palsy, likely reflective of reduced articulator excursion during speech. In a group of individuals with hearing impairment, FCR values increased as severity increased, reaching as high as 1.63 in those with profound loss (Naderifar et al., 2019).

Looking across FCR data for individuals from four families in this study, the average FCR dips below the typical score of 1.0 for some individuals and rises above 1.0 for others. Fam20_PR28 shows the most severe CAS phenotype of all the participants, and her FCR value is the lowest at 0.91. A lower FCR value is indicative of a larger vowel space that is less centralized. An FCR value below 1.0 is also seen for two individuals in Family 37 (BR17 and SI14) and two from Family 30 (BR12 and BR10). Seeing this pattern in many of the participants with a history of CAS diagnosis may be reflective of underdeveloped motor programs, as they use more of the vowel space than is needed for efficient speech. In contrast, all the individuals in Family 47 have FCR values greater than 1.0, indicating a more centralized vowel space in which vowel categories are

clustered more tightly. These individuals do not have CAS but show a phenotype characterized by difficulty with phonology and/or literacy skills. Their more centralized vowel space may be indicative of an underdeveloped phonological system that does not take advantage of the full vowel space to maximize intelligibility.

Figure 5.2

Formant Centralization Ratio (FCR) for All Families



Overall, the adaptation task does show some evidence of reliance on feedback given the degree of adaptation to perturbation. Results from the vowel task support the idea that individuals with CAS may still show some underdevelopment of speech motor programs even after their speech has improved post-treatment.

Research Question 2:

Is there evidence of a motor control biomarker that runs in one or more families? It was hypothesized that low scores in measures of sequential processing would be found in the affected individuals of at least one family.

Research Question 3:

Is there evidence of a perceptual biomarker that runs in one or more families? It was hypothesized that low scores in measures of mental representations, consistent with perceptual feature extraction, will be found in the affected individuals of at least one family.

Research questions 2 and 3 will be discussed together through analysis of behavioral testing across families.

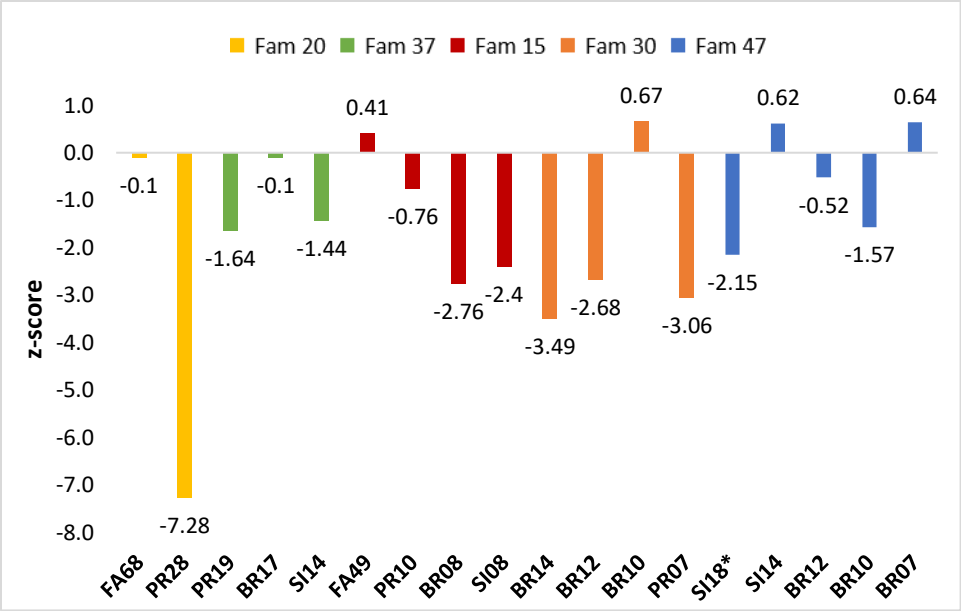
Results for research questions 2 and 3 will be reviewed together below, integrating the discussion of sequential and perceptual processing biomarkers.

Speech Measures

When asked to repeat simple nonwords using the Syllable Repetition Task (SRT), the majority of participants showed at least some difficulties despite the task only incorporating five early-developing phonemes. Even some members of Family 47 showed difficulty despite not having CAS. This could reflect the presence of working memory load in this task, as the stimuli include words up to four-syllables.

Figure 5.3

All Families Nonword Repetition Task (SRT)



On the DDK tasks, only a few participants struggled with the monosyllable condition, with the majority struggling on the disyllable condition. This is consistent with the phenotype of this cohort, given that deficits in the DDK monosyllable condition are usually associated with dysarthria (Thoonen, Maassen, Gabreels, & Schreuder, 1999). Research has demonstrated that those with apraxia generally exhibit a slower rate of DDKs than typical controls (Thoonen et al., 1999). A paired t-test indicated a significant difference between the monosyllabic and disyllabic scores (t=2.11, p=0.03; d=0.46). To further investigate this relationship, a difference score was calculated between the monosyllable and disyllable conditions, with a positive score indicating that they performed increasingly better on monosyllables, and a negative score showing they performed more poorly on disyllables. Across families, this score captures a trend that Family 47 generally shows less difficulty with disyllables. Given that they show

phonological and literacy deficits, this may indicate that the alternating condition of rapid disyllable production is impacted more in CAS individuals, though there is variability across participants. Overall, performance was better for the trisyllable condition. It is uncertain why this is the case, though speculation could suggest that an increasing number of syllables begins to mimic real speech, which they practice every day.

As a group, the participants showed 100% accuracy in their monosyllable productions, 94% accuracy for disyllable productions, and 85% accuracy for trisyllable productions. This suggests that while the trisyllable speed was not below average for the majority of the participants, accuracy did decline as the number of syllables increased, a trend not observed in typical children (Thoonen et al., 1999).

Figure 5.4

All Families Monosyllable DDKs

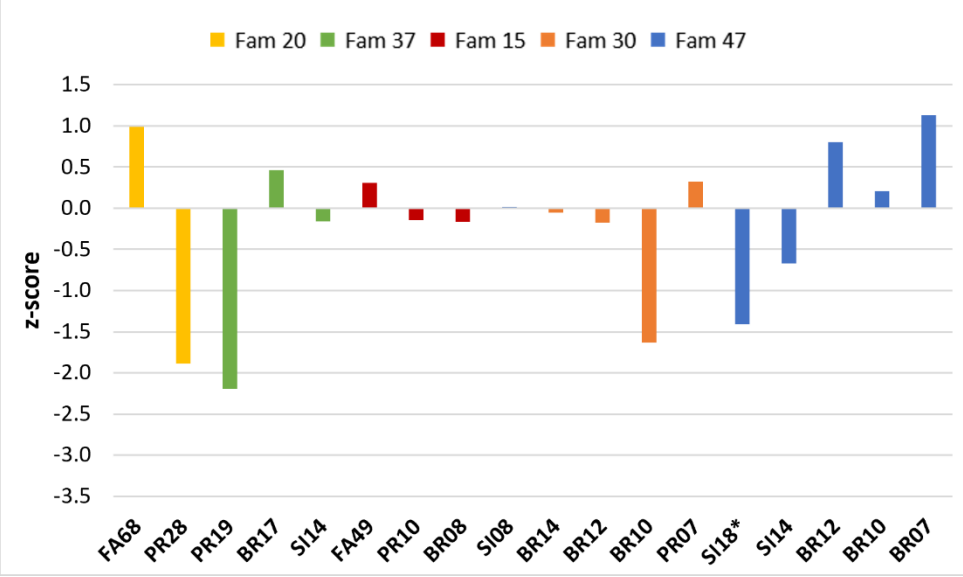


Figure 5.5

All Families Disyllable DDKs

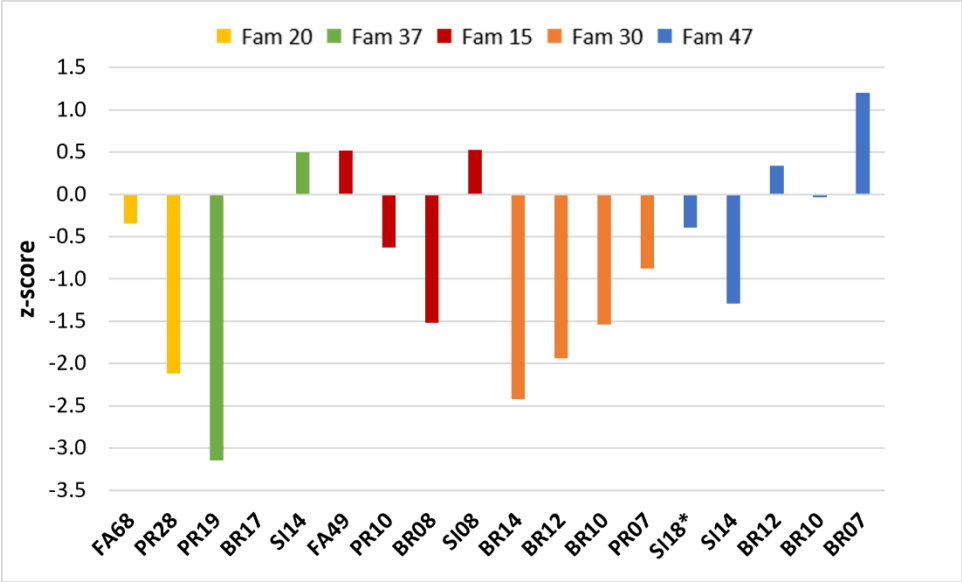


Figure 5.6

All Families Difference Between Monosyllables and Disyllables

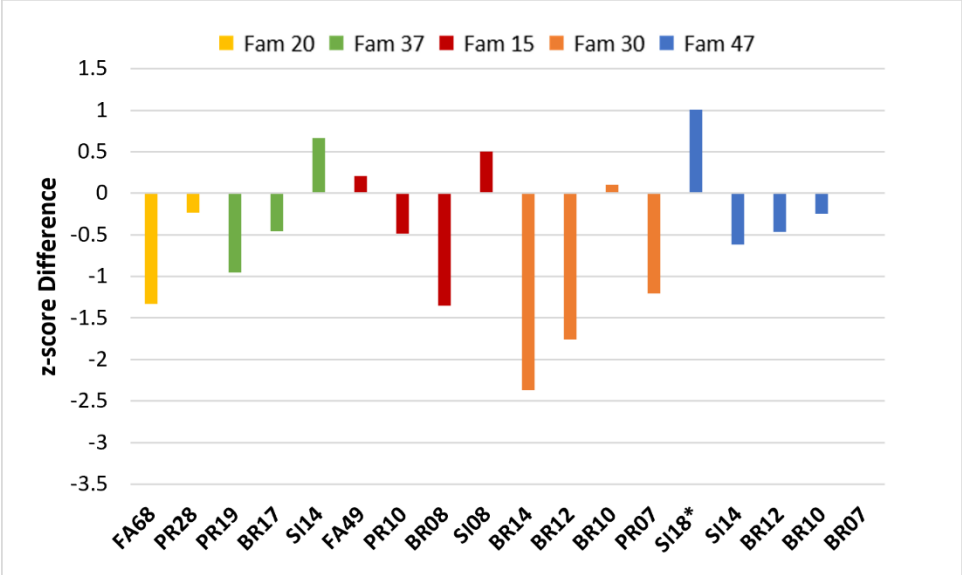
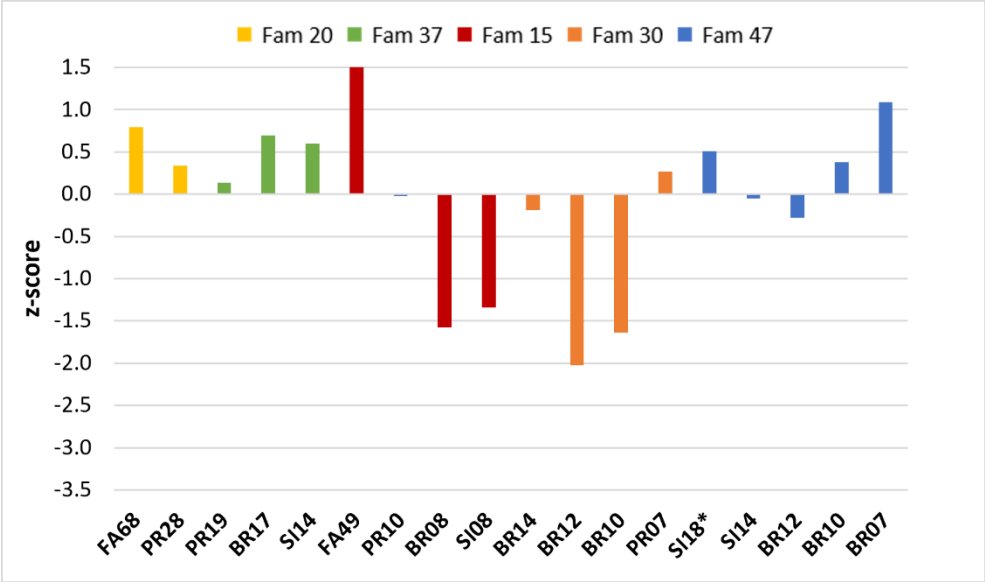


Figure 5.7

All Families Trisyllable DDKs



The speech system was challenged most through the tongue twister task. While this task is not standardized, several observations can be drawn from these results. First, individuals across families struggled more with accurate tongue twister production compared to control sentences. This is undoubtedly due to increased phonemic complexity present within the tongue twisters, but what is unclear is if the difficulty stems only from difficulty with motor planning and production, or if encoding the phonemic complexity added to the task load. Given that Family 47 showed a similar difference score indicates that this task may encompass more than just an isolated challenge to sequential processing.

When the participants did make errors during this task, there was variability in error type, with an interesting pattern in the number of revisions. In general, Family 47, without a CAS phenotype, made far fewer revisions compared to the CAS participants. It

is possible this family may not be aware of their errors when they occur, pointing to deficits in perception. This is supported by data from the adaptation task, which indicated some members of Family 47, as well as Fam37_SI14, showed minimal adaptation in the presence of F1 perturbation. Here they show a lower percentage of revisions to their errors when listening to the tongue twisters.

Figure 5.8

All Families Phoneme Accuracy in Tongue Twisters Compared to Control Sentences

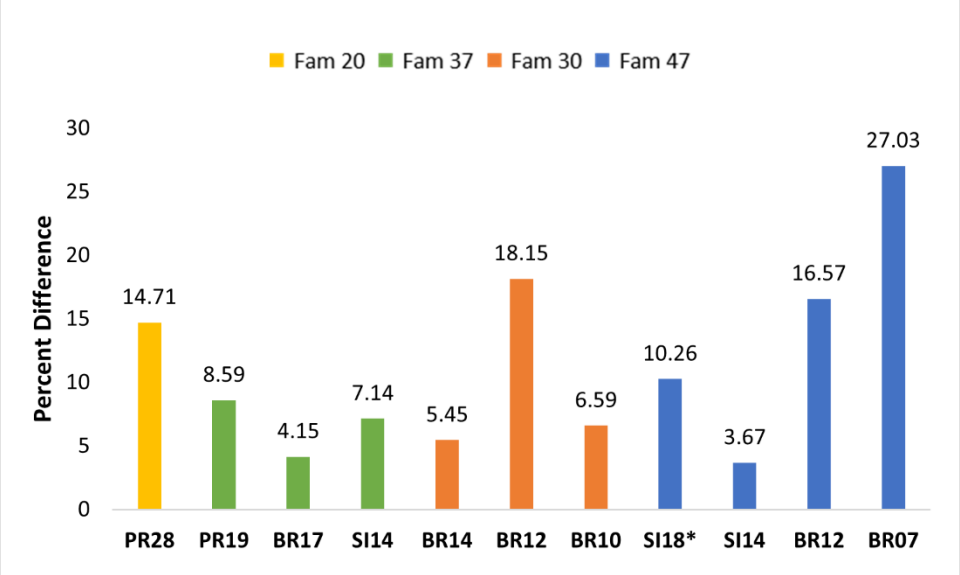
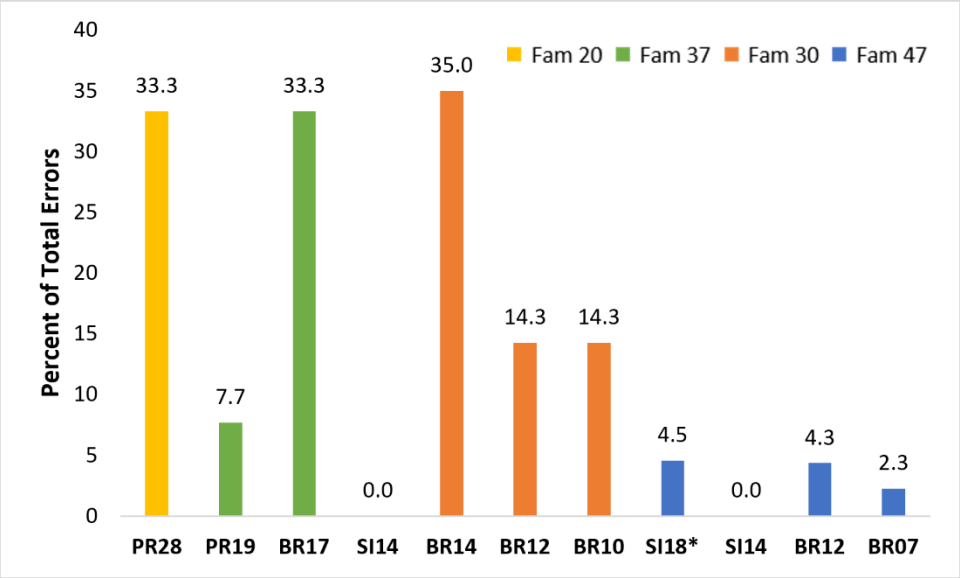


Figure 5.9

All Families Percent Revisions of Total Tongue Twister Errors



Motor Measures

For the repetitive finger tapping task, with left and right hands averaged, most of the participants earned typical scores. The average z-score for each family fell within the typical range, with the exception of Family 15. The father of Family 15 showed a particular deficit in repetitive finger tapping, mainly due to a slower interval with his non-dominant hand. Fam15_SI08 also showed a low score, which would be expected given her history of mild cerebral palsy. Family 47 had the highest family average z-score on this task of 1.25, suggesting strong sequential motor skills.

While there are no available standardized scores available for alternating finger tapping, the members of Families 30 and 47 are well-matched for age, so average alternating finger tapping intervals were explored for these two families. Average

intervals for the left and right hands together were calculated (Figure 5.12), and the data suggest a similar pattern for these two families despite their phenotypic differences.

Figure 5.10

All Families Repetitive Finger Tapping

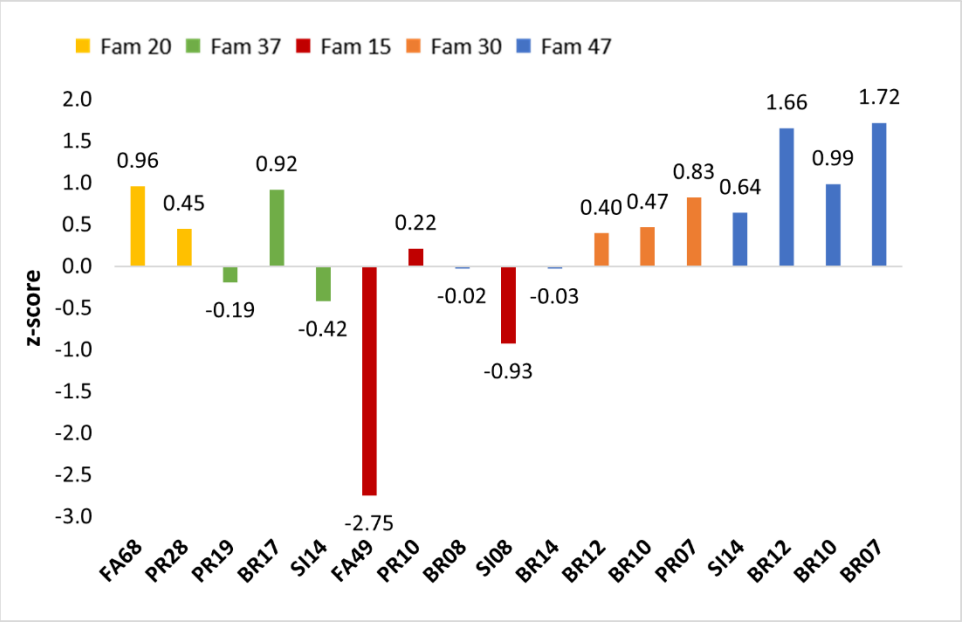
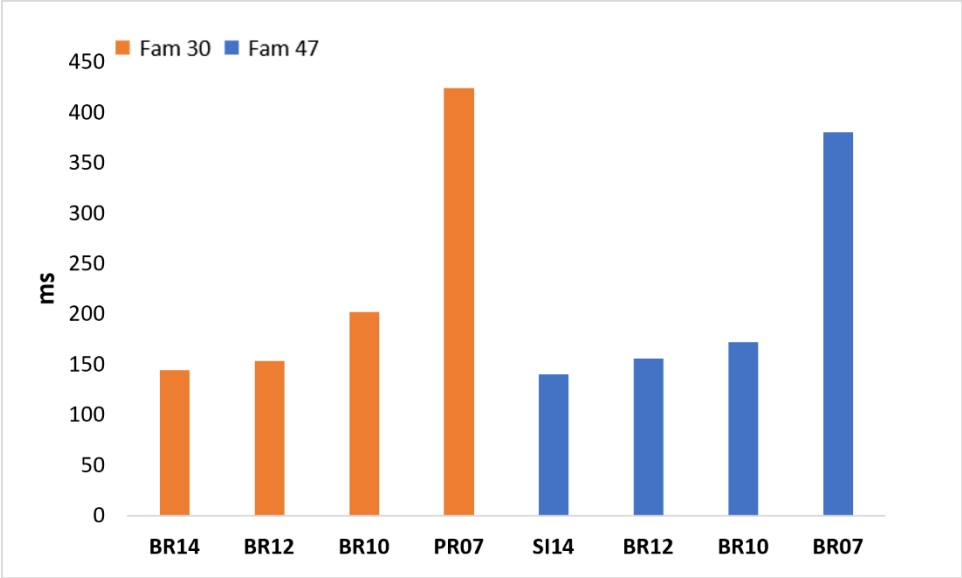


Figure 5.11

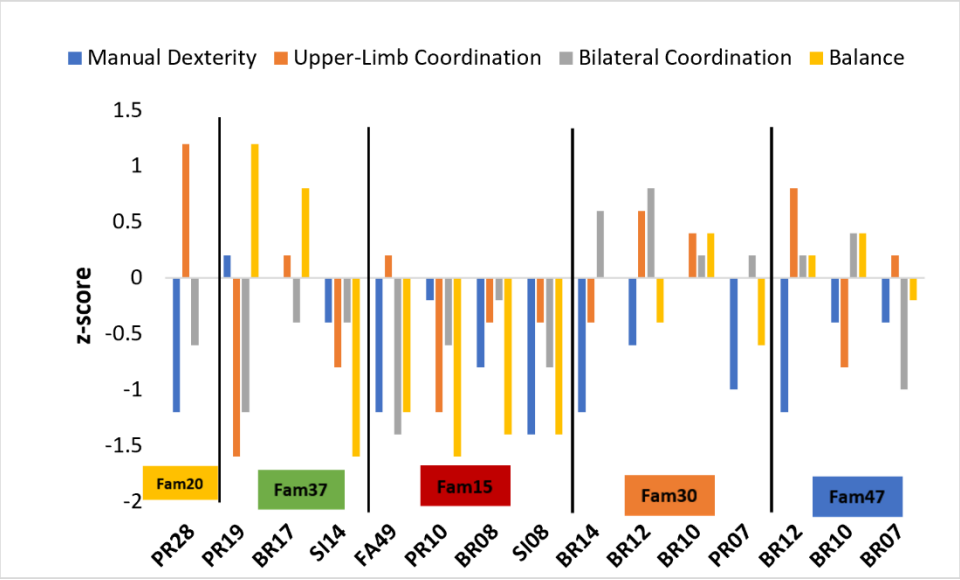
Families 30 and 47 Alternating Finger Tapping



Data from the BOT2 revealed clear motor deficits across all areas for Family 15. The BOT2 subtests include mostly gross motor tasks, with the manual dexterity subtest testing fine motor skills. Across all the families, the participants tended to struggle most with manual dexterity. Families 15 and 37 also demonstrated difficulty with upper-limb coordination. Research has shown that individuals who struggle with speech motor sequencing tasks have also been found to have difficulty with both oral and limb sequencing (Dewey, Roy, Square-Storer, & Hayden, 1988; Iuzzini-Seigel, 2019). The data suggest balance is an issue for Family 15 and for some members of Families 37 and 30.

Figure 5.12

All Families Motor Tasks (BOT2)



An oral mechanism examination was performed using a subset of items from the protocol by Robbins and Klee (1987) (Appendix A). Of particular interest were items requiring sequential processing components. Participants were asked to rapidly alternate production of the /i/ and /u/ phonemes. Individuals that could accomplish the task with regular rate and minimal effort earned a score of 1. Participants were also asked to swallow while smiling to determine the presence of lip pursing during swallowing. Family 15 and the proband from Family 20 showed difficulty across both tasks, whereas Families 37 and 47 performed well across tasks. Most of the members of Family 30 were able to alternate between phonemes, but all struggled to swallow while smiling. It is generally thought that the basic motor pattern for swallowing is developed by around 2 years of age (Guilleminault, Huang, & Quo, 2019). The inability to swallow while smiling could suggest an overreliance on lip contraction to create the necessary pressure

for swallowing (Felício, Folha, Ferreira, & Medeiros, 2010). It is unknown whether individuals with a history of CAS could have subtle difficulties with coordination for swallowing. The existence of swallowing apraxia, defined as difficulty with planning the motor movements of the lips, tongue, and jaw for the oral phase of swallowing (Yun et al., 2019), has been debated in the literature. While swallowing requires motor planning and programming, much of this process is not volitional; however, of the various stages of swallowing, the oral phase is considered more volitional than the subsequent swallowing stages (Daniels, 2000). Future research could investigate any potential relationship between sequential processing deficits and swallowing deficits.

Table 5.1

Oral Mechanism Examination

ID	Alt i/u	Smile Swallow
20_PR28	0	0
37_PR19	1	1
37_BR17	1	1
37_SI14	0	1
15_FA49	1	0
15_PR10	0	0
15_BR08	0	1
15_SI08	0	0
30_BR14	1	0
30_BR12	1	0
30_BR10	0	0
30_PR07	1	0
47_SI18*	1	1
47_SI14	1	1
47_BR12	1	0
47_BR10	0	1
47_BR07	0	0

Language, Reading, and Cognitive Measures

All the families participated in the CASL-2 synonym and antonym tasks. Overall, the families did well on these tasks with the exception of Fam15_BR08, Fam30_PR07, and the three youngest brothers in Family 47. This suggests the possibility of language impairment in these individuals, and the three brothers in Family 47 have received school-based language or literacy support. Because these two subtests do not have the same task design, two additional measures with parallel tasks were added. Thus, Families 30 and 47 also have synonym and antonym scores for the WORD-3 Elementary and WORD-2 Adolescent. Despite the difference in tasks, the only dramatic change was for Fam47_SI14 who performed much worse on the WORD tasks compared to the CASL tasks.

Figure 5.13

All Families Antonyms and Synonyms (CASL-2)

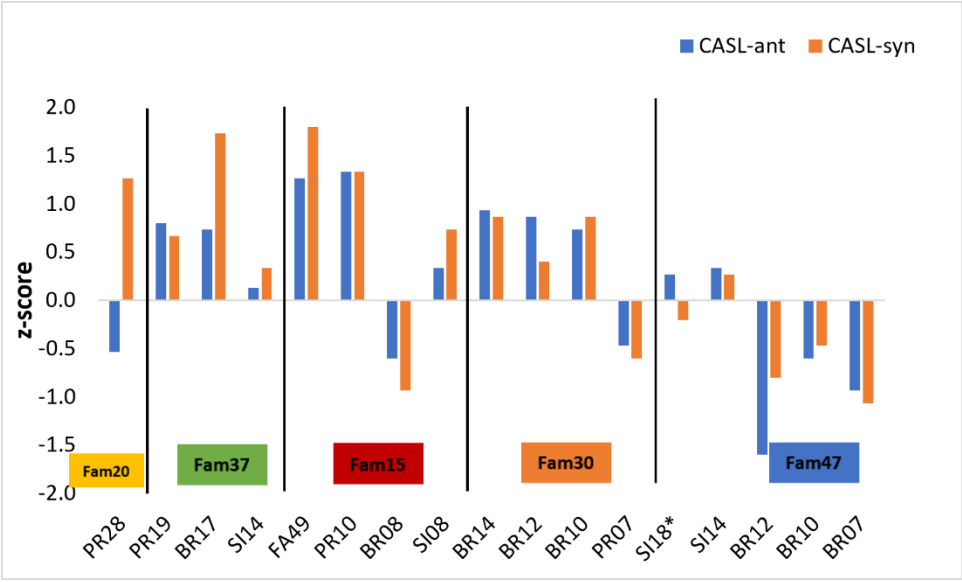
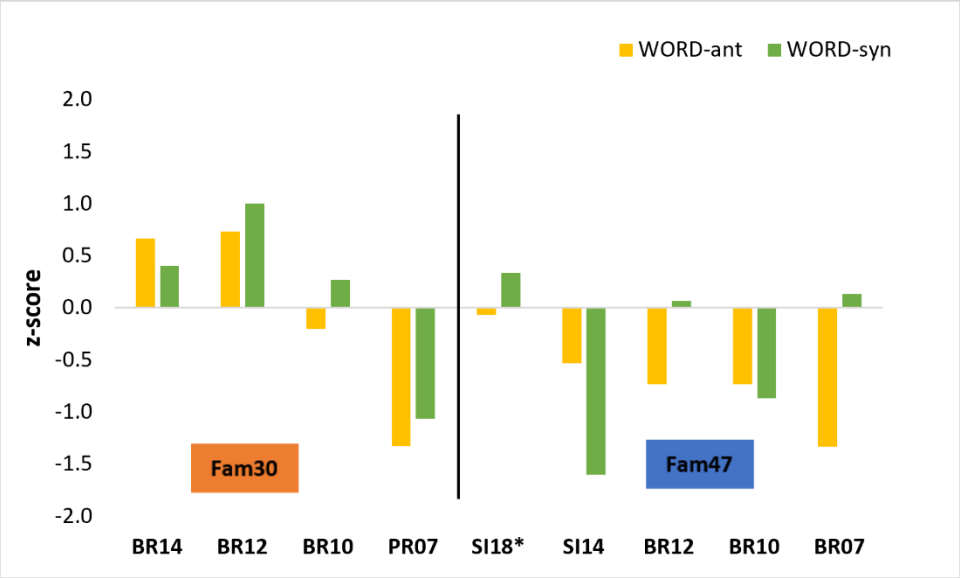


Figure 5.14

Families 30 and 47 Antonyms and Synonyms (WORD Test-2/3)

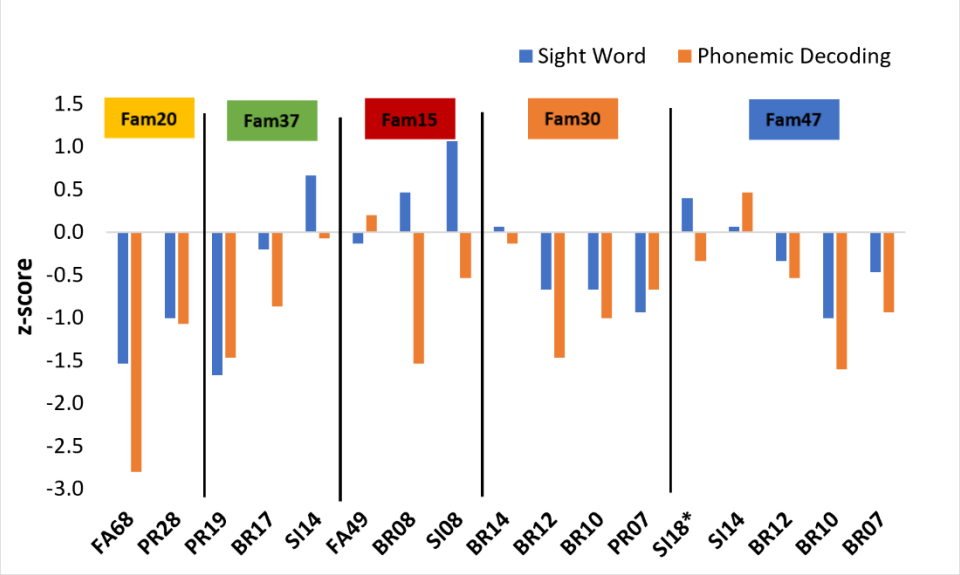


The reading task incorporated a sight word condition and a phonemic decoding condition in which participants read nonwords (e.g., churt, glamp). The scores in these conditions are timed, and thus would be a particular challenge to the individuals with CAS since they would have to integrate reading skills and speech production skills. Thus, low scores may be reflective of reading deficits or may reflect an increased challenge to the speech system. The reading tasks proved difficult for all the families, especially for phonemic decoding which relies heavily on sequential processing (Peter et al., 2018). A paired t-test yielded a significant difference between sight word reading and phonemic decoding (t=2.12, p=0.008; d=0.64), indicating the participants had significantly more difficulty with phonemic decoding. The individuals in Family 20 struggled significantly with both tasks, as did the younger children in Families 30 and 47, and the proband in Family 37. Some individuals struggled primarily with phonemic

decoding, including Fam37_BR17 and the twins in Family 15. In contrast to phonemic decoding, sight word reading leans more heavily on perceptual processing, as the word is learned, stored, and read as a single unit (Miles et al., 2018). While caution must be taken when interpreting these data due to the timing pressure of the tasks, the results suggest that many of the family members struggle with both sequential and perceptual processing. A combination of sequential and perceptual deficits was recently observed in an analysis of spelling errors in adults with dyslexia (Peter, Albert, & Gray, 2020).

Figure 5.15

All Families Reading Tasks (TOWRE)

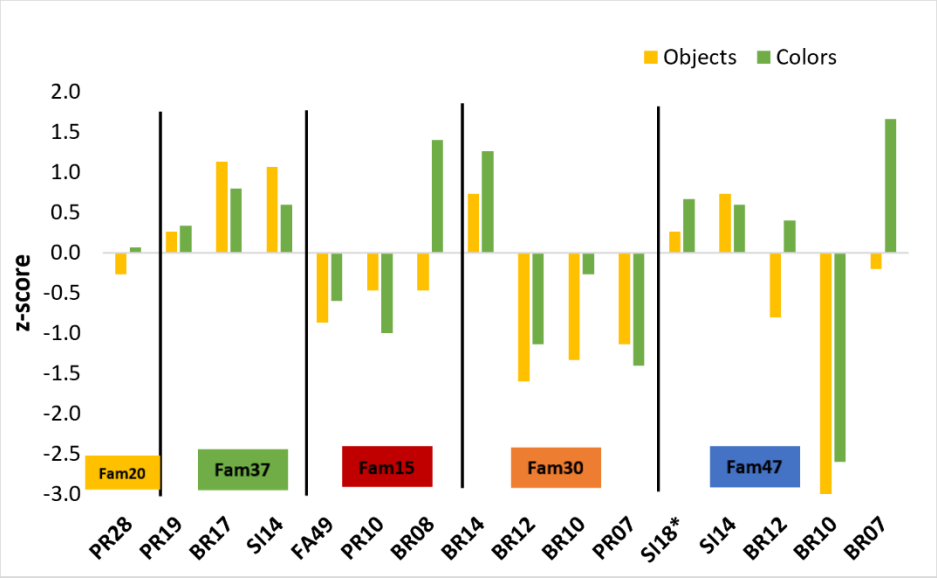


The RAN/RAS examined rapid automatic naming under six different conditions, two of which are discussed here: object and color naming. These subtests were chosen for analysis since they require more perceptual processing compared to letters and numbers, which reflect more automatic processing (Areces, García, González-Castro, Alvarez-García, & Rodríguez, 2018). The RAN/RAS as a whole is highly correlated with

reading ability, yet analyzing these two subtests did not necessarily yield the same results as the sight word task. Fam20_PR28 and Fam37_PR19 performed poorly on both reading tasks, but their scores show average rapid naming. This could suggest a deficit in sequential processing without a co-occurring deficit in perceptual processing. The three youngest children in Family 30 show a different pattern, with low scores for the reading tasks and rapid automatic naming, which could suggest deficits in both sequential and perceptual processing. This pattern also exists in Fam47_BR10, though he does not present with any speech phenotype, but rather may be showing reading and perceptual deficits. In addition, research has indicated that the RAN/RAS can be predictive of attentional issues (Areces et al., 2018), which makes the interpretation less clear for this cohort.

Figure 5.16

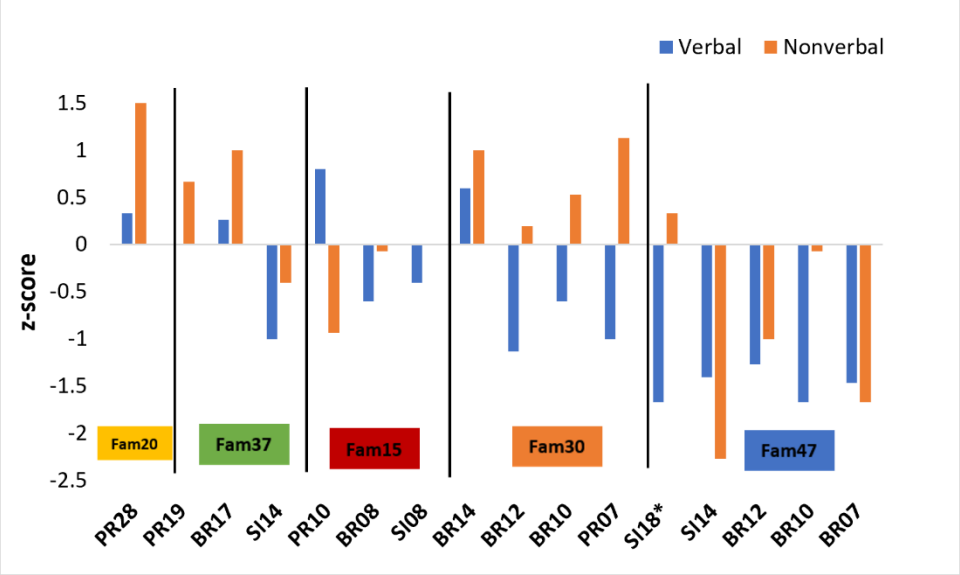
All Families Rapid Automatic Naming (RAN/RAS)



Verbal and nonverbal cognitive skills were assessed using the RIAS-2. As mentioned earlier, Family 47's scores depict a different profile than the others, which may be contributing to their mixed phenotype of phonology, language, and literacy deficits. In Family 30 we see a pattern in the youngest children of distinct differences between verbal and nonverbal skills, with lower verbal scores. This supports the idea that Family 30 may have language and literacy deficits in addition to CAS. For the cohort as a whole, a paired t-test showed a significant difference between verbal and nonverbal scores (t=2.14, p=0.02; d=0.74), which is reflective of the speech-language phenotype observed across families.

Figure 5.17

All Families Cognitive Tasks (RIAS-2)



Investigating sequential and perceptual processing as potential biomarkers related to the speech phenotypes present in these families suggests at least some expression of sequential processing deficit across all the families, with individual variation among

family members. This is most clearly reflected by the scores for the SRT (nonword repetition) and the disyllable DDK tasks. Sequential processing for speech seems particularly prominent in Fam20_PR28, Fam37_PR19, and the children in Families 15 and 30, all of whom have a history of CAS. Contrastively, the children in Family 47 do show some evidence of sequential processing deficit, less so for the speech domain. The exception to this seems to be Fam47_SI18, but since she was tested virtually, these data may be an underrepresentation of her actual abilities. For motor sequential processing tasks, only Fam15_FA49 and his daughter SI08 show below average repetitive finger-tapping rates. With overall motor skills, fine motor tasks were more challenging for the majority of the participants, with individual variation among the balance and gross motor tasks. Given the sequential processing deficits experienced by this cohort and the role of the cerebellum in balance, it would be interesting to examine the specific difference between static and dynamic balance. The BOT2 merges both kinds of balance into one subtest, but it would be useful to know if sequential processing difficulty would correlate more with dynamic balance since such tasks involve active movement, and thus, more sequential planning.

In investigating perceptual processing as a potential biomarker, it seems that some individuals in Families 30 and 47 struggled more with sight-word reading and rapid automatic naming for objects and colors. While Fam20_PR28 and Fam37_PR19 both struggled significantly with sight words, they did not show difficulty with rapid automatic naming, whereas the youngest three family members of Families 30 and 47 did. Additional examination of perceptual processing could help to better differentiate

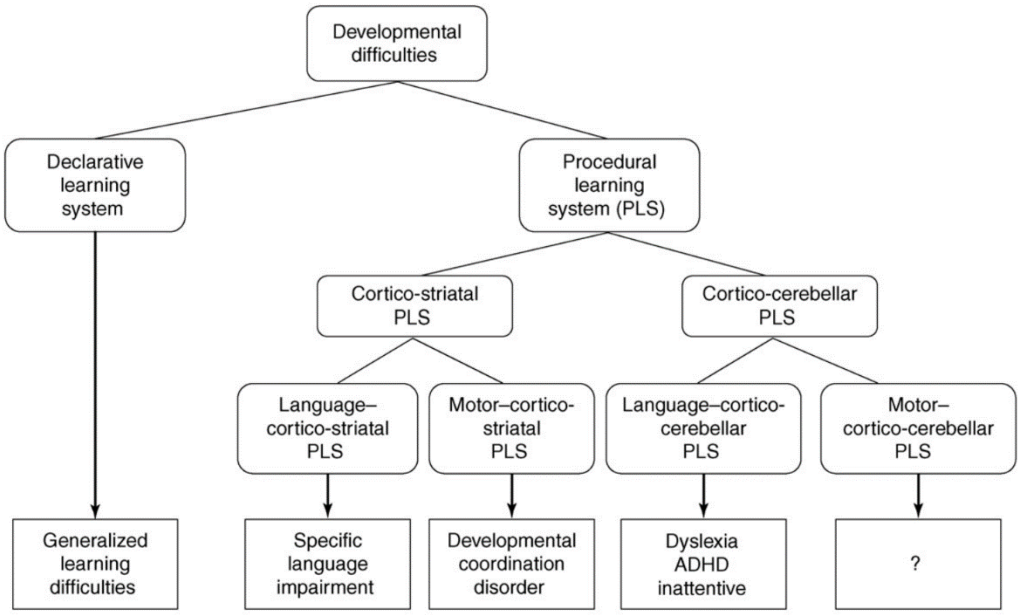
the participants. Thus, it seems like all of the participants with a history of CAS showed signs of sequential processing deficits, but some also showed signs of perceptual processing difficulty as well. Family 47's mixed phenotype shows a few signs of sequential processing difficulty, though this is generally milder than the other families. They do show signs of perceptual processing deficits, particularly for the three youngest boys that were tested.

In summary, it seems that a CAS phenotype is clearly associated with sequential processing deficits, but there is also evidence of co-occurring deficits in perceptual processing for some individuals. Those without a CAS phenotype show milder signs of sequential processing difficulty, though it is still present for some family members, and show stronger evidence of perceptual processing deficits. Work by Nicolson and Fawcett (2007) that has been recently applied to CAS by Iuzzini-Seigel (2019) provides helpful discussion about the procedural learning deficit hypothesis. The neural systems framework developed by Nicholson & Fawcett, 2007 and summarized in Figure 1 from their paper (Figure 5.19 here), illustrates how deficits in procedural learning, as mediated by differing neural circuits, could result in various phenotypic outcomes. This hypothesis essentially subsumes a cerebellar hypothesis within it and visually depicts the rationale for many of the comorbidities observed in these disorders. While the authors of this study were unsure of a disorder that would fit within the motor-corticocerebellar procedural learning system branch, evidence from this dissertation suggests that CAS would be an appropriate fit. Thus, CAS could be described as a disorder of procedural learning, mediated by corticocerebellar circuitry, impacting feedforward control

mechanisms for speech output. This framework can also account for common CAS comorbidities. Implementing such a system for SSDs would have the advantage of describing not just the surface speech phenotype but also the possible neural mechanisms influencing that phenotype.

Figure 5.18

Figure 1 from Nicolson and Fawcett, 2007



Research Question 4:

Is there evidence that familial genetic variants occur in genes whose early expression in the brain corresponds to the cerebellar or thalamic profiles? It was hypothesized that in each family with a familial behavioral biomarker profile, the most likely variants would be in genes that are expressed in the corresponding brain region during early brain development.

As biomarkers influencing speech phenotypes become clearer, the development of newer biotechnologies allows for the deeper investigation of ways the neural mechanisms

themselves are influenced by genetics. Across all trios and families that had genetic data, the following genes of interest were found (Figure 5.2). The majority of the genes were highly expressed in developing brain areas (BrainSpan), and the group as a whole had means similar to known genes of interest for CAS, though the medians were lower (Eising et al., 2018). Rather than the expression rates in these genes dominating for one brain region, it seems that, while some genes have much higher expression rates overall, the distribution of the expression rates remains generally consistent across the three regions. Several genes that differ from this dispersion pattern are *CNTNAP2*, which is highly expressed in the striatum, and *GPR56* and *LAMB1* which are highly expressed in the cerebellum. A LoF *CNTNAP2* variant was found in Family 30, whose members generally present with CAS as well as some language and literacy difficulties. Under the perceptual learning deficit hypothesis, Family 30's phenotype is reflective of deficits associated with both cortico-striatal and cortico-cerebellar neural networks. This corresponds to their genotype, as *CNTNAP2* has high expression rates across all three brain regions, especially the striatum. In contrast, Family 15's genotype shows a variant in *GPR56*, which is highly expressed in all three regions, but especially the cerebellum. Their phenotype is characterized by significant motor deficits across all subtests of the BOT2. Thus, some families do show variants in genes that are expressed more highly within a particular area, though not to the exclusion of other brain regions. In examining differences between cortical and noncortical regions, the expression rates for the genes of interest are slightly lower for the cortical regions in general, with the exception of *NBEA* which has higher expression rates in the cortical regions compared to noncortical. When

examining the known genes of interest from the Eising et al. paper (2018), there are inconsistent expression patterns, with *FOXP2* having higher expression in noncortical regions, *BCL11A* having higher expression in cortical regions, and *ERC1* having steady expression across all brain regions. All of this evidence suggests that the original hypothesis is supported for expression rates in these developing brain regions in general, but there is only minimal evidence supporting that idea that gene expression rates are region-specific.

Figure 5.19

Brain Expression Rates for Genes of Interest

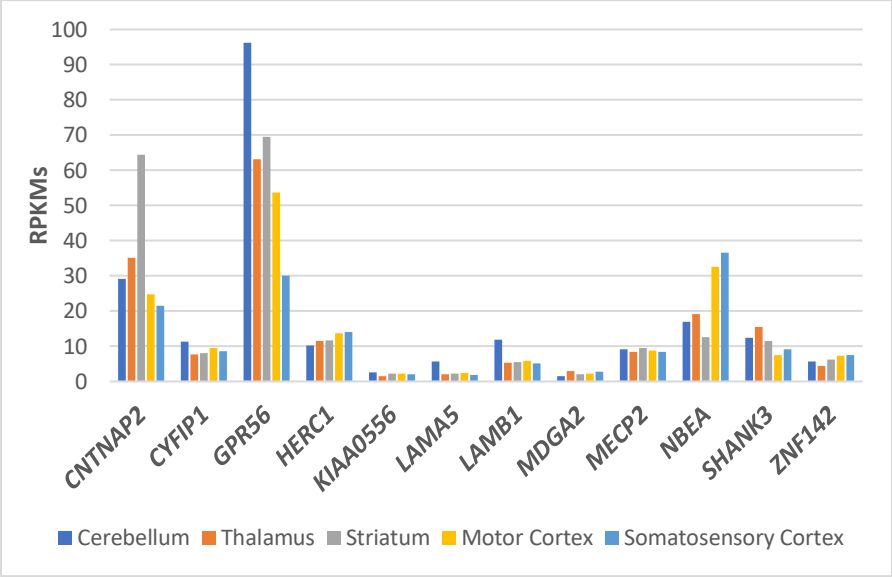


Table 5.2

Variants of Interest for All Families

Gene	**Family**	Expression in Developing **Cerebellum**	Expression in Developing **Thalamus**	Expression in Developing **Striatum**	Expression in Developing **Primary Motor Cortex**	Expression in Developing **Primary Somatosensory Cortex**
CNTNAP2	Fam 30	29.04	35.07	64.45	24.85	21.4
CYFIP1	Trio 13	11.23	7.65	8.09	9.42	8.54
GPR56	Fam 15	96.25	63.21	69.42	53.64	30
HERC1	Trio 17	10.2	11.51	11.6	13.72	14.01
KIAA0556	Trio 46	2.64	1.57	2.19	2.27	2.09
LAMA5	Trio 31	5.74	2.1	2.14	2.31	1.85
LAMB1	Trio 13	11.82	5.33	5.46	5.81	5.15
MDGA2	Trio 46	1.5	2.92	1.96	2.16	2.68
MECP2	Trio 23	9.05	8.31	9.51	8.7	8.37
NBEA	Trio 13, Fam 47	17	19.12	12.54	32.64	36.65
SHANK3	Trio 17	12.43	15.55	11.47	7.51	9.14
TENM3	Fam 30	NA	NA	NA	NA	NA
ZNF142	Trio 31	5.73	4.38	6.18	7.28	7.55
Mean		**17.72**	**14.73**	**17.08**	**14.19**	**12.29**
Median		**10.72**	**7.98**	**8.8**	**8.11**	**8.46**
Known genes of interest for CAS (Eising, 2018)						
FOXP2		18.58	20.14	11.46	1.22	1.46
BCL11A		16.89	8.47	29.58	42.4	46.03
ERC1		13.6	14.42	15.69	17.5	19.09
Mean		**16.36**	**14.34**	**18.91**	**20.37**	**22.19**
Median		**16.89**	**14.42**	**15.69**	**17.5**	**19.09**

Research Question 5:

Are the implicated genes across families in a functional association with each other? It was hypothesized that implicated genes would share functional properties (e.g., cell-cell adhesion), as has been found with other complex phenotypes such as autism spectrum disorder.

The Gene Ontology EnRIchment AnaLysis and VisuaLizAtion Tool (GOrilla) (Eden et al., 2009) was used to explore ontology terms for genes associated with all called variants across all families. A list of genes ranked from highest to lowest CADD score was entered into GOrilla. Genes associated with variants that had no available CADD score were included at the bottom of the input list. Analysis of 4809 genes yielded 39 gene ontology terms related to process, several of which are relevant for a speech phenotype (Table 5.3; Appendix D). Results suggest that many of the genes found in this cohort are related to processes of central nervous system development, including development of crucial brain regions such as the thalamus and superior temporal gyrus. It is interesting to note that proprioception is included in this list, as this function is an important component of feedback control within the DIVA model. String analysis using GeneCards data revealed that, while both *LAMB1* and *LAMA5* are laminin genes involved in cell migration and organization, none of the other genes show evidence of a functional link (Stelzer et al., 2016). Thus, gene ontology shows some evidence of shared functional properties among the genes of interest found for this cohort of participants. While some families had just a single gene of interest that remained after analysis and filtering, others had several. This supports the idea that for complex

phenotypes like communication, multiple genes of smaller effect are important to consider for genetic studies.

Table 5.3

GOrilla Analysis

Gene Ontology Term	Description	P-value
GO:0050877	nervous system process	1.86E-04
GO:0035176	social behavior	2.08E-04
GO:0008038	neuron recognition	2.26E-04
GO:0007413	axonal fasciculation	2.37E-04
GO:0106030	neuron projection fasciculation	2.37E-04
GO:0045163	clustering of voltage-gated potassium channels	2.93E-04
GO:0021955	central nervous system neuron axonogenesis	4.14E-04
GO:0019230	proprioception	4.36E-04
GO:0071109	superior temporal gyrus development	6.54E-04
GO:0021794	thalamus development	6.54E-04
GO:0021761	limbic system development	6.54E-04
GO:0097485	neuron projection guidance	9.23E-04
GO:0007411	axon guidance	9.23E-04

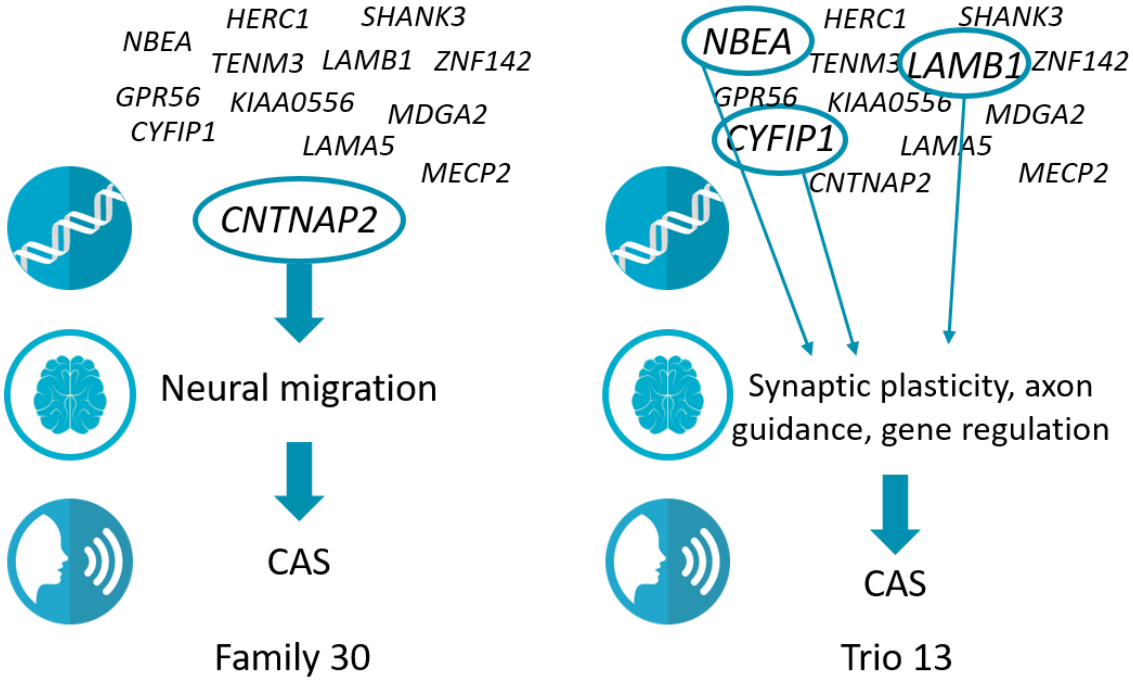
Summary

Investigating the genetic and behavioral biomarkers underlying familial speech sound disorders confirms the heterogeneity of these disorders, even for the subset of individuals with childhood apraxia of speech. Behavioral data reveal that the individuals with CAS displayed clear sequential processing deficits for speech, but the impact on nonspeech domains varied even within biologically related families. Many showed accompanying sequential processing deficits for fine motor skills, with some showing impact on language and literacy as well. Deficits in perceptual processing were also observed for some individuals with CAS, hinting at the possibility of a deeper procedural learning deficit. The family without CAS (Family 47) showed evidence of perceptual processing deficits. Though they demonstrated better sequential processing skills overall,

a few members of Family 47 still showed some difficulty with sequential processing tasks. Data from whole exome sequencing revealed 13 different genes of interest for the participants in this study, with *NBEA* as the only gene of interest appearing in more than one family. This supports the concept of speech as a complex, polygenic trait. It also suggests that the underlying neurological impact affecting behavior may arise from disruption to single genes or sets of many possible genes converging on relevant biological processes (Figure 5.20).

Figure 5.20

Possible Genotype-Phenotype Pathways



Clinical Implications

Providing speech-language pathologists working clinically with evidence to refine their practice as they support the best possible outcomes for their clients is the end goal of research in communication disorders. The results of this study confirm the heterogeneity of SSDs as a group, even within the subset of individuals affected by CAS. The results also demonstrate a pattern that individuals with a history of CAS in essence still experience residual effects of CAS even though their speech phenotype has significantly improved. Participation in therapy has all but eliminated the severity of the surface level speech phenotype, as demonstrated by typical scores on the GFTA-3 and little to no impact on informal conversation. When these individuals have control over their communication, the speech of these individuals likely would not stand out as unusual to the average listener, but when their system is challenged, they still show significant struggle. This suggests that the underlying biomarker is still present and active within their system. This begs the question, "Has remediation truly taken place?" The change to a surface-level speech phenotype is still a significant change that improves quality of life, yet a key question that arises from this study is, "Should the role of a speech-language pathologist be focused on treating the surface phenotype or, in concert with other disciplines, on treating the underlying mechanism as well?" What could this look like? Perhaps an intervention that addresses the sequential processing biomarker could potentiate the clinical interventions already received by clients across multiple domains. One could speculate about the possibility of an intervention using music, since it is also enlists sequential processing in both motoric and symbolic ways, relying on feedforward

and feedback control. Future research could explore sequential processing in CAS individuals who have had significant musical instruction to determine if a deeper level of remediation has occurred.

Another outcome of this study that has clinical implications is that the surface level speech presentation of the children in this study with some remaining speech errors looked similar to children who present with residual articulation errors. Capturing word-level errors with the GFTA-3 yielded low scores for a few of the participants, but overall, their residual errors were present for later-developing phonemes like /r/ and /θ/, common errors seen with an articulation disorder phenotype. Without a thorough case history and deeper testing to examine sequential processing deficits, the CAS children could easily be perceived as having just a few developmental speech sound errors, when in fact their speech acquisition did not follow a developmental trajectory at all. This misidentification could lead to inappropriate treatment approaches, an underestimation of therapy time and intensity needed for remediation, and delayed progress in treatment overall. Along with speech and motor impacts, a number of the participants in this study displayed difficulty with language and literacy skills. As research in the field has begun to demonstrate, more research is needed on how sequential processing impacts language and literacy outcomes, and how intervention could be optimized by addressing potential needs in this area.

Finally, while it may seem that incorporating genetic data into therapeutic decisions is still relegated to the future, the work of this dissertation and other similar studies could eventually help build knowledge of genetic risk for speech disorders, knowledge which could be used to support earlier interventions. This currently happens

on an informal level, as pediatric speech-language pathologists will often take into consideration if a child's older siblings have needed therapy when reviewing eligibility for services. On a more formal level, preliminary proof-of-concept research by Peter et al. (2020) has shown how genetic knowledge can be applied clinically with the aim of mitigating, or even preventing, speech and language disorders. While genetic research has identified some genes of interest for speech and language, it would also be interesting to pursue an investigation of protective genes in the area of communication. More research is needed to investigate genetic and neural underpinnings of speech sound disorders, but as this knowledge grows, it may be possible to refine assessment, potentiate treatment, and hopefully mitigate or even prevent the impact of these disorders on communication.

Study Limitations and Future Directions

The small size of both the behavioral and genetic cohorts was a primary limitation of this dissertation project. Hopefully, post-pandemic, further behavioral testing can take place with additional family members in order to expand the phenotypic profiles for each family. Additionally, it would be helpful to expand the number of genetic samples sequenced for each family. The only unaffected participants for which we had whole exome sequences were the parents of the trios. To further genetic analysis, it would be helpful to have additional exome sequences from some of the unaffected parents within the larger families to help clarify the family genotypes. One planned study that some of these families may participate in involves studying the metabolome of these individuals as it relates to their behavioral phenotype. Another potential study that could be explored

would be developing a therapeutic intervention that targets the level of the sequential processing endophenotype to investigate any possible potentiation of existing therapeutic interventions in those with speech sound disorders.

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APPENDIX A

ORAL MECHANISM EXAMINATION ITEMS,

ADAPTED FROM ROBBINS & KLEE, 1987

Oral Mechanism Examination
*Tasks of interest for sequential processing biomarker

Structure	Task	+/-	Notes
Lips-at rest	Symmetry		
	Relationship (open/closed)		
Lips-oral function	Rounding (/o/)		
	Protrusion (/u/)		
	Retraction		
	*Alternate pucker/smile		
	Bite lower lip		
	Lip seal*		
	Open/close		
Lips-speech function	Rounding on /o/		
	Protrusion on /u/		
	Retraction on /i/		
	*Alternate /u/-/i/		
	Bite lower lip /f/		
	Open & Close lips /ma/		
Teeth	Gaps/Crowding		
	Missing		
	Occlusion		
Tongue	Position at rest		
	Protrusion		
	Elevation to alveolar ridge		
	Anterior-posterior sweep		
	Lateralize tongue-external		
	Frenulum		
	Tongue position for speech		
Velopharynx	Uvula		
	Mallampati		
	Tonsils		
	Vault height		
	Sustain /a/		
	Movement for /a-a-a/		
Swallow	*Lip pursing during swallow		
	*Swallow water while smiling		

APPENDIX B

GENES OF INTEREST FOR SPEECH-LANGUAGE DISORDERS, COMPILED BY DR. BEATE PETER

ABCC13
ANKK1
16p11.2 deletion region
22q11.2 deletion region
ABAT
ABCC13
ACCN1 (ASIC2)
ACSL4
ACTR2
ADGRG1
ADNP
ADSL
AK056897
AK126351
AL157450
ALG11
ALG5
ANKRD12
AP3B2
AP4E1
APOE
ARID1B
ARL17A
ARL17B
ATP13A4
ATP2C2
AUTS2
BAG2
BCL11A
BCL11B
BCR
BDNF
BEND6
BMP4
C2CD3
C4orf21
CACNA2D1
CASK
CCDC136
CCDC148
CDCA7
CDH1
CDH18
CGEF2
CHD3
CHMP1A
CHRNA3
CHRNA7
CLIC2
CLP1
CMIP
CNTN4
CNTNAP1
CNTNAP2
CNTNAP2 (CASPR2)
CNTNAP5
COG4
COL4A2
COMT
CPLX1
CRKL
CSNK1A1
CTNNA3
CTNND2
CTTNBP2
CUL4B
CXorf22
CYFIP1
CYP19A1
DAG1
DAZAP1
DCDC2
DCDC5
DEAF1
DIP2A
DLX1
DLX2
DNAAF4
DNAH14
DNM1
DOCK4
DPYD
DRD2
DST
DYM
DYX1C1
DYX2
DYX3
DYX5
DYX6
DYX8
DYX9
EARS2
ELKS
ELP4
EN2
ERC1 (ELKS)
ERLIN2
EXOSC3
EXOSC8
FAM48 (SUPT2OH)
FGF12
FLCN
FLNC
FMR1
FOXG1
FOXP1
FOXP2
FRMD1
FRRS1L
GABARAP
GABRD
GALT
GATAD2B
GCFC2
GCFC2 (C2ORF3)
GLI3
GLP2R
GMPPB
GNAO1
GNPTAB
GNPTG
GNS
GPT2
GRIN2A
GRIN2A (NR2A)
GRIN2B
GRN
HAT
HGC6.3
HNF1B
IDO2

IFIH1
IMMP2L
ISCA2
KAT6A
KCNAB2
KIAA0319
KIAA1267
KIF25
KIF5C
KLHL15
KMT2D
LHX1
LOC169834 (ZNF883)
LRRC37A
MAP1D
MAPK1
MBD5
MC5R
MCOLN1
MDH2
MECP2
MED13L
MFF
MFSD2A
MID2
MLK7-AS1
MLLT4
MPDU1
MRPL19
MSRA
MUC6
MYO10
MYO19
NACC1
NAGPA
NBEA
NCOR1
NDST4
NEDD4L
NEGR1
NEK8
NFXL1
NIPA1
NIPA2
NIPBL
NOBOX
NOP9
NRXN1
NSF
NSFP1
NUDT16L1
OR6P1
OXR1
PAX6
PCDH11X
PCDH11Y
PCNT
PDE11A
PDK1
PIGA
PIGN
PIGV
PKP4
PLCL1 (PRIP)
PMM2
PNPT1
POMT1
PRDM16
PRIM2
PRMT2
PRPS1
PTEN
PURA
RAB23
RAPGEF
RARS2
RBFOX2
RERE
RFXAP
ROBO1
ROBO2
S100B
SCN11A
SCN9A
SEMA6D
SETBP1
SETD1A
SETX
SH2B1
SIK1
SKI
SLC16A2
SLC25A12
SLC2A1
SLC33A1
SLC9A6
SMAD9
SMARCA2
SMARCE1
SMCR8
SOX5
SPATA5
SPRED2
SRCAP
SRPX2
ST3GAL5
STARD9
STUB1
STXBP1
SYNGAP1
SYNPR
TBC1D24
TBR1
TBX1
TCTN2
TDP-43
TELO2
TM4SF20
TMEM231
TNRC6B
TTRAP
TUBGCP5
UBA5
UBASH3B
UBE3A
UNC80
UQCRQ
UTRN
VWA3B
WDR45
WDR5
ZDHHC15

ZEB2	ZGRF1	ZNF385D
ZFHX4	ZNF277	ZNF737

APPENDIX C

ACCRONYMS AND ABBREVIATIONS

AD=autosomal dominant

AOS=apraxia of speech

AR=autosomal recessive

ASD=autism spectrum disorder

Ben=benign

CADD=combined annotation dependent depletion

CAS=childhood apraxia of speech

CH=compound heterozygosity

CNV=copy number variation

Crblm=cerebellum

DDK=diadochokinetic

DH=dominant hand

Del=Deleterious

DN=*de novo*

DNA=deoxyribonucleic acid

F1=first formant

F2=second formant

FCR=formant centralization ratio

kb=kilobase

LoF=loss-of-function

MOI=mode of inheritance

NDH=nondominant hand

NFE=non-Finnish European

pLI=probability of being loss-of-function intolerant

PoD=possibly damaging

PrD=probably damaging

QC=quality control for exome sequencing

RNA=ribonucleic acid

SIFT=sorting intolerant from tolerant

SLI=specific language impairment

SLP=speech-language pathologist

SNP=single nucleotide polymorphism

SSDs=speech sound disorders

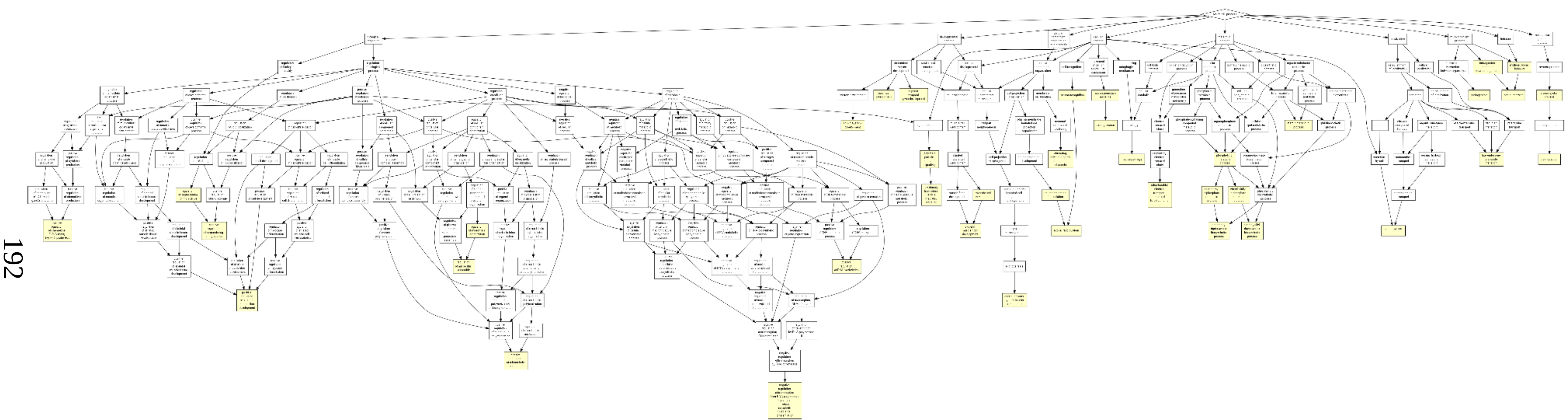
Thal=thalamus

Tol=tolerated

VSA=vowel space area

APPENDIX D

GORILLA BIOLOGICAL PROCESS OUTPUT



APPENDIX E

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