Genetic Variations and Associated Electrophysiological and Behavioral Traits in Children with Childhood Apraxia of Speech

by

Caitlin Vose

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Graduate Supervisory Committee:

Beate Peter, Chair
Li Liu
Gene Brewer

ARIZONA STATE UNIVERSITY

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ABSTRACT

Childhood Apraxia of Speech (CAS) is a severe motor speech disorder that is difficult to diagnose as there is currently no gold-standard measurement to differentiate between CAS and other speech disorders. In the present study, we investigate underlying biomarkers associated with CAS in addition to enhanced phenotyping through behavioral testing. Cortical electrophysiological measures were utilized to investigate differences in neural activation in response to native and non-native vowel contrasts between children with CAS and typically developing peers. Genetic analysis included full exome sequencing of a child with CAS and his unaffected parents in order to uncover underlying genetic variation that may be causal to the child's severely impaired speech and language. Enhanced phenotyping was completed through extensive behavioral testing, including speech, language, reading, spelling, phonological awareness, gross/fine motor, and oral and hand motor tasks. Results from cortical electrophysiological measures are consistent with previous evidence of a heightened neural response to non-native sounds in CAS, potentially indicating over specified phonological representations in this population. Results of exome sequencing suggest multiple genetic variations contributing to the severely affected phenotype in the child and provide further evidence of heterogeneous genomic pathways associated with CAS. Finally, results of behavioral testing demonstrate significant impairments evident across tasks in CAS, suggesting underlying sequential processing deficits in multiple domains. Overall, these results have the potential to delineate functional pathways from genetic variations to the brain to observable behavioral phenotypes and motivate the development of preventative and targeted treatment approaches.

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CHAPTER 1

INTRODUCTION

Childhood Apraxia of Speech: Behavioral Traits and Burden

Childhood apraxia of Speech (CAS) is a severe motor speech disorder that has a substantial impact on a child's ability to communicate functionally across environments. The American Speech and Hearing Association (ASHA) describes CAS as having three main characteristics, including inconsistent speech errors on repeated productions, lengthened and disrupted co-articulatory transitions and inappropriate prosody (ASHA 2007). Additional characteristics of CAS include vowel distortions, lack of differentiation between stressed and unstressed syllables, mis-stressing syllables, and difficulty with multisyllabic words (Shriberg et al., 2011). For purposes of this study, it is important to note that vowel distortions, in particular, are highly unusual in typical speech and delayed speech development at any age. Although these characteristics of CAS have been well established, it has also been suggested that CAS is a multi-level disorder involving auditory/perceptual deficits in addition to deficits in planning/programming of speech (Shriberg, Lohmeier, Strand, and Jakielski, 2012). CAS is remarkably complex, difficult to treat clinically, and places a heavy burden on children and families who are impacted. A better understanding of the biological causes and associations, in terms of genes, brain functions, and behavior, can lead to the earliest possible identification and development of novel and proactive interventions.

Little is known about the etiology of this severe speech disorder, but it has been suggested to be a neurological sensorimotor speech sound disorder (SSD) subtype with a disruption of neurophysiological processes at the level of motor planning and/or

programming of speech movement sequences (ASHA, 2007). CAS may occur secondary to a known neurological injury such as intrauterine stroke or infection (Brown et al., 2000), neurodevelopmental disorder, or genetic mutation (Shriberg, Potter, & Strand, 2011), although in most cases, the cause is unknown (Murray, McCabe, Heard, & Ballard, 2015).

It is well established that children with CAS require extensive periods of intervention due to the severity and complexity of the disorder (ASHA 2007) and intensive treatment is typically recommended to produce maximal outcomes (Rietvield et al., 2015). The cost of treating speech disorders (SD) including CAS is substantial. Slow and limited progress within speech therapy has also been reported in treatment (Maas, Butalla, & Farinella, 2012). Campbell (1999) reported that children with CAS typically require 81% more therapy than children with phonological impairments to achieve functional speech production. Specialized treatment methods are often necessary including motor treatments, linguistic approaches, alternative augmentative communication, and biofeedback such as electropalatography and ultrasound (ASHA, 2007; Gillon & Moriarity, 2007; Hall 2000; Preston, Brick, & Landi, 2013; Preston, Leece, McNamara, & Maas, 2017). In addition to disordered speech production, children with CAS are also likely to experience severe written language difficulties, as well as deficits in phonological awareness, reading, and spelling difficulties (Lewis et al., 2004; Gillion and Moriarty, 2007; McNeill et al., 2009). These deficits often persist into adolescence regardless of gains in speech production (Stackhouse & Snowling, 1992). Children with CAS are also at risk for social and vocational difficulties due to persisting deficits in phonological, semantic, syntactic development and subsequent decreased

reading and writing performance (Lewis et al., 2004; Moriarty & Gillon, 2007). Despite the substantial expense and difficulty of treating CAS, as well as the persisting nature of the disorder, there remains a scarcity of intervention studies for treatment.

Children with CAS are primarily described as having deficits in the planning and/or programming of speech movements (Grigos & Kolenda, 2010; Terband, Maassen, Van Lieshout, & Nijland, 2011; Grigos, Moss, & Lu, 2015; Preston et al, 2014). Planning and programming of speech requires phonological information to be used to create a motor plan that determines the specific speech movements for each phoneme of a spoken word (Van der Merwe, 2009). The execution of this plan leads to the production of the target word. Planning and programming are often used interchangeably in the CAS literature. Here we will be more specific. Following van der Merwe's (1997, 2009) model, planning refers to the movement of the articulatory structures required to achieve motor goals, and programming refers to muscle specific goals such as muscle tone, movement velocity, force, and range. Children with CAS may have impairments in one or both of these processes.

Identifying the core features of CAS has been a topic of controversy in recent years (McCauley & Strand, 2008). For many years, there was no validated list of diagnostic features to differentiate CAS from other pediatric SSD (ASHA, 2007). Established, universally accepted, core features will provide clinicians with a clear diagnostic criteria, increasing the accuracy of this challenging diagnosis. The current difficulty in differential diagnosis interferes with early detection and treatment of CAS. Many speech-language pathologists (SLPs) are hesitant to diagnose CAS because of this problem. In addition, the difficulty of accurately diagnosing CAS has had a detrimental

effect on the emerging research in this area, as there is no single assessment procedure that can be used to identify true positives. Therefore, all research must be reviewed with caution, as there is a lack of agreement on accurate diagnosis (Shriberg et al., 2012).

In a review of published standardized tests available for the diagnosis of motor speech disorders in children, McCauley and Strand (2008) found no assessments that can be considered sufficiently developed to diagnose children with motor speech disorders. Clinicians typically use assessment tools such as informal sampling, published checklists (i.e., "NIDCD Speech and Language Developmental Milestones", 2017), and standardized measures (i.e., Goldman Fristoe Test of Articulation -3; Goldman & Fristoe, 2015). All tests reviewed addressed several major content areas including motor speech function, nonverbal oral motor function, and oral structure. Given the lack of a valid assessment tool for CAS, the authors recommend that clinicians rely on their own clinical judgment and knowledge of the client in addition to the best evidence based test available. The authors also report that an increased number of reviews of standardized tests are needed to determine which tests can be reliably used for the assessment of oral motor function in children. Most standardized speech sound assessments are not normed for very young children. For example, the Goldman Fristoe Test of Articulation -3 (GFTA-3; Goldman & Fristoe, 2015), a widely used standardized articulation test, is normed for ages 2 years and up, meaning it cannot be used to assess speech sound development in the early stages of speech and language development. It is also important to note that standardized assessments may not be possible with children of a young age, as attention and cooperation may not be adequate for the completion of the numerous measures that may be required (Davis & Velleman, 2000).

Recently, there have been several attempts to identify the features that can be accurately utilized in the diagnosis of CAS. In a study conducted by Murray et al. (2015), twenty-four quantitative measures were then taken from several selected tests and examined for their accuracy of predicting CAS in comparison to expert opinion. Four of these measures together were found to have a 91% level of accuracy in predicting an accurate diagnosis of CAS. These measures were: percentage of lexical stress matches (stress placed on syllables within words), syllable segregation (brief or lengthy pause between syllables), accuracy on diadochokinetic tasks (repetitive and alternating syllable repetition), and percentage of phonemes correct (PPC) on a polysyllable test. In addition to these measures, the authors also advocate for the importance of an oral motor assessment to identify structural and neurological deficits (Murray et al, 2015). The measures found to be strong predictors of CAS in this study provide support for the characteristics described in the ASHA position paper, with the exception of inconsistency of errors. According to Forrest (2003), inconsistency is the feature used most frequently by speech therapists to make a diagnosis of CAS. It is clear that additional evidence is needed to support this particular characteristic of CAS. The authors also state that more research is necessary to identify measures that can be used in children with limited verbal output, as the measures described are only predictive for highly verbal children (Murray et al, 2015). A recent series of articles by Shriberg et al. (2017a, 2017b, 2017c, 2017d, 2017e) identified a behavioral measure termed the pause marker (PM) as a robust diagnostic marker of CAS. The PM was found to be highly accurate in distinguishing CAS from SD. This measure uses a behavioral correlate of CAS, inappropriate between-words pause, within connected speech to compute a PM score. The PM is an example of

a measure that has been found to be highly accurate in identifying CAS versus other forms of SSD in children who are producing connected speech, although this measure is awaiting validation by other researchers before it can be widely used as a diagnostic marker.

Although there is emerging evidence for the core features of CAS, clinicians continue to face substantial challenges in the differential diagnosis of the disorder, particularly in the early stages of speech development. Measurable traits in childhood motor speech disorders vary greatly as a function of age, leading to further difficulty in accurate diagnosis (McCauley & Strand, 2008). Many aspects of speech that must be measured in order to fully assess a motor speech disorder may only be apparent at distinct stages of the child's development. A test that measures prosody, for example, may not be useful with a young child with limited verbal output, as the child's prosody patterns might not be apparent. Diadochokinetic rates and multisyllabic words may be indicative of CAS in older children, but cannot be elicited from a very young child. As mentioned previously, many standardized tests are also not appropriate for young children as their attention span and ability to focus on a single task may be limited, making formal assessment of CAS a challenge.

The passage of recent early intervention statutes, including the Individuals with Disabilities Education Improvement Act of 2004, calls attention to the need for early identification and treatment of speech and language disorders in infants and toddlers. SLPs are asked to identify these children as early as possible and provide research-based assessment and intervention (IDEA '04, Part C). Unlike some medical conditions that can be diagnosed at or even before birth based on readily observable or measurable signs

and symptoms, speech disorders can only be diagnosed at an age when deficits become apparent, which, for speech, is typically 2 to 3 years of age. In the case of CAS, children most likely have limited verbal output, making early diagnosis an even greater challenge. It is believed, however, that early identification, if possible, can lead to increased treatment outcomes and better long-term prognosis, as has been demonstrated in Autism Spectrum Disorder (Dawson et al., 2010).

Children with CAS show differences from their peers at various stages of speech development and across many developmental domains. According to an ASHA (2007) report, difficulties with feeding in infancy and early childhood have been reported in CAS, as well as delays in fine and gross motor development. Additional non-speech motor signs of CAS include clumsiness, impaired volitional oral movements, low muscle tone, and hyper- or hyposensitivity of the oral mechanism. Children with CAS are also more likely to develop deficits in academic areas such as reading, spelling, and written expression (Lewis et al, 2004). Regarding behaviorally observable traits, we focus primarily on the role of auditory perception and the ability to process sequential information.

An important area of interest in CAS is the study of speech perception. The development of speech production is believed to be closely linked to the development of speech perception, and both are vital for the development of accurate speech (Whalen, 1999). Computational models of speech sound production provide insight into potential deficits in CAS. The DIVA (Directions Into Velocities of Articulators) model of speech production (Guenther, 2006; Guenther & Vladusich, 2012) depicts a model of feedback and feedforward loops that both play a role in the acquisition of speech. Decreased

performance in either of these two systems results in changes to foundational phonemic representations. Speech production requires the synthesis of auditory, somatosensory, and motor information from various areas of the cerebral cortex, including the temporal, parietal, and frontal lobes, and the cerebellum. According to the DIVA model, when an infant learns to produce speech sounds, imitation and babbling along with the feedback and feedforward loops are utilized to fine tune correct speech production. The feedforward control system begins with neurons associated with a "speech sound map" to activate articulatory control units in the cerebellum and primary motor cortex. The feedback system then provides auditory and somatosensory feedback to help shape the accurate production of the sound. For children with CAS, it is hypothesized that an error is occurring at some point in between the feed-forward and feedback processes in the acquisition of vowel sounds. Although deficits in motor planning and programming are well established in CAS, little is known about the potential underlying perceptual deficits associated with this disorder. For example, children with CAS often do not babble in infancy and early childhood (Highman, Hennessey, Sherwood, & Leitão, 2008). It is not fully understood why this occurs, and whether this relates to motor planning/programming and/or perceptual deficits.

Several studies have shown decreased phoneme perception in CAS. Maassen, Groenen, and Crul (2003) found decreased vowel perception in children with CAS as compared to age-matched controls. This study used two vowel continua in identification and discrimination tasks that showed decreased phonetic processing and auditory processing in children with CAS. Children with CAS have also been shown to have difficulty with rhyming and syllable awareness, suggesting limited phonological

representations (Marquardt, Sussman, Snow, & Jacks, 2002). Zuk, Iuzzini-Seigel, Cabbage, Green, & Hogan (2018) recently determined poor speech perception is not a core deficit of CAS, rather a co-occurring trait. Speech perception was examined in participants with CAS with and without language impairment in comparison to children with speech delay and typically developing peers. Children with CAS with language impairment and children with speech delay and language impairment showed decreased speech perception in comparison to children with CAS without language impairment and typically developing peers. Therefore, it was determined that decreased speech perception is not a core feature of CAS, but is a co-occurring trait that occurs in the presence of language impairment and CAS.

Models of speech and language acquisition suggest that in the early stages of typical development, native language phoneme representations are non-specific (Bernhardt & Stoel-Gammon, 1994). As a child's phonological system develops, underlying representations of phonemes become language-specific (Kuhl & Rivera-Gaxiola, 2008). The loss of the ability to perceive nonnative phonemes is believed to be strongly associated with the success in perceiving native language phonemes. Kuhl (2004) has termed this process the native language neural commitment (NLNC) hypothesis. This theory suggests that in the first year of life a child's brain begins to neurally commit to phonemes distinct to his or her native language. A series of studies utilizing event related potentials (ERPs) and behavioral measures found that the increased ability to detect native phonemes was related to the development of higher level language skills. Contrastively, increased discrimination on non-native phonemes was correlated with decreased language skills at later stages of development (Kuhl et al., 2008; Kuhl,

Conboy, Padden, Nelson, & Pruitt, 2005; Kuhl, 2006). Applying the NLNC hypothesis to speech development, failure to fine-tune the phonological features of a native language may result in difficulty with accurate phoneme retrieval and production (Gierut & Morrissett, 2012). Dogil and Mayer (1998) proposed this theory in relation to acquired apraxia of speech in brain injured adults, in that phonological representations are over specified leading to planning and execution errors. Children with CAS may have a representational impairment for native speech sounds leading to an overabundance of options for articulation. Support for this theory may be may be generated by examining the neural mechanisms underlying speech perception in CAS.

Sequential Processing and CAS

Children and adults with a history of CAS show signs of global sequential processing deficits in motor, cognitive, and linguistic task performance. Sequential processing refers to the processing of complex sequential information, which involves sensory encoding, storing (including sensory, short-term/working, and long-term memory), retrieval, phonological assembly, motor programming, motor planning and motor execution (Levelt, 1999; Levelt, Roelofs, & Meyer, 1999; Stackhouse & Wells, 1997). Deficits in sequential processing can result in errors during encoding, storing, and/or producing the sequence of sounds necessary for spoken language. Peter, Button, Stoel-Gammon, Chapman, and Raskind (2013) investigated a global sequential processing deficit as an endophenotype in a multi-generational family with a history of CAS. The authors hypothesized that sequential processing tasks are present across a variety of tasks – motor, linguistic, and cognitive. Results provide evidence for decreased sequential processing in cognitive processes upstream from motor

programming. Peter and Raskind (2011) found that measures of sequential motor processing during diadochokinetic tasks involving rapid alternating-sequential movements (repetitions of /pata/, /taka/, and /pataka/) and hand motor tasks involving alternating key tapping were highly associated with a history of CAS, in comparison to repetitive diadochokinetic tasks (repetitions of /pa/, /ta/, and /ka/) and repetitive keyboard tasks.

Shriberg (2012) hypothesized that deficits in motor programming for speech may be indicative of overall motor programming and execution deficits. Differences in oral motor control have been observed in children with SSD as compared to typically developing children (Lewis et al., 2011). An emerging body of research supports the idea that the development of speech and language skills follows a similar trajectory as the development of motor skills. In typically developing infants, longitudinal changes in their articulatory movements are highly correlated with early communication development (Nip, Green, & Marx, 2011). Children with speech disorders display a similar, although delayed, trajectory, as gross and fine motor differences are often reported in children with delayed speech and language (Ceremak, Ward, & Ward, 1986). Dewey, Roy, Square-Storer, and Hayden (1988) found that children with CAS have trouble with transitioning between movements within one motor task (e.g., pulling and then turning a knob), but not with repetitive movements. Bradford and Dodd (1996) also found decreased coordination and dexterity for complex movements in children with CAS as compared to children with other speech disorders and controls. These findings provide further evidence for a global sequential processing deficit in CAS.

It is hypothesized that CAS shares an endophenotype with dyslexia due to comorbidity of traits shared between these disorders, including the underlying sequential processing deficit described previously. This hypothesis implies that CAS and dyslexia have a shared genetic etiology which is expressed in the brain. It is well established in the literature that children with CAS and children with dyslexia struggle with tasks requiring sequential processing such as nonword imitiation (Catts, 1986; Shriberg, Lohmeier, Strand, & Jakielski, 2012). Sequential processing deficits in dyslexia have been reported in several studies. Children with dyslexia have been shown to be less accurate than controls when judging phoneme order in consonant clusters, and demonstrated improved accuracy when stimuli were presented at a slower rate (Ray, De Martino, Espesser, & Habib, 2002). Peter, Lancaster, Vose, Middleton, and Stoel-Gammon (2017) investigated a potential shared underlying deficit in the processing of sequential information between adults with a probable history of CAS and dyslexia using non-word repetition, multisyllabic real word repetition, and non-word decoding tasks. Overall results are consistent with a shared persisting sequential processing deficit in both groups and across linguistic and motor tasks. Participants in both groups were found to produce substantially more sequencing errors as compared to substitution errors in the non-word decoding tasks, with omissions as the most prevalent error type (i.e., "sprawn't" (/sprɔnt/) as "spawn't" (/spɔnt/)). This study also provides evidence that sequencing errors in CAS occur not only in the motor speech and hand motor domain, but also during the encoding stage of visual information. For example, a frequently seen error suggesting a visual encoding deficit are [ralut] for "wrault" as if it were spelled "wralut", and [braɪkəl] for /bɝkəl/, as if it were spelled "brycal".

Brain Correlates of CAS

Knowledge of the neural correlates associated with CAS is extremely limited. Furthering this knowledge can lead to meaningful clinical translations, including novel treatment methods and early diagnosis. Unlike acquired adult apraxia of speech, which often occurs following a left hemisphere lesion, CAS does not typically occur in children who have suffered lesions in the left hemisphere (Chilosi et al., 2008). Models of speech production in adults suggest the pre- and primary motor cortices, the cerebellum, and subcortical central loops play crucial roles in speech production (Jürgens, 2002). In the case of CAS, however, understanding of neurobiological markers is still an emerging field.

One possible explanation for the overlap in phenotypes between CAS and dyslexia mentioned previously is a shared deficit in cerebellar function. It is believed that the cerebellum plays a crucial role in cognitive-linguistic tasks, including the processing of sequential information (Marien & Beaton, 2014) in terms of sensory encoding, storing, retrieval, phonological assembly, motor programming, motor planning and motor execution (Levelt, 1999; Levelt, Roelofs, & Meyer, 1999; Stackhouse & Wells, 1997). The cerebellum has been implicated in several of these processes. For example, a case study of a patient with a cerebellar lesion who had selective impairment of verbal working memory (VWM), specifically a deficit in the phonological output buffer. The phonological output buffer is described as a working memory space in which phonological segments are stored temporarily, prior to various output processes (i.e., planning and editing of the procedures needed for speech). This deficit was interpreted as evidence of cerebellar involvement in the planning of speech production at a level that

does not require an overt articulation (Silveri, Di Betta, Fillippini, Leggio, & Molinari, 1998). Chen and Desmond (2005a) demonstrated a superior cerebellar involvement during the encoding phase of VWM tasks. The role of the cerebellum as a detector of change and deviation of sequential events has been demonstrated using magnetoencephalography, providing evidence that the cerebellum plays a role in the processing of a sudden violation of sequence prediction timing (Tesche & Karhu, 2000). Gebhart, Petersen, and Thach (2002) investigated language skills in subjects with cerebellar lesions, including an antonym generation task, noun (category member) generation task, verb selection task, and a lexical decision task. It was found that subjects with right cerebellar lesions were impaired on an antonym generation task in both accuracy and reduction of reaction time with practice. The authors suggest that the deficit in antonym generation may be due to the increased level of processing of this task as compared to the other measures utilized in the study. Overall evidence from sensory (Bower, 1997; Restuccia, Della Marca, Valeriani, Leggio, & Molinari, 2007), motor (Thach, Goodkin, & Keating, 1992) and behavioral (Leggio et al., 2008) domains suggest sequencing processing is the basic function of the cerebellum in language (Molinari, Chiricozzi, Clausi, Tedesco, De Lisa, & Leggio, 2008).

Severe SSD consistent with CAS has been shown to be associated with neurological changes (i.e., Preston et al., 2014; Froud & Khamis-Dakwar, 2012; Liegeois & Morgan, 2012; Liegeois et al., 2003). Neurobiological tools such as electroencephalography (EEG) and event related potentials (ERPs) have been widely used to study speech and language processing in infants and young children (Kuhl, 2004), although these types of studies investigating CAS are limited. EEG is a highly

temporally sensitive measure that records electrical activity of the brain at the level of the scalp. ERPs are time locked analysis of the EEG signal following a stimulus. Auditory mismatch negativity (MMN) is an ERP that reflects the brain's ability to detect changes in auditory stimuli, including discrimination of phonemes (Näätänen, Kujala, & Winkler, 2011). This is an automatic, pre-attentional change-detection response for auditory discrimination of phonemes (Näätänen, 1992; Näätänen, Kujala, & Winkler, 2011; Näätänen & Winkler, 1999). This response is elicited by an infrequent change in a sequence of repetitive auditory stimuli, where a sequence of a "common" stimulus is presented the majority of the time, and an "odd" stimulus is presented infrequently. This response can be elicited without conscious attention and is therefore a useful tool when examining ERPs in children. This is a particularly useful tool in examining EEG responses in children with CAS as MMN is elicited early in auditory processing, and is unlikely to show attentional or cognitive processes which may be related to deficits commonly observed in CAS (ASHA, 2007).

To date, two ERP studies examining CAS have been published. Froud and Khamis-Dakwar (2012) investigated differences in MMN between children with CAS and age matched controls when listening to phonemic and allophonic contrasts. This study was conducted under the assumption of the NLNC theory that CAS is associated with an over specification of phonological representations. An ERP experiment targeting the MMN response was employed to investigate this hypothesis. MMN reflects the brain's ability to detect changes in auditory stimuli, including discrimination of phonemes (Näätänen, Kujala, & Winkler, 2011). The authors discovered an expected MMN response to phonemic sound contrasts (i.e., /ba/ vs. /pa/) but not in allophonic

contrasts (i.e., /pa/ vs. /p^{h}a/) in the typical developing group, whereas the CAS group showed an MMN response to allophonic contrasts but a less mature response in phonemic contrasts. The authors conclude that these findings demonstrate children with CAS have phonological deficits in addition to deficits in motor planning, and they also may have overly specified representations of phonological information.

Preston et al. (2014) examined pre-speech neurolinguistic processes in typically developing children and children with CAS during production of simple and complex words. Findings of this study indicate reduced amplitude of the observed signal in processing of complex words versus simple words in the CAS group. Children in the CAS group also showed different electrophysiological activity in the right hemisphere during speech preparation. Specifically, the CAS group presented with decreased amplitude in activity over the right hemisphere for complex words, relative to simple words, whereas the typically developing group showed now such difference. These findings suggest that children with CAS may utilize different neuronal populations when preparing for speech production, particularly in preparation for more complex word forms.

An fMRI study examining brain activation during a nonword repetition task in four affected members of the KE family (whose affected members presented with SSD consistent with apraxia and a mutation of the *FOXP2* gene which will be discussed in coming sections of this paper) compared to age and gender matched controls found reduced activation in the KE family members in the anterior cingulate, supplementary motor area in the right hemisphere, and left dominant speech execution regions including the precentral gyrus and left rolandic operculum. In addition, under activation of the

cerebellum and putamen were observed. Under activation of the rolandic operculum was specific to nonword repetition tasks, as this was not observed in previous fMRI studies of the KE family. The authors of this study hypothesize that nonword repetition is important in speech learning early in development and may be at the root of severe forms of CAS (Watkins, Gadian, & Vargha-Khadem, 1999; Liegeois, Morgan, Connelly, & Vargha-Kadem, 2011). These results, however, cannot be generalized to all cases of CAS.

Genetic Influences on CAS

Providing further support for the biological basis of CAS are discoveries of genetic differences in this population. Briefly, genetic variations are disruptions of chromosome regions that can affect the functions of an organism. For the purposes of this study, we will focus on *de novo*, or spontaneous mutations. *De novo* refers to a newly occurring genetic change that is not present in the parents. Single nucleotide variants (SNVs), or point mutations, are the most common type of genetic variation, occurring when a single nucleotide in the DNA sequence (adenine (A), thymine (T), cytosine (C), or guanine (G)) is altered. Copy number variations (CNVs) are a type of structural variant involving the alteration (deletion or duplication) of specific regions of DNA. These can either be inherited or *de novo* (Thapar & Cooper, 2013). Various methods can be employed for the discovery of these variants. Whole-genome sequencing (WGS) involves the analysis of all bases (A, T, C, G), and is the most comprehensive collection of an individual's genetic variation (Ng & Kirkness, 2010). Whole-exome sequencing (WES) is a method that examines the protein-coding portion of the genome in search for disease causing mutations.

Genetic studies of CAS and other types of SSD are beginning to emerge. Several studies have investigated SSD in multigenerational families. A groundbreaking discovery was the disruption of the *FOXP2* gene on chromosome 7 in a multi-generational family in the UK, the KE family, that caused a severe SSD, disordered language, and brain abnormalities (Lai et al., 2001; Vargha-Khadem et al., 2001). Extensive testing of affected and unaffected members of the KE family indicated the most sensitive task for determining affectation status is non-word repetition (Liegeois, Morgan, Connelly, & Vargha-Khadem, 2011). This is of particular interest in the case of motor speech disorders as non-word repetition gives a clear picture of speech production and sound sequencing independent of language abilities and semantic understanding. It is a task that requires processing on multiple levels, including auditory perception, phonemic awareness, storage in short-term memory, retrieval, motor planning and programming, and articulatory execution.

Since the discovery of the *FOXP2* gene, additional genes and regions of interest have been found in CAS and related phenotypes, including CNVs and point mutations, both inherited and *de novo* forms. The *CNTNAP2* gene, which is also located on chromosome 7, was found to be related to language impairment (Vernes et al., 2008). This gene is functionally related to the *FOXP2* gene and is also associated with reading deficits (Peter et al., 2011). Vernes et al. (2008) found that *CNTNAP2* is strongly associated with performance on nonword repetition. A syndromic form of CAS has been found in children with galactosemia, who have a mutation of the *GALT* gene, causing impairments including apraxic speech (Shriberg, Potter, & Strand, 2011). Several candidate genes, including *CDH18* and *ZGRF1,* were identified in two large

multigenerational families with severe SSD consistent with CAS (Peter, Wijsman, Matsushita, Chapman, & Raskind, 2016). Of interest is the notion that almost all of the genes identified in this study are highly expressed in the cerebellum, raising the possibility that many genes, when disrupted, interfere with cerebellar function and thus influence behaviors regulated by the cerebellum. A *de novo* heterozygous deletion of the *BCL11A* gene was discovered in a child with a severe SSD consistent with CAS, low muscle tone, and developmental delay (Peter et al., 2014). An additional candidate region linked to CAS is located on chromosome 16p11.2 (Newbury et al., 2012). These studies all provide examples of the heterogeneity of CAS, in that many genetic etiologies may be causal of the disorder.

The analysis of whole exome parent-child trios is a powerful way to detect rare causal variants underlying sporadic disorders (Steinberg, et al, 2015). The trio design allows for increased power with a small sample size as compared to other methods (i.e., genome wide association studies). In addition, the trio design is not susceptible to population stratification due to sampling of cases and controls from populations of different ancestries. Next generation sequencing has substantially reduced the time and cost required to sequence a full genome, increasing the feasibility of whole genome studies (McKenna et al., 2010). Along with this, however, come additional challenges due to significantly increased dataset size (Worthey et al., 2013). Many researchers tackle this challenge through the use of whole exome sequencing, rather than whole genome sequencing. This technique allows us to examine only protein coding regions of an individual's DNA. It is believed that protein coding regions encompass 85% of the known mutations involved in disease related traits (Choi et al. 2009). Therefore, WES is

a powerful tool for detecting pathogenic mutations. Whole exome sequencing (WES) in trios with a proband (affected child) and both unaffected parents has been used successfully to identify *de novo* mutations that were potentially causal for several neurodevelopmental disorders including autism spectrum disorder (ASD) (O'Roak et al., 2011, 2012; Iossifov et al., 2012; Neale et al., 2012), intellectual disabilities (Vissers et al., 2010), and schizophrenia (Roos et al., 2011; Fromer et al., 2014). This design has not yet been utilized for the study of CAS.

Gene-Brain-Behavior Connection

Understanding the relationship between genetic variants and corresponding brain regions has the potential for scientific insights and meaningful clinical translations. In terms of CAS, this understanding may help to explain the puzzling genetic heterogeneity observed in this disorder. Specifically, knowledge regarding the areas of the brain where candidate genes associated with CAS are expressed may lead to a deeper understanding of the neurological differences causing disordered speech. This may help us to understand the convergence of genetic effects and how this relates to the phenotype associated with CAS, via a hypothesized "many genes – focused brain region – underlying deficit" pathway (See Figure 1). The term "many genes" is used here as CAS is genetically heterogeneous, meaning a set of genes can produce similar phenotypes in different individuals. CAS is a complex disorder, as multiple variants in an individual may be causal in the given phenotype. Clinically speaking, a better understanding of the neurological correlates of CAS may lead to improved measures of therapeutic gains. It is possible that brain measures may indicate treatment effects before behavioral effects are

manifested. This is important to recognize, as it may lead to improved understanding of specific treatment techniques and greater clinical outcomes.

Understanding differences in perception in individuals with CAS can also lead to important clinical translations. As mentioned previously, it is unknown whether the vowel errors seen in CAS are a result of perceptual deficits or strictly related to decreased motor planning abilities. This knowledge will give us the ability to develop treatment methods specific to the causal deficit. Similarly, the potential underlying sequential processing deficit is important to understand, as targeting this area in treatment may help to address underlying issues across body systems (i.e., speech, gross motor, fine motor). This also highlights the importance of communication across therapy disciplines (physical therapy, occupational therapy, and speech therapy) as each discipline may be treating the same underlying deficit, in terms of the child's coordination and sequencing abilities.

In summary, increased knowledge of CAS biomarkers through behavioral testing, electrophysiological measures, and genetic analyses will lead to the early identification of infants at risk, drive the creation of earliest preventative interventions during the pre-linguistic stages, and motivate the implementation of individualized intervention programs.

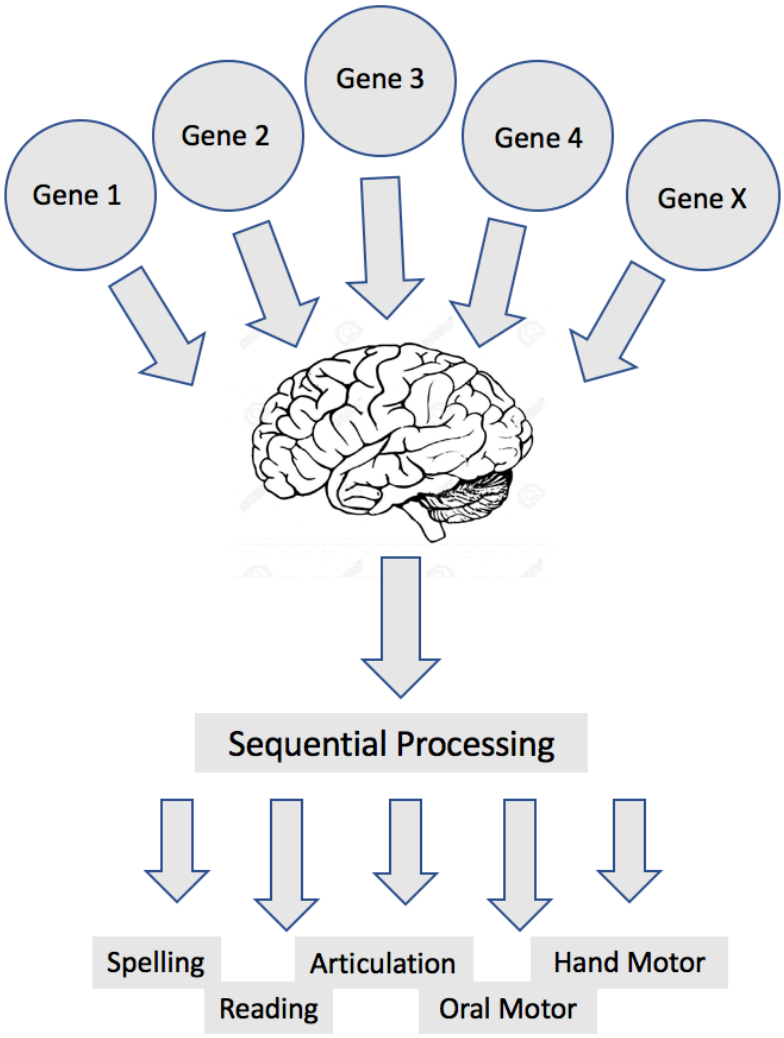

Figure 1. Gene-Brain-Behavior Connection

Research Questions

Although various types of data (behavioral, genetic, brain imaging) have been collected in children with SSD, no study to date has utilized these methods concurrently to create a comprehensive set of characteristics to depict CAS. The present study was designed to illuminate the relationship among behavioral phenotypes, genetic variations, and electrophysiological measures.

To address the current knowledge gaps, the following research questions are addressed:

1. Behavioral Measures
    a. Which of the behavioral traits associated with CAS are observable in the CAS group but not in the control group? Hypothesis: Each participant with CAS will exhibit at least 3 associated traits of CAS, and no observable traits will be present in the control group.
    b. Do the two groups (CAS and typically developing) differ in measures of sequential processing? Hypothesis: There will be a significant group difference regarding these measures.
2. EEG
    a. Do participants with CAS show altered responses to vowel stimuli as compared to typical peers? Hypothesis: As a group, the participants with CAS, but not the typical control participants, will show atypical responses to stimuli in the following conditions:
        i. Native/non-native contrast
        ii. Contrast between vowels in close acoustic proximity
3. Genetics
    a. Do *de novo* CNVs explain the CAS phenotype?
    b. Do *de novo* point mutations explain the CAS phenotype? Hypothesis: *De novo* CNVs and point mutations will explain the CAS phenotype.
    c. In males, do maternally inherited X-chromosome point mutations explain the CAS phenotype?
    d. Is there evidence of a recessive pattern of inheritance?

e. Is there evidence of compound heterozygosity?

4. What are the plausible biological associations among behavioral phenotypes, brain phenotypes, and genotypes? Hypothesis: Discovered genetic variants will be highly expressed in the brain and related to impairments demonstrated in behavioral testing.

General Methods: Participants

This study was conducted with the approval of the University of Washington's institutional review board acting on behalf of Arizona State University's institutional review board. Adults gave written consent, parents gave written permission for their minor children to participate in the study, and school age children gave written assent. Participants were recruited though referral sources including the speech and hearing clinic at Arizona State University (ASU), other local speech therapy clinics associated with ASU, and online via social media outlets. Family history interviews were conducted with at least one adult in each participating family in order to obtain background information regarding speech and language history. Parents also completed a questionnaire regarding each child's educational, developmental, and health history. Affectation status was assigned based on this background information as well as performance on tasks included in the assessment protocol. In order to control for expected developmental differences in neurological activity for the EEG component of the study, all participants were between 9 and 17 years of age. Male and female participants were both eligible to participate. All children were monolingual speakers of English, were right-handed, and had normal hearing and vision. Children with hearing loss and head injury were excluded, as observed speech impairments may be secondary to

these characteristics and not true CAS. Children with a known neurological condition or neurobehavioral disorder (such as autism) were excluded from the study.

CAS Group

This CAS group consisted of 11 children (8 male, 3 female), with an age range from 9;1 (years; months) to 17;11. All affected children had a documented history of CAS, as diagnosed by a speech-language pathologist. All children were monolingual speakers of English, were right handed, and had normal hearing and normal vision.

Control Group (TD)

The TD group consisted of 11 children (7 male, 4 female), who were age matched with the CAS group (range of 9;0 to 17;1). Parent report was utilized to confirm that all children had no speech, language, cognitive, or neurological deficits. In addition, speech samples obtained during testing were analyzed by a certified SLP to ensure that no speech or language impairments were present.

Table 1

Participant Characteristics

Variable	CAS (*n*=11)	TD (*n*=11)	Statistic	Sig. (2-tailed)
Sex Ratio (Male:Female)	0.27	0.36	$\chi^2 = .210$	.647
Age in Months	142.10 (33.39)	146.73 (29.37)	$t = .346$	.733
GFTA-3 Standard Score*	74.55 (30.11)	104.00 (1.26)	$t = 3.241$	.004
KBIT-2 Nonverbal Standard Score*	104.00 (12.95)	110.27 (9.55)	$t = 1.293$	.211

KBIT-2 Verbal Standard Score*	102.82 (12.65)	111.73 (9.41)	$t = 3.005$	.011

Notes. *Mean=100, standard deviation=15. GFTA-3=Goldman Fristoe Test of Articulation-3; KBIT-2=Kaufman Brief Intelligence Test-2.

CHAPTER 2

EXPERIMENT 1: BEHAVIORAL ASPECTS OF CHILDHOOD APRAXIA OF SPEECH AND THE SEQUENTIAL PROCESSING DEFICIT

Method

Measures

Qualitative Measures

Parent Questionnaire. Parents of children in both groups were asked to complete a questionnaire to obtain information regarding birth history, medical history, developmental history and educational history. This questionnaire also included information regarding home experiences with music and reading. See Appendix A for a copy of this questionnaire.

Parent Interview. Parents of children in the CAS group participated in an interview to obtain information regarding family history, parent's educational history, and parent's developmental history. They were also asked to describe their child's development in detail including history of babbling in infancy, onset of first words, difficulty feeding/swallowing, problems with social language/pragmatics, problems with literacy development (learning to read, spell, and write), and difficulties with fine and gross motor tasks.

Core and Associated Traits. To identify core traits of CAS that were present in CAS participants, conversational speech, complex multisyllabic words, DDK rates, and GFTA-3 speech samples were analyzed. Ten traits were selected based on Shriberg et al. (2010). Operational definitions for these traits were created following the model of Zuk, Iuzzini-Seigel, Cabbage, Green, and Hogan (2018). See Appendix C for full definitions. If a trait was observed one or more times, it was classified as present. To identify co-

occurring traits of CAS, results of parent questionnaires and parent interviews were analyzed. Co-occurring traits were adapted from the ASHA (2007) technical report on CAS. If parent report indicated the presence of a co-occurring trait at any point in the child's development, it was classified as present. See Table 1 for characteristics and results.

Speech Measures

Goldman Fristoe Test of Articulation-3. (GFTA-3; Goldman & Fristoe, 2015). The GFTA-3 is a standardized English assessment used for the assessment of speech production. Its norming sample includes children with and without SSD between the ages of 2 years, 0 months and 21 years, 11 months, with separate scores for males and females. Standard scores on the GFTA-3 are based on children's performances on the sounds-in words subtest. This subtest requires participants to complete a picture naming task, and productions of target phonemes are scored as correct or incorrect. Standard scores of 85-115 are considered in the normal range. GFTA-3 samples were also analyzed for the presence of vowel errors. This measure allowed us to detect speech sound errors at the word level and was one assessment used to confirm established traits of CAS in the CAS group.

Complex Multisyllabic Word List. A list of multisyllabic words (MSWs) (Catts, 1986) was administered using a sound file of a male adult speaker. The child was instructed to repeat the words exactly as they are presented. This word list consisted of 20 multisyllabic words with complex phoneme sequences. See Appendix B for a copy of this word list. This measure allows us to examine the accuracy of word repetition in complex word structures and was used as a confirmatory measure of CAS characteristics,

as it is well established in CAS that as word complexity increases, accuracy decreases (ASHA, 2007).

Conversational Speech Sample. A minimum of 3 minutes of conversational speech was recorded for each child. Samples were analyzed for the presence of vowel distortions, voicing errors, distorted substitutions, difficulty achieving initial articulatory configurations or transitionary movement gestures, groping, intrusive schwa, increased difficulty with multisyllabic words, syllable segregation, slow speech rate, and equal stress or lexical stress errors, as these are known characteristics of CAS (Shriberg, et al., 2010).

Language Measures

Clinical Evaluation of Language Fundamentals-5 English. (CELF-5 English; Wiig, Semel, & Secord, 2013). The CELF-5 is a standardized English assessment utilized for the detection of language disorders. Two subtests were selected from this assessment: concepts and following directions (CFD) and sentence assembly (SA). The concepts and following directions subtest requires children to follow auditory directions of increasing length and complexity, involving a variety of modifiers. Sentence assembly requires children to create grammatically correct sentences from short phrases and single words presented visually. Both tasks involve sequential processing abilities and test receptive language (CFD) and expressive language (SA). The population mean is 100, with a standard deviation of 15. Standard scores of 85-115 are considered in the normal range. According to the test manual, the CELF-5 has a high level of reliability and diagnostic sensitivity. This assessment allows us to examine differences in expressive and receptive language abilities. In the CAS group, we would expect higher receptive language as

compared to expressive language as decreased expressive language with intact receptive language is common in this population.

Comprehensive Assessment of Spoken Language. (CASL; Carrow-Woolfolk, 1999). The CASL is standardized English assessment of expressive and receptive language. This test is standardized for ages 3 years, 0 months to 21 years, 11 months. The antonym subtest requires the participant to generate antonyms from words presented verbally by a male speaker. To gain additional information about antonym generation in CAS, this test was also timed. As mentioned previously, Gebhart, Petersen and Thach (2002) found that decreased antonym generation measured by both accuracy and time was characteristic of subjects with right cerebellar lesions. Utilizing the cerebellar hypothesis, we would expect participants with CAS to show decreased skills on this task.

Written Language

Test of Word Reading Efficiency. (TOWRE; Torgesen, Wagner, & Rashotte, 2012). The Sight Word Efficiency (SWE) subtest and the Phonemic Decoding Efficiency (PDE) subtest were administered to assess word reading abilities under timed conditions. In both subtests, the participant is asked to correctly read as many words as possible in 45 seconds. Both SWE and PDE test the participant's ability to process sequential information under time constraints. These tests were utilized to create a difference score (SWE – PDE) and compared in tests of group difference. This measure was calculated in order to observe real word reading skills as compared to non-word reading skills. For example, a higher difference score indicates better real word reading abilities as compared to non-words, where as a negative score indicates better non-word reading abilities as compared to real words.

Woodcock Reading Mastery Tests – Revised. (WRMT-R; Woodcock, McGrew, & Mather, 2001). The Word Attack (WATT) and Word Identification (WID) subtests were selected as measures of word decoding and sight word reading. The WATT subtest includes non-words that follow standard English orthography and must be sounded out in a sequential manner. The WID subtest includes words that follow standard English orthography as well as words that do not. These tests were utilized to create a difference score (WID – WATT) and compared in tests of group difference. Similar to the difference score calculated for the TOWRE, this measure was calculated in order to observe real word reading skills as compared to non-word reading skills.

Wechsler Individual Achievement Test – III. (WIAT-III; Wechsler, 2009). Participants completed the Spelling (SP) subtest from the WIAT-III. In this subtest, participants are given a word, presented verbally, as well as the word used in the context of a sentence, and finally the word repeated once more. This subtest includes words that follow standard English orthography as well as words that do not follow standard English orthography. This test was used as a confirmatory measure, as children with CAS are expected to show decreased spelling abilities. This test also requires high loads of sequential processing, as the words presented must be stored in long-term memory, retrieved, and converted into written sequences of letters.

Phonological Awareness

Comprehensive Test of Phonological Processing. (CTOPP; Wagner, Torgesen, & Rashotte, 1999). The Non-word Repetition (NWR) subtest of the CTOPP was administered using the sound files provided by the manufacturer. This subtest consists of 18 items of increasing complexity presented auditorily. Participants are instructed to

repeat each non-word exactly as it is presented. This test was used as a confirmatory measure as it is well established that children with CAS have difficulty with nonword repetition tasks (Catts, 1986, Shriberg, Lohmeier, Strand, & Jakielski, 2012). We recently showed that adults with a probable history of CAS have persisting difficulties with this task as well (Peter, Lancaster, Vose, Middleton, & Stoel-Gammon, 2017). This task also requires sequential processing in the encoding, storing, retrieving, and motor execution of complex phoneme sequences.

Motor Measures

Keyboard tapping task. Two computer key tapping tasks were used as a measure of manual fine motor skills following the published protocols by Gualtieri and Johnson (2006). A computer program custom-designed with LabVIEW software (National Instruments, Austin, TX) was used to record tapping intervals. The first task administered was repetitive tapping. During this task, the participants were instructed to look at the screen and focus on a large gray circle. They were instructed to start tapping on the spacebar as fast as possible as soon as the gray button on the screen turned bright green to the moment it returned to the gray color, which spanned ten seconds. The onset of the start cue button was randomized between a 2 and 4 second delay, so the children could not anticipate the start of the task. The second task administered, alternating tapping, required the participants to use their index and middle fingers to alternate between tapping on the left and right arrow keys as fast as possible after the gray start cue button turned bright green to the moment it returned to the gray color, 10 seconds in duration.

The experimenter demonstrated one trial for each condition prior to the child initiating the activity. A total of 20 trials were administered for each child, 10 for the

repetitive condition and 10 for the alternating condition. Trials alternated between hands, beginning on the right hand and then switched to the left hand, for a total of 5 trials in each hand. If the child began to press keys other than the spacebar or arrow buttons, the trial was discarded and the trial was repeated.

The LabVIEW program outputs text files from which inter-tap durations in milliseconds were extracted. Outlier inter-tap interval values of greater than 3 deviations in either direction from the mean per participant and task were excluded to control for anomalies in the data or pauses due to external circumstances. The average time in milliseconds between tapping (inter-tap) intervals was calculated for each task and hand. The mean inter-tap interval in milliseconds for each task was recalculated after exclusion of the outliers and used as independent variables in the subsequent analyses.

Diadochokinetic (DDK) rates. This assessment is used to measure speed and regularity of oral movement of articulators (Fletcher, 1972). Slow and imprecise DDK performance is an established core trait of CAS. Conditions included monosyllables (/pa/, /ta/, /ka/), disyllables (/pata/, /taka/), and multi-syllables (/pataka/). Productions of /pa/, /ta/, and /ka/ can be thought of as the repetitive DDK task, and the production of /pata/ and /taka/ as the alternating DKK task. This task requires sequential processing in the motor planning and execution of simple and complex syllable sequences. The /pataka/ task involves an additional challenge in terms of maintaining the more complex pattern. Children with CAS have even greater difficulty with this task, compared to the disyllabic and monosyllabic condition (Rvachew & Matthews, 2017). Following the methods established by Fletcher (1972), participants were instructed to produce each of the conditions as fast as possible. The goal was to obtain 20 repetitions of each of the

monosyllables and 15 repetitions of each of the disyllables to calculate average syllable duration through the use of PRAAT software (version 6.0.26; Boersma and Weenink, 2016). Outlier values of greater than 3 standard deviations from the mean in either direction were excluded from further analysis to control for anomalies in the data or pauses due to external circumstances. Average syllable duration (low numbers indicate rapid syllable repetition rates) was used as the variable of interest in all analyses. Norms for monosyllabic and disyllabic repetitions are available for 6-13 years (Fletcher, 1972). Norms for 13-year olds were used for participants age 14-17 due to the unavailability of norms for this age range. It is possible that these norms underestimate oral motor speeds based on age (Peter, Matsushita, & Raskind, 2011). *Z* scores were calculated individually for monosyllabic and disyllabic measures. In order to better observe motor sequencing abilities, the averaged *z* score from the disyllabic durations was subtracted from the averaged *z* score from monosyllabic durations. If the result was positive (monosyllabic rates were faster than disyllabic rates), it was interpreted as a deficit in motor sequencing. Additionally, the averaged *z* score for the multisyllabic (/pataka/) was subtracted from the *z* score for the disyllabic condition (/pata/, /taka/). This is believed to be a measure of overall executive planning.

Bruininks-Oseretsky Test of Motor Proficiency-2, Short Form. (BOT-2; Bruininks & Bruininks, 2005). The BOT-2 Short Form is a screening tool for overall motor proficiency. This test includes tasks of fine motor precision (e.g., tracing a line), fine motor integration (e.g., copying a star, copying circles), manual dexterity (e.g., stringing blocks), bilateral coordination (e.g., alternating tapping finger to nose with eyes closed), balance (e.g., walking forward on a line), agility (e.g., one legged side hop),

upper limb coordination (e.g., catching a tossed ball), and strength (e.g., push-ups). Point scores from all subtests are combined to calculate a standard score. Standard scores between 85-115 are considered within normal ranges. This assessment was utilized to confirm the presence of decreased fine and gross motor skills in the CAS group as compared to the TD group, as this is a commonly reported co-occurring trait of CAS.

Cognition

Kaufman Brief Intelligence Test-2. (KBIT-2; Kaufman & Kaufman, 2004). The KBIT-2 is a standardized screening test that measures verbal and nonverbal intelligence. The KBIT-2 includes a crystallized (verbal) scale with verbal knowledge and riddles subtests as well as a fluid (nonverbal) scale with a matrices subtest. Scores between 85-115 are considered within normal ranges. According to the test manual, the KBIT-2 has high reliability and validity. This measure was utilized to confirm average intelligence in both groups as well as determine differences in verbal versus nonverbal intelligence.

Statistical Analyses and Reliability

Measures were classified as confirmatory or experimental. Confirmatory refers to measures that account for a known deficit in CAS, including the CTOPP (NWR), DDK multisyllable, CELF-5, SWE, PDE, WID, WATT, and spelling. The CELF-5 was utilized to observe discrepancies between expressive and receptive language measures. For this purpose, a difference score was calculated for each participant by subtracting the expressive language measure (SA) from the receptive language measure (FD). To account for literacy measures, a literacy score was calculated. This was the average of standardized scores for SWE and PDE, WID, WATT, and SP subtests. A difference score was also calculated for the KBIT-2 to observe differences in non-verbal and verbal IQ.

This was calculated by subtracting the standard score for the verbal subtest from the nonverbal subtest. Experimental refers to measures that are less documented in the CAS literature. These are measures we believe to capture sequential processing abilities. To assess sequential processing in reading, difference scores were calculated for each of the reading measures (SWE-PDE, WID-WATT). Experimental measures also included the CASL antonym task and BOT-2. To examine motor sequencing, difference scores were calculated utilizing *z* scores for DDK mono- and multisyllabic tasks. The mulstisyllabic DDK *z* score was subtracted from the disyllabic DDK *z* score. This difference score provides a measure of motor sequencing abilities. A negative score indicates higher *z* scores in general for the multisyllabic condition as compared to the disyllabic condition Keyboard tapping was analyzed utilizing average duration scores (in ms) for the alternating and repetitive tasks (repetitive was subtracted from alternating). Five total measurements were included in the confirmatory analyses (KBIT-2 difference score, CELF-5 difference score, Literacy score, CTOPP NWR, and DDK monosyllabic (/pa/, /ta/, /ka/)– disyllabic (/pata/, /taka/)). Six measurements were included in the experimental analyses (SWE-PDE difference score, WID-WATT difference score, Antonym standard score, BOT-2 standard score, DDK disyllabic (/pata/, /taka/) – DDK multisyllabic (/pataka/), and Keyboard alternating – repetitive (ms)). All measures were analyzed using two-tailed *t* tests to test for group differences. To evaluate the association between sequencing ability during linguistic, hand motor and oral motor tasks, a Pearson correlation coefficient was computed for experimental measures, as these are believed to best capture sequential processing abilities. An experimental measure believed to capture sequential literacy skills was calculated, the Sequential Literacy Score, and used in

correlation analysis. This score is an average of WATT, PDE, and spelling scores as each of these tasks requires sequential processing in the form of serial decoding, as compared to whole word identification.

Statistical significance was determined at $\alpha = 0.05$. Bonferroni adjustments for multiple testing were calculated for confirmatory measures and experimental measures separately. With five comparisons planned in the confirmatory analyses, the Bonferroni-corrected $\alpha = 0.01$. With six comparisons planned in the experimental analyses investigating sequential processing, the Bonferroni-corrected $\alpha = 0.0083$. For correlational analyses, the Bonferroni-corrected $\alpha = 0.0018$. Note that the measures capture related concepts and the independence assumption underlying the Bonferroni correction may not be fulfilled; hence, the Bonferroni correction is excessively conservative.

The author collected all data and completed the initial data reduction and standard analysis. Approximately 15% of data were checked for reliability by an undergraduate student in the Department of Speech and Hearing Science at Arizona State University who was thoroughly trained in the scoring of each measure. Any discrepancies in raw score points were resolved by consensus.

Results

Participant Demographics

Table 1 provides data describing participant groups. Tests of group difference found no significant differences between groups in age, sex, or KBIT-2 non-verbal standard score. As expected, groups were significantly different in articulation as measured by the

GFTA-3 and KBIT-2 verbal standard score. Individual scores can be found in Appendix B.

Qualitative Data

Table 2. *Core and Co-occurring traits of CAS*

Category	CAS Characteristic	C1	C2	C3	C4	C5	C6	C7	C8	C9	C10	C11
Core	Vowel distortions	x			x		x	x				
	Voicing errors	x	x		x							
	Distorted substitutions	x										
	Difficulty achieving initial articulatory configurations or transitionary movement gestures	x			x							
	Groping	x	x		x	x	x			x		x
	Intrusive schwa	x					x	x				
	Increased difficulty with multisyllabic words	x	x	x	x	x	x	x		x	x	x
	Syllable segregation	x	x				x					
	Slow speech rate and/or slow diadochokinetic rates	x	x	x	x	x	x			x	x	x
	Equal stress or lexical stress errors	x	x	x	x	x	x	x	x			x
Total Core Traits		**10**	**6**	**3**	**7**	**4**	**7**	**4**	**1**	**3**	**2**	**4**
Associated	Expressive Language Problems	x		x	x	x	x					x
	Delayed Language Development	x	x	x	x	x	x	x	x	x	x	x
	Problems with Literacy	x	x		x	x	x					x
	Pragmatic Problems	x										
	Gross/Fine Motor Delays		x	x	x		x		x		x	x
	Feeding Difficulties						x	x				x
	Sensory Processing Deficits	x		x	x		x		x		x	x
Total Associated Traits		**5**	**3**	**4**	**5**	**3**	**6**	**2**	**3**	**1**	**3**	**6**

Overall, CAS01 has the highest number of observable CAS core traits. He also presents with multiple associated traits of CAS. CAS06 presents with the most associated CAS traits overall. In general, the younger children (CAS01, CAS02, CAS04 and CAS06) who are ages 9;1 – 9;5 were also found to present with more CAS core traits in comparison

to the older children. In terms of associated traits, delayed language development was the most common, and was present in all CAS participants. Gross/fine motor delays and sensory processing deficits were the next most common secondary characteristics. Only one CAS participant presented with pragmatic problems. No associated traits were observed in TD participants, other than minor difficulty on the MSW list, which is judged to be typical in nature.

Confirmatory Measures

Between-group comparison for the KBIT-2 difference score was trending toward significance but did not meet the Bonferroni corrected alpha level. The negative mean KBIT-2 Difference Score in the TD group indicates overall higher verbal standard scores in the TD participants and higher non-verbal standard scores in the CAS participants. Overall Comparison of Literacy Score between groups was significant, indicating lower literacy skills in the CAS group as compared to the TD group, with the CAS group achieving lower overall scores in areas of reading and spelling. The effect size for this comparison (d = 1.13) was found to exceed Cohens (1988) convention for a large effect (d = .80). CTOPP NWR was also significantly different between groups, with CAS participants receiving lower standard scores as compared to TD participants, and a large effect size of d = 1.33. The CELF-5 Difference Score was not significant between groups. Both the CAS and TD groups demonstrated a negative overall mean, indicating lower receptive language scores as compared to expressive language scores. DDK Multisyllabic *z* scores were highly significant between groups. The CAS group performed slower on average in the multisyllabic condition, as compared to the TD group. See table 4 for between group comparisons for confirmatory measures.

Table 2

T tests Comparing Confirmatory Measures

Variable	Mean CAS	SD	Mean TD	SD	*t*	*p* value
KBIT-2 Diff. Score	5.18	9.14	-1.46	3.96	2.21	.039
Literacy Score	89.80	17.66	109.56	10.68	3.18	.005
CTOPP NWR	6.36	1.63	9.64	2.06	4.13	.001
CELF-5 Diff. Score	-5.46	10.11	-3.18	9.56	0.54	.594
DDK Multisyllables	-3.56	2.20	0.62	0.94	5.81	.001

Note. KBIT-2 Diff Score = Nonverbal subtest – Verbal subtests. CELF-5 Diff Score = SA subtest – FD subtest.

Experimental Measures

Mean scores for SWE – PDE indicate better performance on phonemic decoding (PDE) in the TD group, as a negative score indicates a higher phonemic decoding standard score as compared to sight word reading (SWE). Children in the CAS group, however, performed better on average in the task of sight word efficiency, as the mean score was positive. Comparison between groups was not significant. WID – WATT Difference score was not significant between groups. Both groups had positive difference scores, indicating higher performance in real word reading (WID) versus non-word reading (WATT). Comparison between groups for antonym scores is trending toward significant, with the TD group performing substantially better than the CAS group, and an effect size of d = 1.02. Scores on the BOT-2 reveal higher mean average in the TD group as compared to the CAS group, indicating greater performance on gross and fine

motor tasks. The comparison between groups was significant. Effect size for this comparison was large (d = 1.22). Disyllabic DDK subtracted from monosyllabic DDK difference scores were not significant between groups, with lower difference scores for the TD group, on average, as compared to the CAS group, indicating a smaller difference between *z* scores for the disyllabic and monosyllabic conditions in the TD group, and higher *z* scores in general for the monosyllabic condition as compared to the disyllabic condition in the CAS group. Comparison of repetitive keyboard tapping averages subtracted from alternating averages (measured by inter-tap interval) was trending toward significant between groups, with longer inter-tap intervals in general for the CAS group as compared to the TD group. A large effect size was found for this comparison (d = -.95). Means and standard deviations for all motor tasks can be found in Table 6.

Table 3

T tests Comparing Experimental Measures

Variable	Mean CAS	SD	Mean TD	SD	*t*	*p* value (2-tailed)
SWE - PDE Diff. *	6.46	14.88	-1.09	10.28	-1.38	.182
WID - WATT Diff.*	5.73	11.72	4.09	11.27	-.33	.742
CASL Antonyms*	93.36	23.56	117.64	17.73	2.73	.013
CASL Duration*****	294.82	96.11	191.09	49.03	-3.19	.005
BOT-2**	47.73	11.42	60.73	3.98	3.57	.002
DDK monosyllabic – disyllabic***	1.14	0.97	0.65	.82	-1.28	.216
Keyboard alt – rep ****	244.83	206.77	86.54	46.21	-2.48	.022

* Population mean = 100, SD = 15; **Population mean = 50, standard deviation = 9; ***Population mean = 0, SD = 1; ****Reported in inter-tap intervals (no standardized scores available) *****Reported in seconds (no standardized scores available).

Table 4

Summary of motor results and t tests comparing CAS and TD

	/pa/ Mean (SD)	/pata/ Mean (SD)	/pataka/ Mean (SD)	Repetitive tapping Mean (SD)	Alternating Tapping Mean (SD)
TD	171.87 (34.27)	171.69 (22.34)	186.58 (49.61)	178.70 (24.61)	265.24 (35.48)
CAS	254.85 (96.05)	287.99 (90.12)	390.56 (255.29)	212.27 (61.62)	457.10 (243.40)

Note. Results are reported in milliseconds. For oral motor tasks, this represents the average syllable duration. For the tapping tasks, this represents the average inter-tap duration.

Significant and positive correlations were present among BOT-2 standard scores (measure of gross and fine motor skills), and DDK monosyllable, disyllable, and multisyllable. A significant negative correlation was found between BOT-2 scores and keyboard tasks (both repetitive and alternating). Correlation between BOT-2 scores and antonym time was trending toward significance, indicating more time needed on the antonym task is related to lower scores on the BOT-2. A significant and positive correlation was present among antonym standard score and DDK multisyllable, indicating higher antonym scores are related to faster DDK multisyllable production.

DDK monosyllable was significantly and positively correlated with DDK di- and multisyllable. Alternating and repetitive keyboard tasks were positively correlated with each other, indicating increased repetitive tapping speed is associated with increased alternating tapping speed.

Table 5

Correlations and Significance Level for Experimental Tasks

	Seq. Literacy	Ant. SS	Ant. Time (secs)	BOT-2 SS	DDK Mono	DDK Di	DDK Multi	KB Rep.	KBAlt.
Seq. Literacy	1								
Ant. SS	.577*	1							
Ant. - Time (secs)	-.527*	-.521*	1						
BOT-2 SS	.786**	.568*	-.579*	1					
DDK Monos	.684**	.551**	-.737**	.692**	1				
DDK Di	.676**	.515*	-.784**	.636**	.867**	1			
DDK Multi	-.761**	.690**	-.766**	.797**	.815**	.854**	1		
KB Rep.	-.509*	-.152	.297	-.723**	-.515*	-.471*	-.459*	1	
KB Alt.	-.589*	-.233	.319	-.644**	-.633*	-.563*	-.529*	.834**	1

Note: *significant at p<.05; **significant at p<.0018

Discussion

The purpose of this study was to closely examine the behavioral phenotype associated with CAS, with a specific focus on measures of sequential processing. This was accomplished by comparing the results of behavioral assessments of 11 children with CAS compared to 11 age-matched typically developing peers. Overall, results indicate that children with CAS perform poorly on measures of sequential processing as compared to typical children. In addition, several interesting findings were made in the core and associated traits observed and reported in children with CAS.

In terms of qualitative data, including core and associated traits of CAS, several observations were made. CAS01 demonstrated all ten core traits of CAS, indicating the most severely impacted phenotype. This child did not, however, demonstrate the most associated traits in the CAS group, as no gross/fine motor delays or feeding difficulties were reported. Individual review of this child's BOT-2 scores did reveal below average performance on gross and fine motor measurements. It is possible that this was never discovered over the course of the child's intervention and, therefore, was never brought to the attention of the parents. Although the BOT-2 is a screening tool, given the child's substantially decreased scores, it is surprising that there is not reported history of fine and gross motor delays.

In general, the younger children, ages 9;0 to 9;11 demonstrated substantially more core traits of CAS as compared to the older children. This is to be expected, given the nature of the CAS phenotype to change as a function of age and treatment (McCauley & Strand, 2008). The TD group did not demonstrate any associated traits of CAS at any age, other than minor errors on MSWs, which is to be expected given the complexity of the words included in the task. The most prominent core traits observed overall were increased difficulty with multisyllabic words, slow speech rate/slow DDK, and stress errors. These traits were observed in both younger and older children in the CAS group demonstrating the persisting nature of these deficits. These results are not consistent with the hypothesis that each participant with CAS will exhibit at least 3 associated traits of CAS. Two children in the CAS group, CAS05 and CAS09 demonstrated only one to two associated traits of CAS. It is, however, possible that some of these associated traits were present at some point in the child's development (i.e., sensory processing deficits), and

not properly diagnosed and were therefore not reported by the child's parents during the assessment process.

Overall, confirmatory measures were well aligned with expected differences in CAS and TD. The KBIT-2 difference scores indicated better overall performance on measures of non-verbal IQ as compared to verbal IQ in the CAS group. The comparison between groups did not meet significance level, however. This may be due to the age range of the sample, as a larger difference between verbal and nonverbal IQ may exist in children with CAS at earlier stages of development. It is possible that children with CAS may improve their verbal IQ as they develop better language-based skills, and therefore this sample did not capture the true discrepancy between verbal and nonverbal IQ in CAS and TD due to the large age range of participants.

As expected, the overall literacy measure was highly significant between groups, consistent with previous evidence for decreased literacy skills in CAS (ASHA, 2007; Gillon & Moriarty, 2007; Lewis, Freebairn, Hansen, Iyengar, & Taylor, 2004). This provides important evidence for the need to target literacy issues as early as possible in a child's development in the treatment of CAS. Providing support in the area of literacy development even before the child demonstrates measurable signs of literacy deficits may be appropriate for children diagnosed with CAS, as it is clear that these children often demonstrate substantial and persisting issues in this area. In the experimental measures of reading abilities, SWE – PDE difference scores and WID – WATT difference scores show better real word reading than non-word reading in the CAS group, although neither comparison was found to be statistically significant between groups. Overall mean scores for the CAS group indicate overall decreased ability in all reading tasks as compared to

the TD group (SWE, PDE, WID, WATT). Although individual error profiles were not completed for these tasks, decreased scores in the CAS group on measures of non-word reading may indicate sequencing errors during the encoding stage of visual information as found by Peter, Lancaster, Vose, Middleton, and Stoel-Gammon (2017). Non-word decoding requires the execution of the highly sequential processes of encoding, and storing strings of graphemes in working memory, then converting them into strings of phonemes. This differs from real word reading, in which a word is recognized as a whole. This finding is consistent with the idea that individuals with CAS have sequencing deficits during the encoding and storing the graphemes in working memory. This is similar to the encoding errors found in children and young adults with CAS during non-word repetition (Shriberg et al., 2012). Providing further evidence for the sequential processing deficits in the CAS group is the experimental measure of Sequential Literacy Score. A significant and positive relationship was found between the Sequential Literacy Score and BOT-2 scores, indicating higher scores on the BOT-2 are related to higher scores in sequential measures of reading (PDE, WATT) and spelling. As mentioned previously, the PDE and WATT tasks involve the execution of several highly sequential processes. Similarly, spelling requires high loads of sequential processing, as the words presented must be stored in long-term memory, retrieved, and converted into written sequences of letters. The BOT-2 involves several tasks that require motor sequencing, such as stringing beads on a string and pivoting thumbs and fingers. Additional measures include balance, fine motor integration, upper limb coordination and strength. As this measure does not provide individual standardized scores for each subtest, conclusions regarding sequential processing should be made cautiously. However, experimental

between group comparisons and correlational analyses may indicate a sequential processing deficit may be an underlying factor in the decreased sequential literacy and fine and gross motor issues observed in CAS.

Examination of NWR scores indicates low skills in the CAS group, whereas the TD group demonstrated age appropriate skills. These results are also consistent with prior research showing decreased NWR skills in children and adults with CAS (i.e., (Button, Peter, Stoel-Gammon, & Raskind, 2013; Peter, Lancaster, Vose, Middleton, & Stoel-Gammon, 2017; Peter, Button, Stoel-Gammon, Chapman, & Raskind, 2013; Shriberg, Lohmeier, Strand, & Jakielski, 2012). Non-word repetition is a task that allows us to observe speech production and sound sequencing independent of language abilities. This task requires the individual to store the stimulus in working memory prior to the assembly and execution of motor programs. As such, it is expected that individuals with CAS would perform poorly.

In terms of oral motor and hand motor skills, overall the TD group performed better than the CAS group, as expected. The confirmatory measure of DDK multisyllabic (/pataka/) was significant between groups, showing decreased oral motor planning skills in the CAS group. This is expected as the accurate production of three syllable types, /pa/, /ta/, and /ka/ in a sequential and rapid manner requires intact and efficient motor planning abilities. The experimental difference measure of disyllabic *z* score subtracted from monosyllabic *z* score was positive for both the CAS and TD groups, although the CAS group had a higher difference score, on average, indicating better performance in the monosyllabic DDK condition as compared to the disyllabic DDK condition, as expected. This comparison between groups was not significant, however. The keyboard

difference measure revealed a higher difference score for the CAS group, indicating longer inter-tap intervals for the alternating task as compared to repetitive. This is consistent with previous findings of decreased alternating versus repetitive hand movements in CAS (Peter & Raskind, 2011).

The antonym task was an experimental measure to examine potential sequential processing issues in the form of word generation. In line with the cerebellar hypothesis of sequential processing deficit in CAS, we would expect this task to be more difficult for participants in the CAS group as compared to the TD group, as antonym generation has been found to be decreased in patients with cerebellar lesions (Gebhart, Petersen, & Thach, 2002). This hypothesis was confirmed, as a significant between-group difference was found in both time and standard score on the antonym generation task. Correlation of antonym standard score and time required to complete the antonym task was another interesting finding. Participants with CAS do not show higher accuracy with longer duration, but instead show both decreased accuracy and longer duration in comparison to typical peers. This suggests that they are not using additional time as a compensatory strategy to achieve higher accuracy in this task. It should be noted that four participants with CAS demonstrated expressive language deficits. Because of this, antonym generation may have been impacted, both in standard score and reaction time. However, review of individual scores indicates participants with intact expressive language skills and CAS also demonstrate below average antonym generation and slower time of completion as compared to typical participants, indicating impaired antonym generation in the presence of age appropriate expressive language. Antonym measures were also found to be correlated with BOT-2 scores and the measure of sequential literacy. These

correlations provide further evidence for an underlying sequential processing hypothesis, as all measures are believed to involve sequential processing skills.

A role for the cerebellum in the sequencing of incoming patterns and outgoing responses has been suggested (Braitenberg et al., 1997; Ivry, 1997; Mauk et al., 2000). This has been investigated across modalities, including sensory (Bower, 1997), motor (Thach et al., 1992), and behavioral (Leggio et al., 2008). In terms of the antonym task specifically, this task requires multiple aspects of sequential processing including auditory encoding and processing of words presented, recalling an antonymous word from memory, and transcoding the spoken response. The comparison of standard scores between groups was trending towards significance, and the difference in duration between groups was highly significant. providing further support for the cerebellar hypothesis in sequential processing and CAS and are in line with previous evidence demonstrating cerebellar involvement in antonym generation tasks.

Overall, these findings as a whole suggest a global sequential processing deficit apparent across tasks in CAS. They also support a model of CAS in which deficits exist in sequential processing across domains, as evidenced by speech, language, reading, spelling, fine/gross motor, oral motor, and hand motor tasks. CAS is a multifaceted and complex disorder involving a range of characteristics that change as a function of age, although many of these deficits persist after speech production skills have been resolved.

Clinical Implications

The errors observed in older children with CAS, including increased difficulty with multisyllabic words, slow speech rate and/or slow DDK, and stress errors may provide useful clinical guidance for SLPs working with children in the later stages of CAS

treatment. Therapy goals and treatment methods must be tailored specifically to the child's current level of functioning, with special attention to the changing nature of CAS over time. As speech sound errors begin to resolve, and some of the core features observed in the younger children in the CAS group begin to resolve (i.e., vowel errors, intrusive schwa), new therapy goals targeting the traits typically observed in older children with CAS should be developed. These include targeting appropriate stress and intonation and strategies to increase accurate production of complex words and sentences. In this way, these areas of difficulty can be addressed prior to the child being discharged from speech therapy.

The overall observation that children with CAS demonstrate sequential processing deficits across tasks provides insight to a potentially causal underlying deficit that needs to be addressed early in treatment and by all disciplines of professionals working with the child (SLP, occupational therapist, physical therapist, music therapist, etc.). A team approach is crucial in the treatment of CAS as it is typically a multi-deficit and complex disorder. Communication between specialists should be a priority, as each discipline may be working on the same underlying deficit. It is possible that various techniques may be used across disciplines to provide the most effective and efficient therapy possible. The findings in the area of potential cerebellar dysfunction is also important to consider in treatment planning for CAS. Targeting fine and gross motor tasks related to cerebellar control in physical and occupational therapy may translate to improved skills in the area of speech and language.

Incidental Findings

Review of individual articulation patterns in CAS participants lead to several incidental findings. Aside from CAS01 and CAS04, who demonstrated several speech sound error patterns, all other residual errors observed in children with CAS were during the production of prevocalic /r/ and rhotic vowels. The persisting nature of /r/ distortions suggests a high level of motor planning and programming necessary for accurate production. This is a pattern commonly observed in typical speech development, but at much younger ages, and in children with articulation disorders. It is possible that as children with CAS develop, and participate in intensive therapy, they go through a series of stages. It appears from this sample, that children with CAS initially look disordered, with highly atypical speech patterns. As they mature in their speech sound development they present with residual articulatory errors (i.e., /r/ distortion) and demonstrate characteristics similar to a child with an articulation disorder, rather than CAS. These patterns may contribute to the difficulty in distinguishing CAS from other speech sound disorders, particularly when CAS is not diagnosed in the early stages of speech development. Viewing CAS in this way may help us to form a new way of looking at diagnosis and treatment. Rather than approaching CAS as a disorder with a single set of characteristics, it can be thought of as a spectrum. An example of this type of spectrum is our current view of autism spectrum disorder (ASD). A child diagnosed with ASD will present with her or his own unique set of characteristics related to the disorder. In this way, we can view each child with CAS as having their own unique set of characteristics, which will change as a function of age and duration of treatment. As demonstrated in this sample, older children with CAS demonstrate more consistent error patterns than those who are younger. It is important to consider these changes in the progression of CAS in

the evaluation process and throughout the course of treatment as goals and treatment methods need to be specific to each individual child and his or her current level of functioning.

Limitations and Future Directions

The age range of participants in this study is important to note, as it creates a wide range of observable traits and variety of performance on standardized and non-standardized measures. It is possible that this study does not capture the severity of traits that may be present in CAS both in speech and language measures and measures across modalities. It is important to recognize that features of CAS change over time, and the variation between a 9-year-old child with CAS and 17-year-old child with CAS is substantial. Examining a narrower age range may allow us to better capture the CAS phenotype. Similarly, examining CAS with language impairment and without language impairment may provide more insight into the true underlying deficits of the disorder. Children with CAS have been shown to differ in studies of auditory perception when compared between children with and without language impairment (Zuk, Iuzzini-Seigel, Cabbage, Green, & Hogan). Examining measures of sequential processing in this way may lead to interesting findings.

In the antonym generation task, we did not include a comparison measure, such as synonym generation. In the future, examining the antonym task in comparison to synonym generation would be useful, in order to determine if the deficits relate solely to antonyms or if they are present across tasks. Due to the extensive testing completed during assessment sessions, additional measures could not be completed, but should be considered in the future.

Our findings in the area of gross and fine motor deficits in CAS and the relationship of these skills to measures of sequential processing must be interpreted with caution. The assessment tool used, the BOT-2, is a screening measure, and does not provide individual scores for subtests. As some of these subtests such as alternating motor movements and stringing beads involve more sequential processing, it may be more meaningful to examine individual tasks, rather than a composite standard score. In addition, the balance task may provide useful information to contribute underlying cerebellar deficits. Utilizing more extensive measures of gross and fine motor skills in future studies may lead to interesting clinical translations.

CHAPTER 3

EXPERIMENT 2: PERCEPTION OR PRODUCTION? AN EEG STUDY OF VOWELS IN CAS

Methods

Participants

See methods section of Experiment 1 for participant information. One child from the CAS group was unable to complete EEG testing (CAS04), and one child's data was removed from analysis due to movement artifact (CAS01), leaving a total of 9 children in the CAS group and 11 in the TD group.

Stimuli

Three sets of auditory stimuli were generated, consisting of vowel contrasts. The first contrast to be presented was between acoustically adjacent, phonemically discrete phonemes, /ɛ/ and /I/, with /ɛ/ being the standard and /I/ as the oddball (close proximity contrast; CP). The second contrast included a native sound and a non-native sound, with /u/ as the standard and /y/ as the oddball (native/non-native contrast; N/NN). All vowels were recorded by a female native speaker of English using a Zoom recording device (Handy Recorder H2). The recordings were edited using Praat sound analysis software (Boersma, 2001) to ensure the same duration for all sounds (500 ms) and loudness levels (75dB). All contrasts were presented using E-Prime 3.0 software (Psychology Software Tools, Pittsburgh, PA).

EEG Recording and Experimental Procedure

While the stimuli were presented, EEG measures were recorded using a 128-electrode Electrical Geodesics, Inc., EEG system (Electrical Geodesics; Tucker, 1993). Children were seated comfortably in a sound-attenuated booth in front of a monitor

displaying a fixation cross with an approximate eyes-to-screen distance of 50 cm. Auditory stimuli were presented through insert earphones (Etymotic Research, ER-1). While stimuli were presented, EEG was recorded with sensor impedances maintained below 40 kΩ, sampling at 1000 Hz via a high-input impedance amplifier. Stimuli were trains of vowel standards in each condition (80%) with semi-randomly interspersed deviant sound (20%), for a total of 250 presentations per condition, with a 200 ms inter-stimulus interval. This ratio of standard to deviant was maintained throughout each 10-stimuli block. Measures were taken to prevent artifacts at the time of recording, including proper net placement, identifying external sources of noise (i.e., air conditioners, electrical output in the room), and regularly checking for good impedance.

EEG Pre-processing

Data preprocessing was completed using Net Station Review version 5.4.1.1 and Net Station Tools version 5.4.1.1 The recorded EEG signal was filtered offline using a 30-Hz low-pass filter and a high-pass filter set to 0.1 Hz.

The recording was segmented into 800ms epochs (100 ms pre-stimulus, and 700ms post-stimulus). These epochs were time locked to the stimulus. Each epoch was examined for evidence of artifact, including eye blinks, head movement, and bad channels. The data was first visually inspected to remove bad channels and artifacts manually. Automatic artifact rejection was then completed, marking bad channels as channels having deviations of 200 μV or greater. Eye blinks and eye movements were measured as deviations between pairs of channels (channel 8/26 and channel 25/127). If a channel was determined bad for more than 40% of the recording, it was removed from analysis. Missing or lost channels were interpolated with spherical-spline interpolation,

using a weighted average of surrounding trials, while adjusting the average so that the total voltage over the head remains neutral. Following artifact rejection, one subject (CAS01) was removed from further analyses secondary to lack of usable data due to movement artifact.

Data was re-referenced to average mastoids. The signals were then baseline corrected. The baseline that was used is the measurement of neural activity prior to stimulus onset (i.e., first 100-200ms before the stimulus is presented). This level of activity is the baseline to which all other data points were compared. The data points across these baseline periods were averaged and subtracted from the rest of the ERP signal. Data was then montaged to a group of sensors over frontal regions, which is the expected region for an MMN response, following the montage used by Froud and Khamis-Dakwar (2012) (see Figure 1).

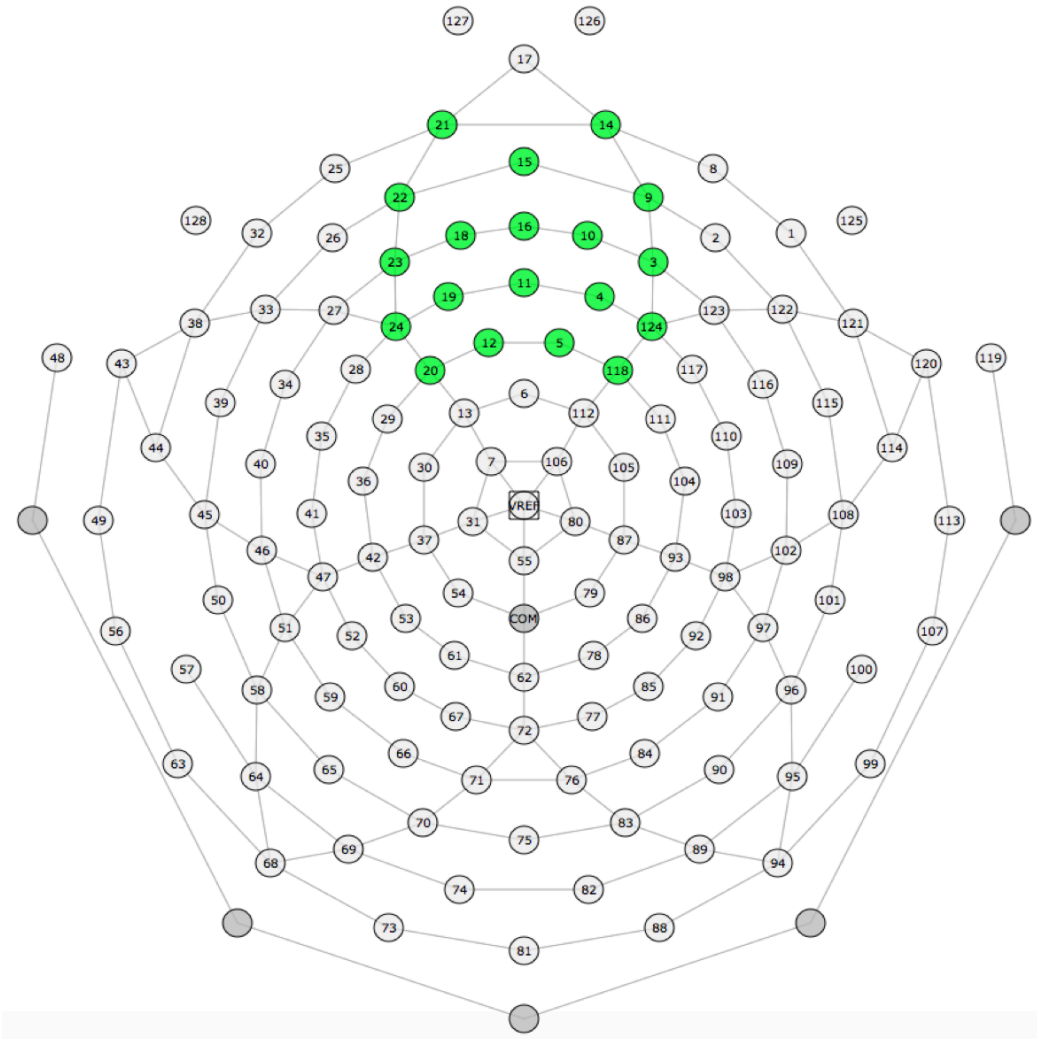

Figure 3. MMN Electrode Montage

Next, the data was averaged across segments separately for each participant, and for each stimulus type (i.e., standard and oddball). Latency variability is an important issue to consider, as changes in latency across trials can make it difficult to detect a neural response in a given waveform. To counteract this problem, an area measure was utilized, calculating the mean amplitude, rather than peak amplitude. This measure is an average of the areas under the curve for each of the individual trials, meaning it was less impacted by delays in response, or latency variability as compared to measures of peak

amplitude (Luck, 2005). Average amplitude was then averaged across groups for each condition.

Statistical Analyses

Statistical extraction was completed for average amplitude in each condition (standard and oddball) for each comparison (N/NN and CP) between 100ms post stimulus onset to 300ms post stimulus onset to capture the MMN time window. A difference score between standard and oddball was computed for each participant for each comparison (standard was subtracted from oddball). This calculation was utilized to complete *t* tests to test for group differences. Statistical significance was determined at a = 0.05. Bonferroni adjustments for multiple testing were calculated. With two comparisons planed in the analyses, the Bonferroni-corrected a = 0.025. The first author collected all data and completed preprocessing and statistical analyses.

Results

In general, the average amplitude for the expected MMN time window for the CAS group was more negative in the N/NN condition, and the TD group showed more negative responses to the CP condition. Mean and standard deviation for each condition can be found in Table 6.

Table 6

Mean and Standard Deviation of Recorded Average Amplitudes in Each Condition

Condition		CAS	TD
Native/Non-native	**Common**	**-1.97 (2.19)**	**0.40 (1.94)**
	Odd	**-4.24 (2.89)**	**0.93 (2.14)**
Close Proximity	**Common**	**2.36 (0.80)**	**-1.12 (0.99)**
	Odd	**0.81 (1.98)**	**-2.42 (2.16)**

Note. Mean and standard deviation reported in μV.

Comparison of difference scores (average amplitude of common stimuli subtracted from the average amplitude of the odd stimuli) were compared using *t* tests between groups (CAS and TD). Means and standard deviations for difference scores can be found in Table 7. The difference calculation for the native condition was found to be significant between groups. The CAS group showed a more negative response to the odd stimulus in this condition, as expected, which resulted in a greater difference score as compared to the TD group. Closer examination of the grand averaged waveforms for the N/NN condition (see Figure 2) reveal a noticeable negativity in the odd condition throughout the majority of the expected MMN time window (150-250ms). The TD group does not show this difference in negativity, as the common and odd waveforms are notably similar (see Figure 3). This difference in negativity in the CAS group is interpreted as evidence of a MMN response to the odd vowel. In general, these findings indicate that in the N/NN condition, an MMN-like response was elicited for the CAS group, but not the TD group in the predicted time window.

Table 7

Mean and Standard Deviation of Difference Calculations and t tests

Condition	CAS	TD	*t*	p-value
Native/Non-native Diff Score	-2.27 (2.58)	0.53 (1.37)	2.50	.029
Close Proximity Diff Score	-1.56 (1.53)	-1.56 (1.53)	.28	.788

Note. Mean and standard deviation reported in μV.

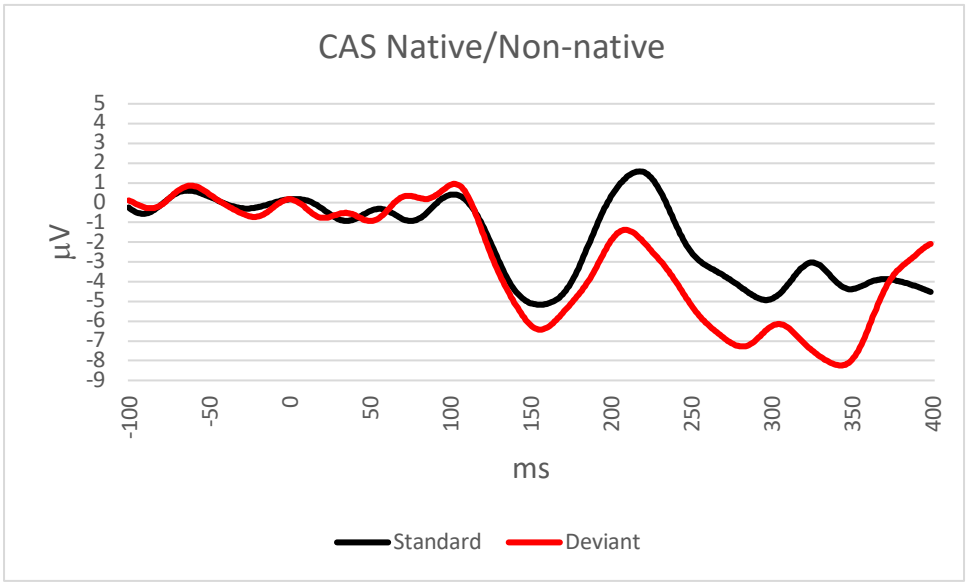

Figure 4. Grand-averaged responses to N/NN vowels at frontal electrode sites for CAS group

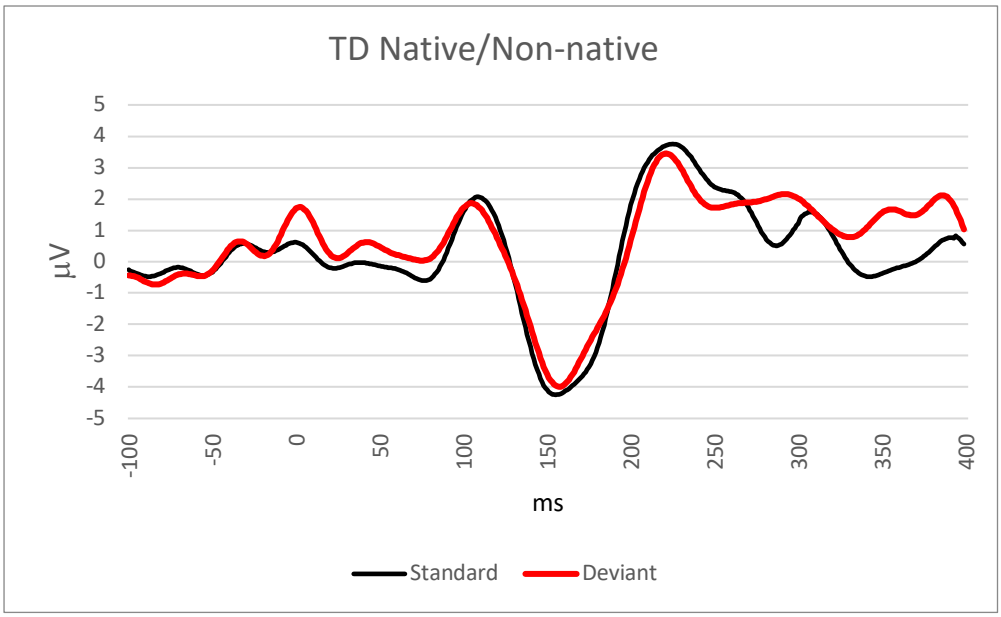

Figure 5. Grand-averaged responses to N/NN vowels at frontal electrode sites for TD group

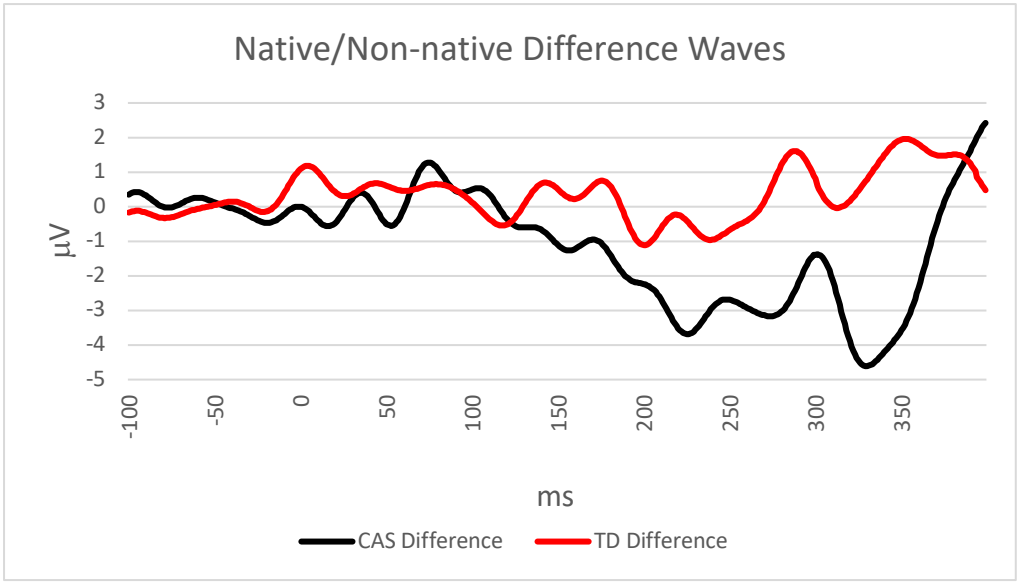

Figure 6. Difference waves for N/NN condition at frontal electrode sites for TD and CAS

The difference calculation for the CP condition was not significant between groups. Both groups showed a more negative response to the odd stimulus in this condition in the expected MMN time window. Examination of the grand averaged wave

forms for the TD group reveal a noticeable and consistent difference in negativity throughout the expected MMN time window (see Figure 5). Both groups show a peak negativity around 150ms. This difference in negativity is interpreted as an MMN response in both groups.

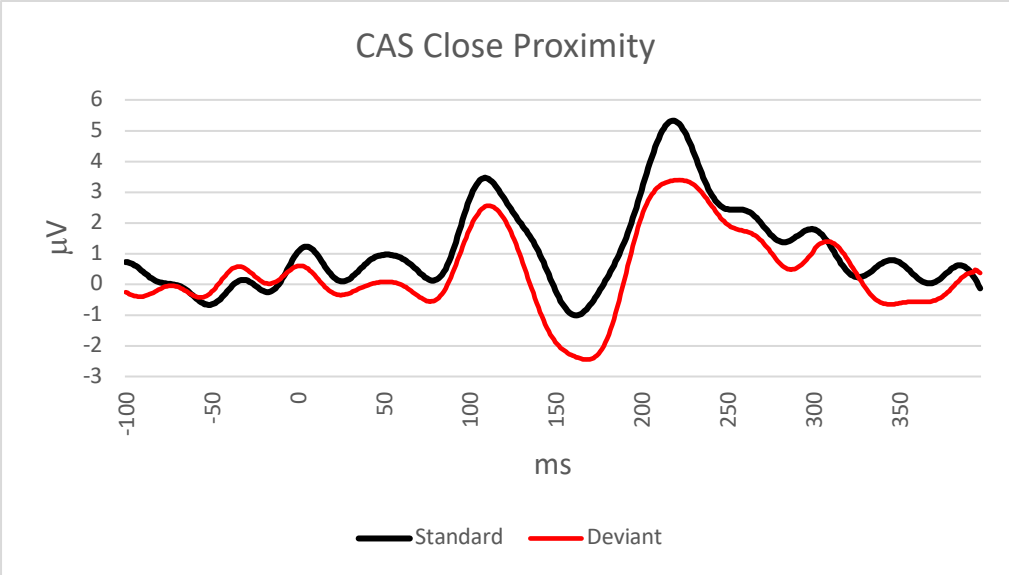

Figure 7. Grand-averaged responses to CP vowels at frontal electrode sites for CAS group

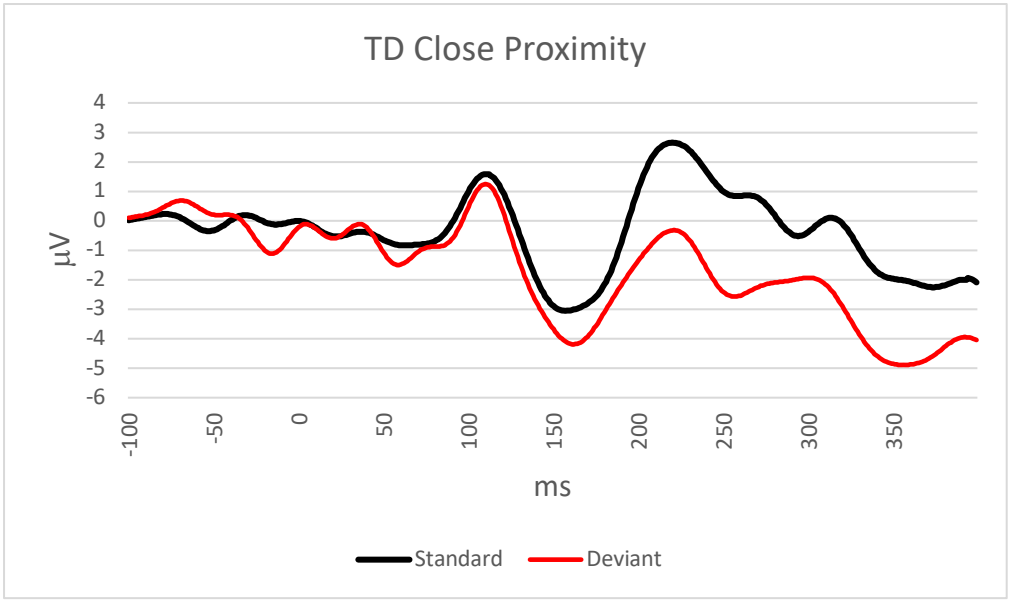

Figure 8. Grand-averaged responses to CP vowels at frontal electrode sites for TD group

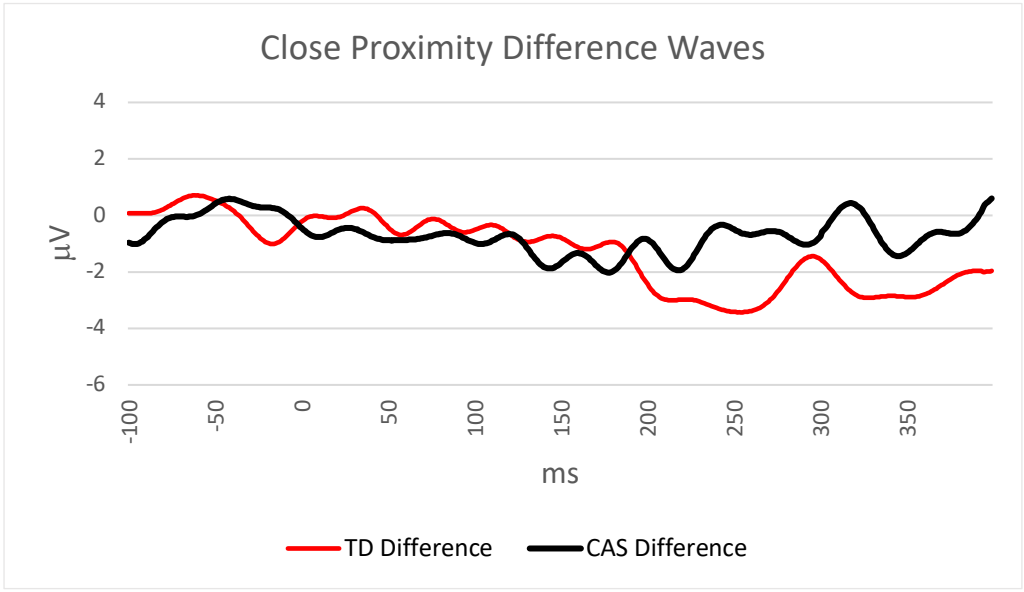

Figure 9. Difference waves for CP vowels at frontal electrode sites for TD and CAS

Discussion

This study investigated ERP responses to vowel contrasts in children with CAS, specifically to evaluate the presence of an altered MMN response in response to native

and non-native vowel contrasts. An altered MMN response has been found in children with CAS in phonemic and phonetic contrasts (Froud & Khamis-Dakwar, 2012). Using an oddball paradigm, EEG recordings were collected from nine children with CAS and ten age matched peers.

In the CP condition, with /ɛ/ being the standard and /I/ as the oddball, the CAS and TD groups showed similar MMN responses. No statistically significant differences were found between the difference calculation (odd stimulus – common stimulus) of mean amplitude of the expected MMN response time window of 100-300 post stimulus onset between groups. Both the CAS and TD groups showed the expected MMN response to the oddball (/I/) contrast. This is contradictory to the study conducted by Froud and Khamis-Dakwar (2012), as they found an atypical response to language-specific phonemes in the CAS group, and an appropriate MMN response to language-specific phonemes in the TD group. The findings of this study suggest that children with CAS, particularly in the age range targeted (9-17 years), are able to appropriately distinguish between vowels that are present in their native language, as would be expected in typically developing children.

In the N/NN contrast with /u/ as the standard and /y/ as the oddball, the TD group did not show an MMN response to the oddball (/y/) contrast. The CAS group, however, demonstrated a more negative response to the oddball, the expected MMN response. This finding is similar to the findings of Froud and Khamis-Dakwar (2012), as the CAS group shows an over-specification to non-native phonemes. The over-specification found in native and non-native consonants is consistent with an over-specification to native and

non-native vowels. Both findings suggest that CAS should not be considered purely a disorder of motor planning, as there is evidence for speech processing deficits as well.

Also relevant to these findings is the recent behavioral study targeting speech perception in CAS (Zuk, Iuzzini-Seigel, Cabbage, Green, & Hogan, 2018) that found poor speech processing is not a core deficit of CAS. It was, however, found to be associated with decreased language skills in this population. Four of the nine children included in this EEG study currently have deficits in expressive language. The over-specification of response to the N/NN contrast in the CAS group may be evidence of poor phoneme perception as a co-occurring trait of CAS, secondary to expressive language deficits. This also coincides with the NLNC hypothesis that failure to neurally commit to phonemes distinct to the child's native language in the first year of life leads to decreased language skills at later stages of development. The decreased language skills often observed in children with CAS may be related to this issue. This is also in line with prior research showing that failure to fine-tune the phonological features of a native language may result in difficulty with accurate phoneme retrieval and production (Gierut & Morrissett, 2012), potentially contributing to the decreased speech production observed in CAS.

To further investigate the idea that decreased speech perception is related to language deficits, post-hoc inspection of individual averaged waveforms for CAS participants with and without language impairments was completed. This included four children with CAS who currently present with expressive language difficulties. Further investigation revealed no noticeable differences between children in the CAS group with language deficits, and children without. This may be due to limited sample size, or due to

the nature of the task. This oddball paradigm focused on native and non-native vowel sounds, similar to the contrast of native and non-native consonants in the study competed by Fround & Khamis-Dakwar (2012). The study completed by Zuk, Iuzzini-Seigel, Cabbage, Green, & Hogan (2018) investigated a continuum of native consonants and vowels (/da/ and /ga/). It is possible that sounds from the child's native language are processed appropriately (when no co-occurring language deficits exist), but non-native sounds are not, therefore, these studies are not investigating the same underlying speech processing deficit. If the deficit is specific to the NLNC theory of failure to fine tune perception to one's native language, studies of native language contrasts will not target the underlying issue.

Clinical Implications

The findings of this study have the potential for meaningful clinical translations. As CAS appears to involve speech processing issues, as demonstrated in both consonants and vowels, it is important to incorporate perceptual tasks into the treatment of children with CAS, particularly when children present with co-occurring language deficits. Providing targeted input in the form of auditory bombardment may assist children with perceptual deficits to develop more specific representations of speech sounds, particularly in the early stages of speech and language development. It is imperative that clinicians working with children who have CAS consider all aspects of the child's speech and language development, not just the accuracy of speech production. It is well established that children with CAS present with phonological processing deficits as well as deficits in reading and spelling (Lewis et al., 2004; Gillion and Moriarty, 2007; McNeill et al., 2009). Paying close attention to deficits in each area and understanding the potential

underlying processing deficit can help clinicians move toward speech therapy that is tailored specifically to the needs of each child with CAS.

Incidental Findings

Several important findings were discovered over the course of data collection and analysis. Children in the CAS group were observed to have greater difficulty participating in EEG data collection as compared to the TD group. Many of these children have known sensory processing difficulties (CAS01, CAS03, CAS04, CAS06, CAS08, CAS10, and CAS11), as is common in CAS. Several of the children with CAS (CAS06, CAS10, and CAS11) reported that listening to the presentation of repetitive sounds caused discomfort (headache). No children in the TD group reported discomfort, and all were able to tolerate EEG testing without complication. CAS01 was removed from analyses completely due to movement artifact and inability to tolerate the net for the duration of testing. Child 4 in the CAS group refused to participate in EEG testing. These findings demonstrate the impact of sensory processing issues in a participant's ability to participate in EEG testing and should be considered when designing experiments. The availability of sensory tools (such as a weighted blanket for sensory input or a footstool for body stability) as well as frequency of breaks from testing should be adapted to meet the needs of children with sensory processing issues.

Limitations and Future Directions

This study included children ages 9 to 17 with a history of CAS. This age range was selected in order to target children who would most likely be able to successfully complete EEG testing, which requires participants to remain as still as possible in order to reduce movement artifacts, making it a difficult task for young children. It is possible,

however, that because of this age range, this study did not completely capture vowel perception in CAS, as many children who participated in this study have participated in extensive speech therapy and have improved their speech production skills to reach age appropriate limits, with most having no or very few vowel errors observed. It may be possible that targeting a younger population of children with CAS would better capture N/NN and CP vowel perception. Comparison of ERP responses in children with and without resolved speech articulation skills may also lead to interesting findings and clinical translations. The CAS phenotype varies greatly as a function of age, meaning closer examination of EEG responses across narrower age ranges may lead to more conclusive results regarding the underlying neural processes in this population. In addition, two of the most severely affected children, as judged by behavioral measures (CAS01 and CAS04), had either unusable data due to movement artifact or were unable to participate in EEG testing. Including data from these participants may have led to even more drastic differences between groups. In addition, studying children with CAS with and without co-occurring language impairment may also lead to more meaningful findings, as differences in phoneme perception have been identified between these two subgroups. Due to the limited sample size, this study was unable to examine specific subgroups in CAS, although this is an interesting concept for future ERP studies in this population. In the future, examining intra-individual variability may be a useful approach to gain a deeper understanding of ERP responses in CAS, particularly when the sample size is small.

Following artifact rejection, only 9 children with CAS were included in the study, and 10 TD participants. Many trials had to be removed from analysis, particularly in the

CAS group, due to movement artifact. Although measures were put in place to eliminate this issue to the greatest extent possible, dealing with a pediatric population in an EEG study presents additional challenges. A larger sample size would allow us to better examine EEG responses, potentially based on subgroups of clinical presentation. As mentioned previously, investigating ERP responses to native and non-native phonemes in children with CAS with and without co-occurring language deficits may lead to additional clinical implications and increase our understanding of underlying perceptual deficits in CAS. Finally, conducting trials in which the stimuli (standard and deviant) are reversed, and comparing these results to the initial trials may provide meaningful insight into the reliability of these measures in CAS.

CHAPTER 4

EXPERIMENT 3: GENETIC VARIATION IN A TRIO WITH CAS

Method

Participants

One child was selected from the CAS participants described in Experiment 1 for genetic testing. CAS01 was selected based on a negative family history of speech and language disorders as well as severity of the observed CAS phenotype. Below is a description of the child's clinical presentation and developmental background.

Patient Report. CAS01, the proband, is a male aged 9;3, born at 39-weeks gestation via planned cesarean section with a birth weight of 3,581 g. The child's mother reportedly used Levothyroxine during pregnancy secondary to hypothyroidism. Infancy was negative for feeding problems, sleeping problems, ear infections, abnormal head size, or other medical complications. No delays were reported in gross or fine motor development, although toilet training was delayed (not fully completed until 5 years of age). First words were delayed, occurring at 24 months of age, and first sentences used at age 5. Babbling in infancy was limited.

Early intervention for speech and language development was initiated at age 27 months. At this time, his communication abilities were characterized by delayed receptive and expressive language skills, and single words were beginning to emerge. Expressive vocabulary was estimated to be approximately 10 words at this time. CAS01 was diagnosed with CAS at age 4;2 based on a limited speech sound inventory, inconsistent vowel production, decreased intelligibility, and inconsistent speech sound production.

Assessment at age 9;3 included standardized measures of speech articulation (Goldman and Fristoe, 2000), untimed reading of words and non-words (WRMT-R; Woodcock, McGrew, & Mather, 2001), timed reading of words and non-words (TOWRE; Torgesen, Wagner, & Rashotte, 2012), gross and fine motor testing (BOT-2; Bruininks & Bruininks, 2005), spelling (WIAT-III; Wechsler, 2009), non-word repetition (CTOPP; Wagner, Torgesen, & Rashotte, 1999), expressive and receptive language (CELF-5 English; Wiig, Semel, & Secord, 2013), and antonym generation (Carrow-Woolfolk, 1999). Non-standardized assessment included hand motor keyboard tapping tasks measured by inter-tap intervals (Gualtieri and Johnson, 2006), DDK measurements during rapid syllable repetitions of monosyllables and multisyllables (Fletcher, 1972), multisyllabic word production, and conversational speech sampling (for full description of behavioral tasks see methods section of Experiment 1). Results of testing were consistent with delayed expressive and receptive language, delayed literacy abilities, and speech production consistent with CAS. Speech traits consistent with CAS included: vowel errors ([pɛg] for [pɪg]), voicing errors ([su] for [zu]), inconsistent production of words ([saɪdʌ] and [paɪdʌ] for "spider"), intrusive schwa ([rɪŋʌ] for [rɪŋ]), dysprosody (flat intonation), oral groping, difficulty with multisyllabic words, and incorrectly placed lexical stress ("lion" with stress on the second syllable and "guitar" with stress on the first syllable). CAS01 also replaced /r/ with [w] or vowels, which is a pattern commonly observed in typically developing children at younger ages. The Kaufman Brief Intelligence Test-2 (KBIT-2; Kaufman & Kaufman, 2004), a measure of verbal and nonverbal intelligence, was also administered. This assessment indicated slightly below average verbal and nonverbal intelligence. CAS01 demonstrated low DDK *z* scores, as

demonstrated by *z*-scores of -2.65 for monosyllables and -3.15 for disyllables. Although norms are not available for keyboard tapping, inter-tap intervals were judged to be substantially higher than average, indicating decreased hand motor skills. See table 8 for a summary of behavioral measures.

Table 8. *Summary of Behavioral Measures*

Measure	**Standard Score**	**Percentile**
Goldman-Fristoe Test of Articulation Third Edition*	40	0.1
Woodcock-Johnson Reading Mastery Tests: Word Identification*	63	1
Woodcock-Johnson Reading Mastery Tests: Word Attack*	61	0.5
Test of Word Reading Efficiency: Sight Word Efficiency*	68	2
Test of Word Reading Efficiency: Phonemic Decoding Efficiency*	66	1
CELF-5 Concepts & Following Directions*	55	0.1
CELF-5 Sentence Assembly*	55	0.1
KBIT-2 Verbal	82	12
KBIT-2 Nonverbal	84	14
CTOPP Non-word Repetition***	5	5
CASL Antonyms*	42	0.1
BOT-2****	30	2
Mean diadochokinetic speeds (z score): Monosyllables**	-3.15	0.8
Mean diadochokinetic speeds	-2.65	0.4

(z score): Disyllables**

Note. * Population mean = 100, standard deviation = 15 ** Population mean = 0, standard deviation = 1 ***Population mean = 10, standard deviation = 3 ****Population mean = 50, standard deviation = 9

Exome Sequencing and Variant Analysis

DNA samples were collected via saliva samples using OraGene® kits (DNA genotek, Ottawa, Canada). Both parents and the proband provided saliva samples. Standard laboratory procedures were used for DNA extraction. DNA extraction was completed at the Translational Genomic Research Institute in Phoenix (TGen; Phoenix, AZ). Exome sequencing was performed at the University of Washington Center for Mendelian Genomics.

Initial quality control (QC) was completed including DNA quantification, gender validation assay, and molecular "fingerprinting" with a 63-SNP OpenArray assay derived from a custom exome SNP set. This 'fingerprint' was 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.

A 96-well plate format was utilized for library construction and exome capture. 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. Library concentration was determined by fluorometric assay and molecular weight distributions verified on the Agilent Bioanalyzer (consistently 150 ± 15bp) and underwent exome capture using the Roche/Nimblegen SeqCap EZ v2.0 (~36.5 MB target). 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 HiSeq sequencer.

The NWGC processing pipeline consists of the following elements: (1) base calls generated in real-time on the HiSeq4000 instrument (RTA 2.7.6) (2) demultiplexed, unaligned BAM files produced by Picard ExtractIlluminaBarcodes and IlluminaBasecallsToSam and (3) BAM files aligned to a human reference (hg19hs37d5) using BWA (Burrows-Wheeler Aligner; v0.7.10) (Li and Durbin 2009). 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 was 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 were then subject to the following steps: (1) "duplicate removal" is performed, (i.e., the removal of reads with duplicate start positions; Picard MarkDuplicates; v1.111) (2) indel realignment is performed (GATK IndelRealigner; v3.2-2) and (3) base qualities recalibrated (GATK BaseRecalibrator; v3.2-2).

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 and flagged using the filtration walker (GATK) to mark sites of lower quality/false positives.

All sequence data were subject to a QC protocol. This included an assessment of: (1) total PE75 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.

An automated pipeline was utilized for annotation of variants derived from exome data, the SeattleSeq Annotation Server (http://gvs.gs.washington.edu/SeattleSeqAnnotation/). This publicly accessible server returned 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.

CNVs were identified using CoNIFER (Copy Number Inference from Exome Reads). Reads from each exome sample were split into consecutive 36mers, up to two per read, and mapped using the single-end mode of mrsFast (Hach et al., 2010), allowing for up to two mismatches per 36mer. Reads were aligned to a concatenated hg19 reference genome. CoNIFER v0.2.2 (Krumm et al., 2012) was utilized to process each exome sample separately. RPKM values were calculated for 194,080 probes and exons targeted

by the Nimblegen EZ Exome v2.0 exome sequenc enrichment platform. The –svd option was set to 20, and default CoNIFER settings were used for all other options. The raw SVD-ZRPKM values were exported and used for further analysis. DNACopy (Venkatraman & Olshen, 2007) and CGHCall (van de Wie et al., 2007) were utilized for segmentation and assignment of deletion or duplication probabilities to SVD-ZRPKM values. Default options for CGHCall were used, and only "deletion" and "duplication" were allowed as called states. Raw CNV calls were filtered to exclude those primarily n duplicated or repetitive gions of the genome as well as for duplicated processed pseudogenes. False positives were reduced by eliminating calls with low signal strength. Individual CNV calls passing filter were grouped into similar CNV regions (CNVRs) using pairwise distances between all CNVs based on a modificed reciprocal overalp (RO) heuristic. Plots were reviewed for duplications and deletions, genes and genomic features (based off of the RefSeq set), and other calls and ESP calls.

Exomes were further analyzed using GEMINI (Paila, Chapman, Kirchner, & Quinlan, 2013), a genome mining software utilizing multiple annotation sources for exploring genetic variation. GEMINI annotates the VCF file using several annotation sources, including ENCODE, OMIM, dbSNP, KEGG, Gerp, CADD, and HPRD. Following annotation, several filters were utilized to filter out any low-quality variants. This included genotyping quality score (20 or greater), impact severity (low impact removed), and read depth (6 or greater). GEMINI was used to analyze exomes for *de novo,* compound heterozygous, autosomal recessive, x-linked, and x-linked *de novo* modes of inheritance. Variants were filtered by allele frequency (variants found in >15% of the population were removed as CAS is a rare speech disorder). If the remaining

variant list was greater than 100, variants were searched for speech and language genes of interest (see Appendix C). These are genes that have been implicated in speech and language or related disorders, such as dyslexia. These procedures resulted in a manageable list of candidate causal genes. Variants of interest were verified with the Integrative Genomics Viewer (IGV) (Robinson et al. 2011, Thorvaldsdottir, Robinson & Mesirov 2013). Variants were further investigated using GeneCards, and descriptions for each variant of interest was modified from GeneCard information.

Finally, pathway analysis was completed using DAVID bioinformatics analysis tool (Huang et al., 2007) version 6.8. This allows us to identify functionally related gene groups and visualize pathway maps, providing further understanding of the variants found during the previous analyses. Bonferroni correction of p values was applied to control for multiple testing.

Results

CNVs. Results of the CoNIFER analysis, utilized to identify CNVs, are reported below. Two *de novo* CNVs were identified on chromosome 14. CoNIFER plots show raw data in the form of red bars (upward bars indicate duplication, downward bars indicate deletion), genes in the region are plotted in purple (gray areas indicate known processed pseudogenes), other calls and ESP calls in black, and CNVs in this CNVR in green.

The two *de novo* CNVs present in the proband are duplications. Figure 7 shows a duplication located on *CDC42BPB*, an gene that is reported to encode a member of the serine/threonine protein kinase family, and has been associated with mytonic dystrophy, a subtype of muscular dystrophy (Meola & Moxley, 2004). Figure 8 shows a duplication

located on *DYNC1H1*, a gene that encodes a member of the cytoplasmic dynein heavy chain family.

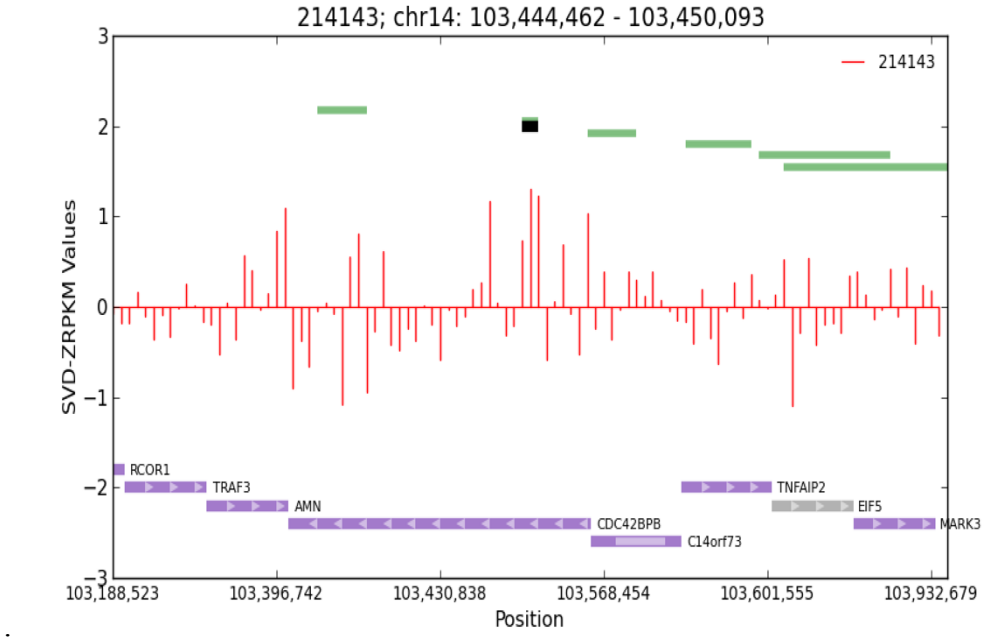

Figure 10. CoNIFER results, chromosome 14 (1).

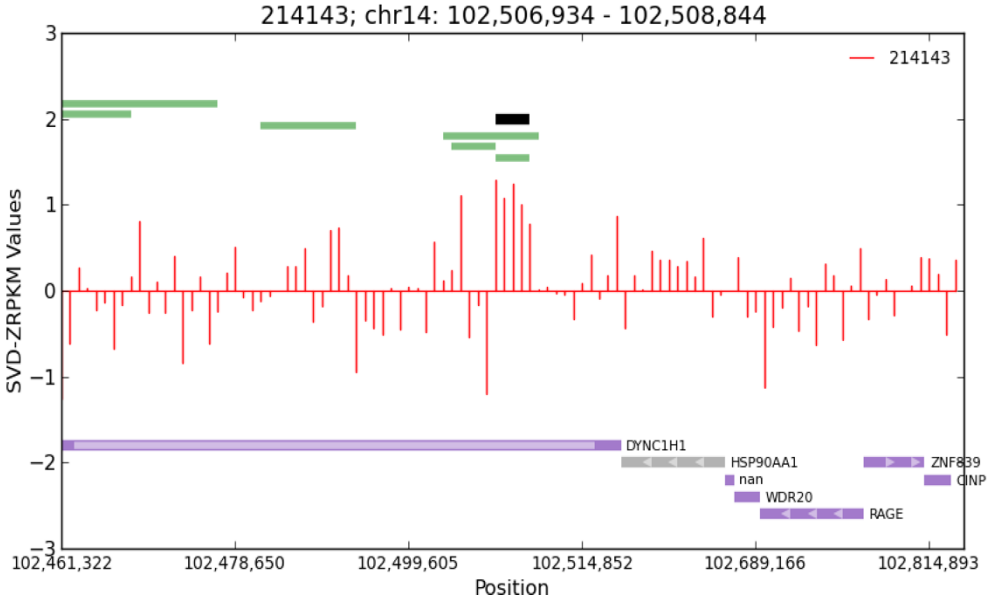

Figure 11. CoNIFER results, chromosome 14 (2).

De Novo Variants. After filtering for allele frequency, 18 *de novo* variants remained, and 3 were verified in IGV (see Table 10). None of the remaining genes were included on the Speech and Language Genes of Interest list. All three were further investigated using GeneCards. These genes were all missense variants. *LAMA5* is associated with encoding of the vertebrate laminin alpha chains and a homozygous sequence variant in *LAMA5* has been associated with a failure of neuromuscular transmission and central nervous system (CNS) manifestations (Maselli et al., 2017). Given the CAS phenotype and the suspected *de novo* model of inheritance, this was selected as the primary gene of interest.

Table 9

De Novo Variants

Gene	Cytoband	Start-End	Impact Severity	CADD Scaled	GERP	Allele Freq. (gnomad_nfe)	Description
OVOS2	chr12p11.21	31282764-31282765	Med.	3.3	2.97	0.3795	Serine-type endopeptidase inhibitor activity
LAMA5	chr20q13.33	60921246-60921247	Med.	22.7	2.69	0	Encodes one of the vertebrate laminin alpha chains
CTAGE4	chr7q35	143882672-143882673	Med.	3.25	0	0.2562	Involved in nucleotide binding

X-Linked Variants. 19 variants passed filtering under the x-linked recessive model and all were verified in IGV (see Table 11). None of these appeared on the Speech and Language Genes of Interest. All variations are missense variants with the exception of

SLC7A3 in which a missense variant and splice variant were discovered. *SLC7A3* has been associated with ASD in males (Nava et al., 2015). Another interesting x-linked variant is located on *NLGN4X* which has been linked to ASD and mental retardation (Jamain et al., 2003; Lawson-Yuen et al., 2008). No variants were found when an x-linked *de novo* inheritance model was implemented.

Table 10

X-Linked Recessive Variants

Gene	Cyto band	Start-End	Impact	CADD Scaled	GERP	Allele Freq. (gnomad_nfe)	Description
GDPD2	chrXq 13.1	69645626-69645627	Med.	11.24	0.148	0.00026231	May have a role in osteoblast differentiation and growth
IGSF1	chrXq 26.2	130419321-130419322	Med.	23.9	3.867	0.0009999	Thought to participate in the regulation of interactions between cells
MCTS1	chrXq 24	119739319-119739320	Med.	26.2	4.920	0.00101194	Anti-oncogene that plays a role in cell cycle regulation
NLGN4X	chrXp 22.32	5811531-5811532	Med.	22.7	3.230	0.00508484	May be involved in the formation and remodeling of central nervous system synapses
MAGEC3	chrXq 27.2	140985242-140985243	Med.	11.39	-0.367	0.00778026	MAGE family member C3; directs the expression of tumor antigens
SPANXD	chrXq 27.2	140785713-140785714	Med.	0.14	0	0.01557447	Encodes differentially expressed testis-specific proteins
NXF5	chrXq 22.1	101096929-101096930	Med.	23.7	2.180	0.02782318	Could be involved in the export of mRNA from the nucleus to the cytoplasm.
MOSPD2	chrXp 22.2	14929374-14929375	Med.	18.7	3.960	0.03152643	Promotes migration of primary

							monocytes and neutrophils, in response to various chemokines
FAM133A	chrXq21.32	92964616-92964617	Med.	25.2	1.38	0.0601674	Cancer/Testis Antigen
FAM122C	chrXq26.3	133963267-133963268	Med.	0	-1.62	0.06361106	Family With Sequence Similarity 122C
EFHC2	chrXp11.3	44171952-44171953	Med.	4.67	2.920	0.06818032	May be involved in the development of epilepsy
SPANXD	chrXq27.2	140785695-140785696	Med.	0.03	0	0.07149762	See above
EFHC2	chrXp11.3	44094684-44094685	Med.	0	-3.600	0.07560484	See above
SRPX	chrXp11.4	38009120-38009121	Med.	23.1	5.170	0.07740851	May be involved in phagocytosis during disk shedding, cell adhesion to cells other than the pigment epithelium or signal transduction
SLC7A3	chrXq13.1	70146474-70146475	Med.	0.49	4.150	0.08534149	Encodes a member of the solute carrier family 7, a sodium-independent cationic amino acid transporter
SLC7A3	chrXq13.1	70146033-70146034	Med.	9.16	2.450	0.10609595	See above
BCORL1	chrXq26.1	129147372-129147373	Med.	4.98	2.710	0.1102897	Transcriptional corepressor that is found tethered to promoter regions by DNA-binding proteins
ARL13A	chrXq22.1	100242535-100242536	Med.	7.12	1.610	0.12859109	ADP-ribosylation factor-like protein 13A
MAP3K15	chrXp22.12	19482475-19482476	Med.	22.5	3.640	0.89440328	Plays an essential role in apoptotic cell death triggered by cellular stresses

Autosomal Recessive Variants. Initial GEMINI analysis produced 658 autosomal recessive variants. Eighty-six variants remained after filtering for allele frequency, and 85

were verified in IGV. Three of these variants were located on genes implicated in speech and language disorders (see Table 11). One variant on *VWA3B* (position 98828388) is a splice variant. A homozygous mutation of *VWA3B* has been associated with cerebellar ataxia and intellectual disability (Kawarai et al., 2016)

Table 11

Autosomal Recessive Variants

Gene	Cytoband	Start-End	Impact Severity	CADD Scaled	GERP	Allele Freq. (gnomad_nfe)	Description
DNAH14	chr1q42.12	225156456-225156457	Med.	2.78	-5.070	0.09319866	Axonemal dynein heavy chain
DNAH14	chr1q42.12	225373071-225373072	Med.	9.46	-0.12	0.10425479	See above
VWA3B	chr2q11.2	98928428-98928429	Med.	12.43	-4.400	0.06381061	Thought to function in transcription, DNA repair, ribosomal and membrane transport
VWA3B	chr2q11.2	98828388-98828389	Med.	1.54	-10.7	0.0748086	See above
FRMD1	chr6q27	168463623-168463624	Med.	10.85	-1.930	0.07397425	FERM domain

Compound Heterozygous Variants. Initial GEMINI analysis revealed 1,698 compound heterozygous variants, and 905 remained after filtering for allele frequency. Filtering by Speech and Language Genes of Interest indicated 18 variants remaining for further investigation, wich were verified in IGV (see Table 12). The primary gene discovered through investigation of the compound heterozygous inheritance model is *PCNT*, which has been associated with dyslexia (Poelmans et al., 2009). Two variants on this gene were

splice variants, as well as one splice variant on *ATP2C2*. All others were missense variants. *LAMA5* also appeared in the compound heterozygous model.

Table 12

Compound Heterozygous Variants

Gene	Cytoband	Start-End	Impact Severity	CADD Scaled	GERP	Allele Freq. (gnomad_ nfe)	Description
ATP2C2	chr16q24.1	84494251 - 84494252	Med.	4.39	0.505	0.13950314	Nucleotide binding and calcium-transporting ATPase activity
LAMA5	chr20q13.33	60909315 - 60909316	Med.	23.4	1.42	0.0174	Encodes one of the vertebrate laminin alpha chains
PCNT	chr21q22.3	47744201 - 47744202	Med.	6.3	-0.333	0.06410552	Important for normal functioning of the centrosomes and cycle progression
PCNT	chr21q22.3	47766112 - 47766113	Med.	1.13	3.27	0.06090289	See above
PCNT	chr21q22.3	47773176 - 47773177	Med.	6.57	1.4	0.12516115	See above
PCNT	chr21q22.3	47786523 - 47786524	Med.	14.28	1.82	0.13836989	See above
PCNT	chr21q22.3	47808678 - 47808679	Med.	24.3	-3.08	0.13085808	See above
PCNT	chr21q22.3	47817315 - 47817316	Med.	23.8	5.46	0.002356	See above
PCNT	chr21q22.3	47831508 - 47831509	Med.	0	2.58	0.12375242	See above
PCNT	chr21q22.3	47831844 - 47831845	Med.	0.14	-10.6	0.06244939	See above

PCNT	chr21q22.3	47836121 - 47836122	Med.	0.05	-1.49	0.07469247	See above
PCNT	chr21q22.3	47836205 - 47836206	Med.	0	3.54	0.07463916	See above
PCNT	chr21q22.3	47836394 - 47836395	Med.	0	-0.739	0.0748035	See above
PCNT	chr21q22.3	47836546 - 47836547	Med.	0.5	1.32	0.07430142	See above
PCNT	chr21q22.3	47838111 - 47838112	Med.	None	0.062	0.07054943	See above
PCNT	chr21q22.3	47841940 - 47841941	Med.	0	1.33	0.07468475	See above
PCNT	chr21q22.3	47841988 - 47841989	Med.	17.95	-7.69	0.060671	See above
PCNT	chr21q22.3	47848458 - 47848459	Med.	18.99	0.662	0.07552517	See above
PCNT	chr21q22.3	47856006 - 47856007	Med.	23.2	4.42	6.56E-05	See above

Discussion

The purpose of this study was to examine genetic variations related to CAS through full exome sequencing of an affected child and his unaffected parents. Examination of variants remaining after filtering lead to several interesting findings. Overall, the *LAMA5* gene is the primary gene of interest, given the CAS phenotype, and patient and family history collected at the time of evaluation. Due to the negative family history reported by the proband's parents, the *de novo* mode of inheritance is believed to be the primary mode of inheritance for the CAS phenotype in this child.

The *LAMA5* gene encodes one of the vertebrate laminin alpha chains. Laminins are a family of extracellular matrix glycoproteins and have been implicated in several

biological processes including cell adhesion, differentiation, migration, signaling, neurite outgrowth and metastasis. Review of the current literature on *LAMA5* provides further support for this gene as the primary gene of interest in the proband. Most notably, a severe deficit of neuromuscular transmission was reported in a patient with a homozygous variant in *LAMA5* (Maselli et al., 2017). This patient presented with muscle weakness, myopia, and facial tics. This patient also presented with weakness of the facial, tongue, and soft palate muscles. Investigation of the neuromuscular junction (NMJ) in this patient revealed underdeveloped nerve terminals. The authors concluded that deficient support of cell adhesion and neurite outgrowth caused by the mutant laminin a5 was most likely causal for the disordered phenotype seen in the patient. It is believed that signaling pathways in the NMJ are impacted by mutant laminin a5, but this mutation most likely does not impact other tissues (i.e., heart, lung) as severely. In terms of motor speech disorders, damage to the neuromuscular junction most often results in a dysarthric speech, rather than apraxic speech, although cases of patients with neuromuscular junction disorder and cerebellar ataxia have been reported (Lorenzoni et al., 2008). Cerebellar ataxia is a motor disorder with a speech phenotype similar to the motor dyscoordination seen in CAS. Patients with dysarthria typically demonstrate imprecise consonant production, distorted vowel production, severely decreased intelligibility of speech, decreased vocal intensity or excessive loudness, monopitch, and inappropriate stress (ASHA, n.d.), whereas patients with cerebellar ataxia often present with severely disordered speech, including abnormal prosody and difficulty with rapid alternating movements such as DDK (Ryan & Engle, 2003). There exists a clear overlap in characteristics between dysarthria, cerebellar ataxia, and CAS. It is suspected that the

potential mutant laminin a5 plays a role in the disordered speech patterns seen in CAS01. Additionally, *LAMA5* is reported to be most highly expressed in tissues of the kidney and cerebellum. Given the CAS phenotype, and suspected underlying deficit in sequential processing, it is expected that the genetic variants associated with CAS would be highly expressed in the cerebellum.

Although *LAMA5* is the primary gene of interest for this trio, several other variants likely contribute to the complex phenotype observed. An autosomal recessively inherited variant on *VWA3B* was found in the proband. This gene has been associated with cerebellar ataxia (Kawarai et al., 2016), similar to *LAMA5,* and may be a contributing factor to the severity of the proband's delays observed across behavioral assessment tasks. In addition, several genes discovered are associated with ASD, including x-linked variants on *NLGN4X* (Jamain et al., 2003; Lawson-Yuen et al., 2008) and *SLC7A3* (Nava et al., 2015). ASD and CAS are often comorbid, with an increased frequency of children with apraxia in the population of children with autism (Tierney et al., 2015). Deficits in praxis are reported in children with ASD, leading to impaired acquisition and performance of a variety of motor skills (Dowell, Mahone, & Mostofsky, 2009; Dziuk et al., 2007; Gernsbacher, Sauer, Geye, Schweigert, & Hill Goldsmith, 2008). Genetic influences contributing to the motor deficits seen in CAS may also play a role in the praxis deficits seen in ASD. Both disorders are strongly heritable, and both have underlying linguistic impairments. This suggests the possibility of commonality in underlying genetic mechanisms, although this area is highly controversial in both the CAS and ASD literature.

Another relevant finding to the CAS phenotype is the variant discovered on *ATP2C2*. A SNP on this gene has been found to have a significant association with nonword repetition (NWR; Newbury et al., 2009). Decreased NWR is common in CAS and was found to be severely impaired in the proband. This task involves phonologic short-term memory, and involves a high level of sequential processing, both of which appear to be severely delayed in the proband. Similarly, measures of real word and non-word reading were found to be impaired. This makes the discovery of a variant on *PCNT* an interesting finding as well. *PCNT* is associated with dyslexia, another disorder that is commonly co-morbid with CAS. It is believed that CAS and dyslexia have a shared underlying sequential processing deficit (Peter et al., 2017), evident in NWR tasks in both groups.

Overall, these findings suggest multiple genetic variations likely contribute to the severely affected phenotype in the proband. Assessment of speech, language, reading, fine motor, gross motor, and oral motor skills indicate severe impairments across domains, contributing to the likelihood that the observed phenotype is not caused by a single genetic anomaly. In general, these findings provide further evidence of heterogeneous genomic pathways associated with CAS.

Clinical Implications

With a more advanced knowledge of the biological causes of CAS, we can move toward the development of proactive and tailored interventions. In this way, treatment can focus on prevention rather than remediation. Treatment of CAS is extremely lengthy and costly, particularly when it is not diagnosed until a child is 2 years or older, as is common practice currently. By this time, the child has most likely missed extensive

periods of speech practice in the form of babbling and is demonstrating marked frustration surrounding communication due to impaired ability to communicate basic wants and needs. By developing proactive methods for the earliest possible intervention (i.e., babble therapy and parent education) for children at genetic risk for speech and language delays, we may enhance speech and language development from birth, leading to substantially improved outcomes and decreased cost of treatment.

Limitations and Future Directions

This study was restricted to exomic data, meaning variations outside of the coding regions were not detected. Future studies of full genome trios in CAS may provide more meaningful evidence towards biological causes of the disorder. Although variants in this study provide interesting and novel findings, they do not equate to causality. Further evidence is needed to make conclusions regarding genetic variations associated with CAS. In addition, only CAS was examined in this study. Including an additional disorder group for control purposes would provide further validity for these findings.

Analysis of the compound heterozygous mode of inheritance resulted in a large quantity of variants. Because of this, variants were filtered for genes that have been previously implicated in speech and language and related disorders. It is possible that a novel gene of interest may have been discovered in the group of variants that was missed in this study.

Finally, family history was provided by parents only, as no extended family members were available to participate in this study. It is possible that speech and language disorders were present in past generations and/or in extended family members but not reported, therefore impacting our suspected mode of inheritance for the proband.

CHAPTER 5

INTEGRATED DISCUSSION: THE GENE-BRAIN-BEHAVIOR CONNECTION

Overall, the results of this study provide evidence that CAS is a multifaceted disorder with deficits across multiple developmental domains. The focus of the behavioral component of this study was an underlying sequential processing deficit in CAS. The hypothesized sequential processing deficits were evident in speech, language, reading, spelling, fine/gross motor, oral motor, and hand motor tasks. It is suspected that these underlying issues may have an association with cerebellar function. In order to further understand the relationship between CAS, sequential processing, and the cerebellum, several factors must be examined, including potential biomarkers causal to the CAS phenotype.

Although EEG testing did not directly target cerebellar function, as this is an area better examined by other neuroimaging modalities, results found here may have interesting implications for the cerebellum, sequential processing, and CAS. It is hypothesized that the cerebellum plays a role in sensory prediction and the generation of expectancies for sequences of sensory information (Bower, 1997; Ramnani, 2006; Wolpert, Miall, & Kawato, 1998). In studies of the MMN response in patients with cerebellar degeneration, altered MMN responses have been detected (Moberget et al., 2008). The MMN task included in this study showed an altered MMN response in children with CAS as compared to typically developing children. Although the tasks used to elicit an MMN are not comparable across studies, there appears to be a relationship between MMN response and cerebellar dysfunction. Future studies of MMN responses in

CAS utilizing tasks shown to elicit differences in patients with cerebellar dysfunction may provide insights to this hypothesis.

Providing additional support for association between he cerebellum, sequential processing, and CAS are the genetic findings of this study. The primary gene of interest, *LAMA5,* is highly expressed in the cerebellum. Reported phenotypes of patients with *LAMA5* mutations indicate decreased oral motor coordination consistent with CAS. In addition, the discovery of a mutation on *ATP2C2* in the proband further supports the idea of underlying sequential processing issues, as this gene is associated with NWR (Newbury et al., 2009), a task requiring high levels of sequential processing.

Experimental behavioral measures included in this study were selected to investigate underlying sequential processing issues. Correlations between the BOT-2, antonym generation task, and sequential literacy measure demonstrate a possible underlying deficit which impacts all of these developmental domains. The BOT-2 includes a measure of balance, which is related to cerebellar function. Children with CAS were found to have significantly decreased skills on the overall BOT-2 measure as compared to the TD group. Although standard scores for individual tasks are not available, it would be expected that scores solely examining balance to be decreased in this population, as overall measures of fine and gross motor skills were low. Further evidence is needed in areas of gross motor specifically targeting CAS in order to gain further knowledge in this area. Antonym generation accuracy and time provided an additional measure related to sequential processing and potentially related to cerebellar function, as has been reported previously (Gebhart et al., 2002). Our findings provide

further support for the relationship between sequential processing and antonym generation.

Relating these findings to models of motor speech production (i.e., the DIVA model), cerebellar sequencing abilities have been associated with feedforward control in the motor domain. Desmond et al. (1997) hypothesized that the cerebellum may play a role in computing the difference between actual and indented phonological rehearsal. This information may be used to update the feedforward command to the frontal lobe. If the cerebellum is impaired, this feedforward input may be insufficient, forcing the speaker to rely heavily on sensory feedback. Impaired feedforward motor control is one hypothesis for the speech difficulties in CAS, and children with CAS have been shown to rely more heavily on auditory feedback (Iuzzini-Seigel, Hogan, Guarino, & Green, 2015). In very early stages of speech development, children use auditory and sensory feedback to develop the neural programs necessary to control production of newly acquired sounds, and repeated productions help to fine tune the feedforward commands (Guenther, 2006). If feedforward commands are impaired, and the child must rely more heavily on auditory feedback. It is possible that this may contribute to the over specification of phonemes in CAS that was observed in the EEG component of this study. Children with CAS may miss the period of fine tuning that occurs in typically developing children, due to an underlying deficit in feedforward commands, potentially secondary to differences in cerebellar function.

Vowels represent another area that can be related to each component of this study. Vowel errors are common in CAS (ASHA, 2007), and in this study, altered vowel perception in children with CAS was demonstrated through EEG measures. Errors in

vowel production were confirmed in several participants in the CAS group through behavioral testing. In terms of genetic findings, the phenotype of individuals with a mutation in the *LAMA5* gene may include vowel distortions, whether this is related to dysarthria, cerebellar ataxia, or CAS. These findings suggest that the vowel errors observed in CAS are not solely related to decreased motor planning and programming but may be due to multiple underlying causes. It is also apparent that vowel production is a crucial component to assess when evaluating children with CAS.

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APPENDIX A

PARENT QUESTIONNAIRE – GENETICS OF SPEECH AND LANGUAGE

Parent Questionnaire – Genetics of Speech and Language

Directions: There are seven parts to this questionnaire. Part I asks for general background information about your family. Part II asks about your child's educational history. Part III asks about your child's developmental history. Part IV asks about your child's health history. Part V asks about your family's health history. Part VI asks about home experiences with reading and writing. Part VII asks about referral source. Use the back of the paper if you need more space to answer.

I. Background Information

A. Child's Ethnic Background:

1. Hispanic or Latino? Yes____ No_____
2. Racial Category:

_____Asian-American
_____Black-American
_____Caucasian (European-American) if possible specify country (ex: Sweden,
Ireland_____
___
_____Hispanic
_____Native American
_____Pacific Islander
_____Middle Eastern
_____More than one race (please specify):
_____

3. Ethnicities of Grandparents (use categories above)

Mother's mother:_____
Mother's father_____
Father's mother_____
Father's father_____

B. Was your child adopted? Yes____
No____
Is your child a foster child? Yes____
No____

C. Mother's Highest Level of Education (check one). (Note: if a child is adopted, indicate the adoptive mother's highest level of education.)

_____Less than high school
_____High School
_____Community college or vocational training after high school
_____College (degree_____and area of study_____)
_____Graduate degree (masters degree, doctoral degree, law degree, medical degree, etc. (explain)_____
_____Unknown

D. Father's Highest Level of Education (check one). (Note: if a child is adopted, indicate the adoptive father's highest level of education.)
_____Less than high school
_____High School
_____Community college or vocational training after high school
_____College (degree_____and area of study_____)
_____Graduate degree (masters degree, doctoral degree, law degree, medical degree, etc. (explain)_____
_____Unknown

E. Mother's Occupation_____

F. Father's Occupation_____

G. Household. Describe who is currently living in the home with the child. Include adults and children.

H. What is the primary language spoken in the home?_____
What other languages are spoken in the home?_____
Is your child monolingual?_____
bilingual?_____
trilingual?_____(choose one)

II. Educational History

A. What schools has your child attended?

Grade	School

B. Has your child ever repeated a grade? Yes_____
No_____
Ifyes,when?,why?_____
_____
_____

C. Has your child ever received any special services? If so, write in the grades in which each kind of service was received.

SPECIAL EDUCATION	SPEECH &HEARING	PHYSICAL THERAPY	OCCUPATIONAL COUNSELING THERAPY

_____ _____ _____
_____ _____

D. Does your child have an Individualized Education Plan (IEP) for special education resource room help?

Yes____
No____
For what subjects do they receive help?Reading_____
Writing_____
Math_____

E. Does your child get Chapter 1 services for Reading?Yes_____
No_____

III. Developmental Health History. Answer these questions as they pertain to this child.

A. Were there any problems with the pregnancy?

	No	Yes	Unknown	Explain
Illnesses	_____	_____	_____	_____
Cocaine, Heroin, Other Drugs	_____	_____	_____	_____
Alcohol (daily, avg. amt., max amt.)	_____	_____	_____	_____
Cigarettes (avg amt, max)		_____	_____	_____
Medications (name)	_____	_____	_____	_____
Other (explain)	_____	_____	_____	_____

B. Difficulties with the birth?

	No	Yes	Unknown	Explain
Prolonged Labor	_____	_____	_____	_____
Prematurity (# of weeks?)		_____	_____	_____
Low Birth Weight	_____	_____	_____	_____
Neonatal Hospitalization	_____	_____	_____	_____
Jaundice	_____	_____	_____	_____
Other (explain)	_____	_____	_____	_____

C. Problems during infancy and preschool years?

	No	Yes	Unknown	Explain
Feeding Problems	_____	_____	_____	_____
Sleeping Problems	_____	_____	_____	_____

Ear Infections	_____	_____	_____	_____
Abnormal Head Size	_____	_____	_____	_____
Other (explain)	_____	_____	_____	_____

D. Was there delay in any of these areas?

	No	Yes	Unknown	Explain
Walking	_____	_____	_____	_____
Talking Single Words (age first words were spoken)	_____	_____	_____	_____
Talking Sentences (age first used sentences)	_____	_____	_____	_____
Social/Emotional Behavior		_____	_____	_____ _____
Toilet Training	_____	_____	_____	_____

E. Did your child have problems with articulation (understandable pronunciation of words)

No	Yes	Unknown	Explain
____	___	_____	_____

F. Does your child have an attention problem?

	No	Yes	Unknown	Explain
ADD Diagnosed by a Doctor		_____	_____	_____ _____
Distractibility	_____	_____	_____	_____
Trouble Staying on Task	_____	_____	_____	_____
Trouble Switching Tasks	_____	_____	_____	_____
Hyperactivity	_____	_____	_____	_____

G. Are there or were there problems with:

	No	Yes	Unknown	Explain
Fine Motor Coordination (finger, hand)	_____	_____	_____	_____
Gross Motor Coordination (delay, stuttering)	_____	_____	_____	_____
Speech or Language	_____	_____	_____	_____
Vision or Hearing	_____	_____	_____	_____
Bowel or Bladder Training		_____	_____	_____ _____
Mathematics	_____	_____	_____	_____
Other Medical Problems	_____	_____	_____	_____
Behavioral or Emotional Problems	_____	_____	_____	_____

H. Are there or were there problems with responses to sensory stimuli such as touch, sights, or sounds?

	No	Yes	Unknown	Explain
Irritability	_____	_____	_____	_____
Fascination		_____	_____	_____
_____				
Frustration	_____	_____	_____	_____
Other:	_____	_____	_____	_____

IV. Child's Health History

	No	Yes	Unknown	Explain
Allergies	_____	_____	_____	_____
Depression/Mania	_____	_____	_____	_____
Medication that might affect		_____	_____	_____
_____				
test scores (for instance, antidepressants, tranquilizers, antiseizure medications, Ritalin)				
Obsessive-Compuls. Disorder		_____	_____	_____
_____				
Schizophrenia	_____	_____	_____	_____
Seizure Disorder/Epilepsy		_____	_____	_____
_____				
Severe Tics	_____	_____	_____	_____
Other Chronic Illness (specify		_____	_____	_____
_____				

V. Family Health History

Is there a family history of:

	No	Yes	Unknown	Explain
Birth Defects	_____	_____	_____	_____
Severe Tics	_____	_____	_____	_____
Other developmental disorders____		_____	_____	_____
_____				
(for instance, pervasive developmental disorder)				
Mental Retardation	_____	_____	_____	_____

VI. Home Experiences with Music and Reading (please use back of page if needed)

A. Music

1. How would you describe your child's music abilities?

_____ extremely gifted in music

_____ definitely shows talent in music
_____ average when it comes to musical talents
_____ not very musical
_____ extremely unmusical
_____ other (please explain):
_____

2. How does your child feel about music?
_____ absolutely loves it
_____ likes it
_____ I can't tell
_____ does not particularly care for music
_____ hates music; tries to get away from it
_____ other (please explain):
_____

3. Has your child ever had individual music lessons?
_____ yes
_____ no
If yes, please indicate
singing lessons or instrument lessons: _____
if instrument, which one: _____
how old your child was when s/he started: _____
how long your child took these lessons: _____
how many times per week s/he had lessons: _____
how long each lesson was: _____
how much your child practiced at home: _____ (minutes per week)

4. Has your child ever had group music lessons?
_____ yes
_____ no
If yes, please indicate
singing lessons or instrument lessons: _____
if instrument, which one: _____
how old your child was when s/he started: _____

how long your child took these lessons: _____
how many times per week s/he had lessons: _____
how long each lesson was: _____
how much your child practiced at home: _____ (minutes per week)

5. Does your child sometimes start singing just for fun?

_____ yes
_____ no
If yes, about how many times per day does that happen? _____

6. When your child sings by him/herself, how would you describe his/her PITCH?
_____ always perfect pitch
_____ very good pitch in general
_____ sometimes the pitch is off
_____ much of the song is off pitch
_____ totally off pitch; difficult to recognize the tune
_____ other (please explain):
_____

7. When your child sings by him/herself, how would you describe his/her RHYTHM?
_____ always perfect rhythm
_____ very good rhythm in general
_____ sometimes the rhythm is off
_____ in much of the song, the rhythm sounds off
_____ totally wrong rhythm; difficult to recognize the tune
_____ other (please explain):
_____

8. Please describe the music instruction your child receives at school as part of the regular school program:
how many times per week: _____
how long each time: _____

singing or instrument: _____

9. Please describe the experience with music your child has in the context of home and family, if you have not yet described this elsewhere in this questionnaire:

_____
_____
_____
_____
_____

10. Any other comments or insights you might have regarding your child's experience with music:

B. Reading

1. What kinds of reading activities does your child do at home?

2. Please estimate how much time your child spends reading at home in a given week.

3. Have you ever helped your child with his or her reading?_____

   If so, please explain how.

4. Please estimate how much time per week you help your child with reading.

   _____less than 10 minutes
   _____10-30 minutes
   _____30-60 minutes
   _____more than 60 minutes

APPENDIX B

INDIVIDUAL SCORES FOR DEMOGRAPHIC DATA

Subject ID	KBIT Verbal SS	KBIT %	KBIT Nonverbal SS	KBIT %	GFTA SS	GFTA - %
TD01	123	94	118	88	103	58
TD02	110	75	109	73	106	66
TD03	121	92	118	88	103	58
TD04	117	87	121	92	105	63
TD05	101	53	103	58	105	63
TD06	128	97	126	96	103	58
TD07	102	55	100	50	102	55
TD08	101	53	102	55	103	58
TD09	105	63	97	42	105	63
TD10	109	73	113	81	105	63
TD11	112	79	106	66	104	61
CAS01	82	12	84	14	40	0.1
CAS02	108	70	114	82	101	53
CAS03	109	73	110	75	103	58
CAS04	86	18	87	19	42	0.1
CAS05	107	68	111	77	88	21
CAS06	84	14	87	19	40	0.1
CAS07	108	81	114	82	57	0.2
CAS08	94	88	117	87	103	58
CAS09	108	70	105	63	102	55
CAS10	97	79	118	88	104	61
CAS11	104	61	97	42	40	0.1

Note. SS = Standard Score

APPENDIX C

OPERATIONAL DEFINITIONS FOR CAS CHARACTERISTICS (ADAPTED FROM ZUK, IUZZINI-SEIGEL, CABBAGE, GREEN, & HOGAN, 2018)

1. Vowel distortions: An error in which the vowel is substituted for another phoneme OR in which the vowel is recognizable as a specific phoneme but it is not produced exactly correctly (e.g., not a prototypical production, may sound like it's in between two vowels).
2. Voicing errors: A sound is produced as its voicing cognate (e.g., a /p/ that is produced as a /b/).
3. Distorted substitution: A consonant production error in which a speech sound is recognizable as a specific phoneme but it is not produced exactly correctly (e.g., an /s/ that is produced with lateralization or dentalization).
4. Difficulty in achieving initial articulatory configurations or transitionary movement gestures: Initiation of utterance or initial speech sound may be difficult for child to produce and may sound lengthened or uncoordinated. Also, child may evidence lengthened or disrupted coarticulatory gestures or movement transitions from one sound to the next.
5. Groping: Prevocalic (silent) articulatory searching prior to onset of phonation.
6. Intrusive schwa (e.g., in clusters): A schwa is added in between consonants. For example, it may be inserted in between the consonants in a cluster (e.g., /blu/ becomes /bə̲lu/). This is NOT considered a "vowel error."
7. Increased difficulty with multisyllabic words: The participant has a disproportionately increased number of errors as the number of syllables increases (as compared to words with fewer syllables).
8. Syllable segregation: Brief or lengthy pause between syllables, which is not appropriate.
9. Slow speech rate and/or slow DDK: Speech rate is not typical. It is slower during production of part (e.g., zziiiiper/zipper) or the whole word (e.g., toommmaatoo/tomato), or decreased speed on DDK tasks.
10. Equal Stress or lexical stress errors: An error in which the appropriate stress is not produced correctly. For example: conDUCT versus CONduct have different stress patterns. It is considered an error if the stress is on the wrong syllable.

APPENDIX D

SPEECH AND LANGUAGE GENES OF INTEREST

Source	*Gene*	Chr.	CytoBand	Locus (hg19)
Gialluisi	*NEGR1*	1	1p31.1	71,868,625-72,748,405
Gialluisi	*DNAH14*	1	1q42.12	225,117,356-225,586,996
Graham & Fisher	*BCL11A (CTIP1)*	2	2p16.1	60,684,329-60,780,633
Gialluisi	*ACTR2*	2	2p14	65,454,829-65,498,390
Laffin et al.	*SPRED2*	2	2p14	65,537,985-65,659,656
Graham & Fisher	*MRPL19*	2	2p12	75,873,909-75,889,334
Graham & Fisher	*GCFC2 (C2ORF3)*	2	2p12	75,889,832-75,938,111
Gialluisi	*VWA3B*	2	2q11.2	98,703,595-98,929,410
Laffin et al.	*CCDC148*	2	2q24.1	159,023,162-159,092,681
Laffin et al.	*PKP4*	2	2q24.1	159,313,476-159,537,940
Laffin et al.	*AK126351*	2	2q24.1	159,514,849-159,591,514
Laffin et al.	*HAT*	2	2q31.1	172,778,935-172,848,600
Laffin et al.	*MAP1D*	2	2q31.1	172,864,804-172,945,587
Laffin et al.	*DLX1*	2	2q31.1	172,950,208-172,954,401
Laffin et al.	*DLX2*	2	2q31.1	172,964,166-172,967,478
Laffin et al.	*PDK1*	2	2q31.1	173,420,779-173,463,862
Laffin et al.	*AL157450*	2	2q31.1	173,587,917-173,600,934
Laffin et al.	*CGEF2*	2	2q31.1	173,686,315-173,917,620
Laffin et al.	*MLK7-AS1*	2	2q31.1	174,062,441-174,146,764
Laffin et al.	*CDCA7*	2	2q31.1	174,219,561-174,233,718
Laffin et al.	*PDE11A*	2	2q31.1	178,487,977-178,937,482
Graham & Fisher	*PLCL1 (PRIP)*	2	2q33.1	198,669,426-199,014,608

Gialluisi	*TM4SF20*	2	2q36.3	228,226,874-228,244,022
Gialluisi	*CNTN4*	3	3p26.3-p26.2	2,140,550-3,099,645
Gialluisi	*ZNF385D*	3	3p24.3	21,462,490-21,792,816
Graham & Fisher	*SCN11A*	3	3p22.2	38,887,260-38,992,052
Graham & Fisher	*FOXP1*	3	3p13	71,003,865-71,180,092
Graham & Fisher	*ROBO2*	3	3p12.3	77,089,294-77,699,114
Graham & Fisher	*ROBO1*	3	3p12.3	78,646,388-79,817,059
Spinorerebellar	*RUBCN*	3	3q29	197,395,738-197,463,797
Graham & Fisher	*NFXL1*	4	4p12	47,849,258-47,916,633
Peter 2016	*C4orf21 (ZGRF1)*	4	4q25	113,460,489-113,558,151
Graham & Fisher	*CTNND2*	5	5p15.2	10,971,952-11,904,110
Peter 2016	*MYO10*	5	5p15.1	16,662,016-16,936,385
Peter 2016	*CDH18*	5	5p14.3	19,473,155-19,988,353
Peter 2016	*NIPBL*	5	5p14.3	36,876,861-37,065,921
Julie Miller	*HOMER1*	5	5p14.3	78,669,647-78,809,659
Gialluisi	*CSNK1A1*	5	5q32	148,875,457-148,931,115
Graham & Fisher	*DCDC2*	6	6p22.3	24,171,983-24,358,280
Graham & Fisher	*KIAA0319*	6	6p22.3	24,544,332-24,646,383
Laffin et al.	*DST*	6	6p12.1	56,322,785-56,819,426
Laffin et al.	*BEND6*	6	6p12.1	56,819,773-56,892,142
Laffin et al.	*BAG2*	6	6p11.2	57,037,104-57,050,012
Laffin et al.	*RAB23*	6	6p11.2	57,053,582-57,087,078
Laffin et al.	*PRIM2*	6	6p11.2	57,182,422-57,513,376
Spinorerebellar	*SNX14*	6	6q14.3	86,215,215-86,303,629

Gialluisi	*UTRN*	6	6q24.2	144,612,873-145,174,170
Gialluisi	*MLLT4*	6	6q27	168,336,080-168,597,552
Gialluisi	*KIF25*	6	6q27	168,418,553-168,445,769
Gialluisi	*HGC6.3*	6	6q27	168,376,604-168,377,619
Gialluisi	*FRMD1*	6	6q27	168,456,464-168,479,839
Julie Miller	*CAMK2B*	7	7p13	44,256,749-44,365,230
Graham & Fisher	*AUTS2*	7	7q11.22	69,063,905-70,257,885
Gialluisi	*CACNA2D1*	7	7q21.11	81,579,418-82,073,031
Graham & Fisher	*IMMP2L*	7	7q31.1	110,303,106-111,202,573
Graham & Fisher	*DOCK4*	7	7q31.1	111,366,164-111,846,462
Gialluisi	*ZNF277*	7	7q31.1	111,846,643-111,983,989
Graham & Fisher	*FOXP2*	7	7q31.1	114,055,052-114,333,827
Gialluisi	*FLNC*	7	7q32.1	128,470,483-128,499,328
Graham & Fisher	*CNTNAP2 (CASPR2)*	7	7q35	145,813,453-148,118,088
Gialluisi	*MSRA*	8	8p23.1	9,911,830-10,286,401
Laffin et al.	*AK056897*	8	8q11.23	54,427,731-54,436,491
Julie Miller	*NTRK2*	9	9q21.33	87,283,466-87,638,505
Laffin et al.	*LOC169834 (ZNF883)*	9	9q32	115,759,400-115,774,472
Laffin et al.	*RAPGEF*	9	9q34.13	134,452,157-134,585,229
CAS	*SETX*	9	9q34.13	135,136,827-135,230,372
Spinorerebellar	*PMPCA*	9	9q34.13	139,305,116-139,318,213
Gialluisi	*CTNNA3*	10	10q21.3	67,679,725-69,455,949
Julie Miller	*CREM*	10	10p11.21	35,416,385-35,501,886
Gialluisi	*DCDC5*	11	11p14.1-p13	30,900,091-31,128,507
Gialluisi		11	11q11-q12.1	

Spinorerebellar	*ATM*	11	11q22.3	108,093,559-108,239,826
Gialluisi	*UBASH3B*	11	11q24.1	122,526,398-122,685,187
Graham & Fisher	*ERC1 (ELKS)*	12	12p13.33	1,100,404-1,605,099
Julie Miller	*GRIN2B*	12	12p13.1	13,714,410-14,133,022
Graham & Fisher	*GNPTAB*	12	12q23.2	102,139,275-102,224,645
Spinorerebellar	*SACS*	13	13q12.12	23,902,962-24,007,867
Laffin et al.	*RFXAP*	13	13q13.3	37,393,339-37,403,740
Laffin et al.	*SMAD9*	13	13q13.3	37,418,968-37,494,409
Laffin et al.	*ALG5*	13	13q13.3	37,523,908-37,573,504
Laffin et al.	*EXOSC8*	13	13q13.3	37,574,678-37,583,751
Laffin et al.	*FAM48 (SUPT2OH)*	13	13q13.3	37,583,451-37,633,850
Bartlett 2004		13	13q21.1-q22.2	chr13:57,044,422-77,086,521
Graham & Fisher	*NOP9*	14	14q12	24,769,098-24,774,374
Gialluisi	*CHRNA7*	15	15q13.3	32,322,686-32,462,384
Graham & Fisher	*DYX1C1*	15	15q21.3	55,722,506-55,800,432
Spinorerebellar	*STUB1*	16	16p13.3	730,115-732,768
Graham & Fisher	*GNPTG*	16	16p13.3	1,401,900-1,413,352
Graham & Fisher	*NAGPA*	16	16p13.3	5,074,845-5,083,942
Laffin et al.	*ABAT*	16	16p13.2	8,768,444-8,878,432
Laffin et al.	*PMM2*	16	16p13.2	8,891,670-8,943,194
Graham & Fisher	*GRIN2A (NR2A)*	16	16p13.2	9,847,265-10,276,263
Laffin et al.	*CARSHP1 (CDH1)*	16	16q22.1	68,771,195-68,869,444
Graham & Fisher	*CMIP*	16	16q23.2	81,478,775-81,745,367
Graham & Fisher	*ATP2C2*	16	16q24.1	84,402,133-84,497,793

Gialluisi	*GABARAP*	17	17p13.1	7,143,738-7,145,753
Peter 2016	*GLP2R*	17	17p13.1	9,729,381-9,793,022
Peter 2016	*NCOR1*	17	17p12-p11.2	15,933,408-16,118,874
Peter 2016	*FLCN*	17	17p11.2	17,115,527-17,140,502
Peter 2016	*SMCR8*	17	17p11.2	18,218,594-18,231,370
Peter 2016	*NEK8*	17	17q11.2	27,055,832-27,069,784
Gialluisi	*ACCN1 (ASIC2)*	17	17q11.2-q12	31,340,106-32,483,825
Spinorerebellar	*CACNA1G*	17	17q21.33	48,638,429-48,704,832
Peter 2016	*ANKRD12*	18	18p11.2	9,136,751-9,285,983
Graham & Fisher	*SETBP1*	18	18q12.3	42,260,863-42,648,475
Gialluisi	*DAZAP1*	19	19p13.3	1,407,584-1,435,682
Gialluisi	*ZNF737*	19	19p12	20,720,798-20,748,626
Gialluisi	*ABCC13*	21	21q11.2	15,646,120-15,673,692
Gialluisi	*PCNT*	21	21q22.3	47,744,036-47,865,682
Gialluisi	*DIP2A*	21	21q22.3	47,878,862-47,989,926
Gialluisi	*S100B*	21	21q22.3	48,018,531-48,025,035
Gialluisi	*PRMT2*	21	21q22.3	48,055,507-48,085,155
Gialluisi	*RBFOX2*	22	22q12.3	36,134,783-36,424,585
Gialluisi	*CXorf22*	X	Xp21.1	35,937,851-36,008,269
Graham & Fisher	*PCDH11X*	X	Xq21.31	91,090,460-91,878,228
Graham & Fisher	*SRPX2*	X	Xq22.1	99,899,163-99,926,296
Graham & Fisher	*PCDH11Y*	Y	Yp11.2	4,924,131-5,610,264