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Susannah Jaeger More
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The Dissertation (or Treatise) Committee for Susannah Jaeger More Certifies that
this is the approved version of the following dissertation (or treatise):

Analysis of the Caudate Nucleus and Attention in Children with 18q-
Treated with Growth Hormone

Committee:

Margaret Semrud-Clikeman, Supervisor

Timothy Keith

Daniel Robinson

Marie Suizzo

Jannine Cody
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Analysis of the Caudate Nucleus and Attention in Children with 18q-
Treated with Growth Hormone

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Susannah Jaeger More, Ph.D.
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Supervisor: Margaret Semrud-Clikeman

This study investigated the caudate nucleus, attention, and externalizing problems in children with 18q- before and after growth hormone (GH) treatment. Children with 18q- were referred for participation in this study by the Chromosome 18 Clinical Research Center. Thirteen participants with 18q- and 12 controls participated in the study. Volumetric analysis of the caudate nucleus using MRI revealed no significant differences in caudate volume and asymmetry among the 18q- participants pre and post treatment and controls. The 18q- participants and controls showed caudate symmetry (L = R), which is contrary to the L > R asymmetry expected in controls and post growth hormone participants. The 18q- participants, pre and post treatment, showed greater attention problems, although mild, than the control group. There was no difference in externalizing problems among the groups. No change in behavioral functioning with GH treatment was found in the 18q- participants. Caudate volumes were found to decrease with age in controls, but this age-related decrease was not observed in 18q- participants. This finding suggests a disruption in the neural system that likely has functional
implications requiring further investigation. Results of this study will contribute to a
greater understanding of the relationship between brain morphology and cognitive and
behavioral functioning in 18q-.
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Chapter 1: Introduction

18q- is a disorder resulting from a partial deletion of the long arm (q) of chromosome 18. The clinical presentation is variable, but individuals with deletions of 18q often have dysmyelination of the brain, growth failure with abnormal growth hormone production, and developmental delays (Gay et al., 1997; Ghidoni et al., 1996; Miller, Mowrey, Hopper, Frankel, & Ladda, 1990; Strathdee, Zackai, Shapiro, Kamholz & Overhauser, 1995). Much of the research describes the clinical features of 18q- (Cody et al., 1999), but fewer studies have focused on behavioral functioning and neuroanatomical correlates of the disorder.

Symptoms of attention-deficit/ hyperactivity disorder (ADHD) have been reported in individuals with 18q-, including inattention, disinhibition, hyperactivity, and aggressive behavior (Hansen & Herlin, 1994; Mahr et al., 1996). Children with ADHD have shown structural differences of the caudate nucleus when compared to typically developing children. Smaller left caudate volumes and reversed caudate asymmetry have been reported in children with ADHD (Hynd et al., 1993; Filipek et al., 1997). This asymmetry was related to the severity of disinhibition and externalizing problems (Semrud-Clikeman et al., 2000). Individuals with 18q- have shown dysmyelination of the brain, including the caudate, based on magnetic resonance imaging (MRI) scans (Cody et al., 2005; Kline et al., 1993; Marwaha et al., 1992). The behavioral implications of dysmyelination of the caudate in 18q- are not understood. Considering structural differences of the caudate are related to ADHD symptoms, dysmyelination of the caudate in 18q- may also be related to problems with inattention, hyperactivity, and impulsivity.
Further research is needed to understand the relationship between dysmyelination of the caudate and attentional factors in children with 18q-.

Preliminary findings have identified changes in T1 weighted scans suggestive of increased myelination in the caudate following growth hormone treatment in children with 18q- (Cody et al., 2005). Growth hormone and insulin-like growth factor-I (IGF-I), a mediator of growth hormone action, have receptors in the caudate (Adem et al., 1989; Burman, Hetta & Karlsson, 1993). IGF-I has been shown to affect myelin formation (De Vellis, 1990). Because IGF-I values are often below normal in children with 18q-syndrome (Hale et al., 2000), and growth hormone affects the production of IGF-I, growth hormone treatment may lead to increased myelination of the caudate.

Growth hormone receptors have been found in the caudate (Adem et al., 1989; Burman et al., 1993), suggesting that growth hormone may affect cognitive functions, such as attention and inhibition (Heilman, Voeller, & Nadeau, 1991). Growth hormone also has been implicated in affecting dopamine activity, a neurotransmitter that affects attention and inhibition and is supplied to the caudate (Deijen, Boer, Blok & Veen, 1996; Ernst et al., 1999). Improvements in attention have been observed in children with growth hormone deficiency following growth hormone treatment (Soares et al., 1999; Van Dan et al., 2000). The effects of growth hormone on the caudate and attentional factors in 18q- has not been investigated.

The purpose of this study is to gain further understanding of the brain-behavior relationship in 18q- through the investigation of the caudate and attention problems. This study will also contribute to the understanding of the effects of growth hormone on the caudate, attention, and externalizing behaviors such as aggression and hyperactivity, potentially assisting in the treatment planning for individuals with 18q-. Further understanding of the attention and behavior problems and effects of growth hormone
treatment on children with 18q- will help medical professional, parents, and educators make appropriate educational and behavioral plans for children with 18q-.

In the current study, caudate volumes of 18q- participants and controls will be evaluated on MRI scans. Attention and externalizing behaviors will be assessed through a behavior rating scale completed by parents. Based on previous findings, it is expected that individuals with 18q- will show smaller caudate volumes and will not show the caudate asymmetry expected in typically developing controls. Participants with 18q- are also expected to have greater problems with attention and externalizing behaviors. Those children with 18q- who qualify for growth hormone treatment are hypothesized to show improvements in attention and externalizing behaviors following growth hormone therapy. The children treated with growth hormone are also hypothesized to show an increase in caudate volume and caudate asymmetry, reflecting the asymmetry of the control group. An inverse relationship between caudate volume and asymmetry and externalizing behavior ratings is also expected. Overall, this study will contribute to the understanding of development in 18q- and to the effects of growth hormone on brain maturation, attention, and externalizing behaviors.
Chapter 2: Review of the Literature

Characteristics of 18q-

18q- is a disorder resulting from the partial deletion of the long arm of chromosome 18. 18q- was first described by DeGrouchy, Roper, and Salamon (1964) and is estimated to occur in approximately 1/40,000 live births with a female to male ratio of 1.7/1 (Cody et al., 1997). The phenotypic features of individuals with 18q- vary widely. The more common characteristics include dysmyelination of the brain, developmental delay, growth deficiency with abnormal growth hormone production, and hearing impairment (Ghidoni et al., 1997; Jayarajan, Swan, & Patton, 2000; Miller et al., 1990; Strathdee et al., 1995; Wertelecki & Gerald, 1971). Growth retardation, developmental delay, mental retardation, hypotonia, flat nasal bridge and tapered fingers have been reported in over 80% of individuals with 18q- (Felding, Kristoffersson, Sjorstrom, & Noren, 1987; Wilson, Towner, Forsman, & Siris, 1979). Other common characteristics include delayed expressive language and proximally placed thumbs (Cody et al., 1999; Gay et al., 1994; Miller et al., 1990). Table 1 lists common features observed in individuals with 18q deletions (Beck et al., 1998; Cody et al., 1999, Gay et al., 1998; Hale et al., 2000).
Table 1

*Characteristics of Individuals with 18q-*

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>% of Individuals</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dysmyelination of the central nervous system</td>
<td>97</td>
</tr>
<tr>
<td>Speech Failure</td>
<td>91</td>
</tr>
<tr>
<td>Hypotonia</td>
<td>79</td>
</tr>
<tr>
<td>Decreased of absent deep tendon reflexes</td>
<td>76</td>
</tr>
<tr>
<td>Foot deformities</td>
<td>74</td>
</tr>
<tr>
<td>Hearing loss</td>
<td>70</td>
</tr>
<tr>
<td>Mental retardation (IQ&lt;70)</td>
<td>68</td>
</tr>
<tr>
<td>Gait abnormalities</td>
<td>68</td>
</tr>
<tr>
<td>Growth hormone deficiency</td>
<td>68</td>
</tr>
<tr>
<td>Proximally placed thumbs</td>
<td>65</td>
</tr>
<tr>
<td>Atretic/Stenotic ear canals</td>
<td>64</td>
</tr>
<tr>
<td>Tremor</td>
<td>62</td>
</tr>
<tr>
<td>Short stature</td>
<td>61</td>
</tr>
<tr>
<td>Microcephaly</td>
<td>53</td>
</tr>
<tr>
<td>Optic nerve hypoplasia</td>
<td>23</td>
</tr>
<tr>
<td>Autistic features</td>
<td>20</td>
</tr>
</tbody>
</table>

*Note.* From the Chromosome 18 Clinical Research Center at the University of Texas Health Science Center at San Antonio, 2000.

**GENETICS AND 18Q-**

The position of a gene on the long arm (q) or short arm (p) of chromosome 18 is designated using digits based on the bands of the stained chromosome (see Figure 1). The bands are counted from the centromere to the tip or telomeric region (ter) of the chromosome. An individual, for example, may have a deletion or breakpoint at 18q21.1. The first number and letter (q) represents the long arm of chromosome 18. The next digit (21) determines the major band (e.g., 18q21) and the subsequent digits represent the sub-bands (e.g., 18q21.1). 18q- is the term for a terminal deletion of the long arm of chromosome 18 (Strathdee, Harrison, Riethman, Goodart, & Overhauser, 1994), although
interstitial deletions have also been reported (Schinzel et al., 1991; Wilson & Al Saadi, 1989; Wilson et al., 1979). Brkanac, Cody, Leach, and DuPont (1998) analyzed DNA from 35 patients who were originally identified as having terminal deletions of chromosome 18, but further analysis identified three subjects with interstitial deletions.

Figure 1. Chromosome 18 Ideogram. Adapted from National Center for Biotechnology Information website http://www.ncbi.nih.gov.

Strathdee et al. (1995) investigated 26 patients having terminal deletions with breakpoints ranging from 18q21.1 to 18q22.3. Abnormal or delayed myelination, developmental delay, decreased growth, and behavioral problems were common findings in this population, although no correlation between deletion size and the severity of the resulting phenotype was found. A following study reported that the critical region for 18q- maps to 18q23 (Strathdee et al., 1997). Kline et al. (1993) hypothesized that the size of the deletion, with breakpoints ranging from 18q21.3 to 18q22.2, may be related to the clinical phenotype in seven subjects. Lower limb abnormalities, hypotonia, and some
dysmorphic facial features mapped to the most distal part of 18q while microcephaly and moderate to severe mental retardation mapped to the more proximal areas of 18q. Cody et al. (1999) showed a correlation between the extent of 18q deletion and head circumference, external ear length, and proximal or anomalous thumbs. Leach et al. (1999) found that individuals with the most proximal breakpoints on chromosome 18q had severe mental retardation, microcephaly, and were unable to walk. This region also contains the gene coding for DCC (deleted in colon cancer), which has been shown to be involved in neuronal migration and to interfere with the ability to walk in mouse models. The DCC gene is hypothesized to be associated with the severity of neurological development in the 18q- phenotype in individuals with large deletions (Leach et al., 1999).

Mahr et al. (1996) studied 27 individuals with abnormal myelination, behavioral problems, and cognitive deficits who had breakpoints ranging from 18q21.2 to 18q22.3, but found no relationship between deletion size and phenotype. Hansen and Herlin (1994) reported a case of a boy with a terminal deletion and breakpoint at 18q22.1, who presented with deficits in attention and motor skills. Wilson et al. (1979) described a girl with a deletion at 18q21.3, who presented with irritability, low frustration tolerance, mental retardation, and small stature. Additional studies of individuals with breakpoints ranging from 18q21 to 18q22.3 report dysmyelination, mental retardation, developmental delays, and abnormal growth (Felding et al., 1987; Miller et al., 1990; Ono, Harada, Yamamoto, Onoe, & Okada, 1994; Vogel et al., 1990; Weiss et al., 1991; Wilson et al., 1979).

Myelin basic protein (MBP) is important for normal myelogenesis, which occurs primarily in the first 18 months of life (Kamholz et al., 1987; Wrabetz et al., 1990). Oligodendrocytes are support, or glial, cells of the brain that form a covering of
myelin on nerve cells to speed nerve impulses (Kolb & Whishaw, 1996). The gene for myelin basic protein (MBP), involved in cerebral myelination, is expressed by oligodendrocytes prior to myelin formation (De Vellis, 1990) and maps to 18q23 near the telomere of chromosome 18q, the area often deleted in 18q- syndrome (Kamholtz et al., 1987; Saxe, Takahashi, & Hood, 1985). Individuals with abnormal white matter have been found to be deficient for one copy of the MBP gene. In contrast, people with interstitial deletions of chromosome 18q with two copies of the MBP gene were found to have normal white matter (DuPont et al., 1995). A chromosomal analysis of children with abnormal myelin revealed deletions ranging from a proximal breakpoint at 18q21.1 to more distal breakpoints at 18q23 (Gay et al., 1997). The individuals with abnormal MRI scans were found to be missing a two megabase region that contains one copy of the gene that encodes for MBP, suggesting that incomplete cerebral myelination in 18q- is related to the haploinsufficiency of the gene MBP (Gay et al., 1997). Haploinsufficiency occurs when a single copy of a gene cannot provide sufficient protein production to assure normal function. Thus, deletion of one copy of the MBP gene may be related to neurological abnormalities found in 18q- (Strathdee et al., 1995; Weiss et al., 1991). Cody et al. (1997) found that 29 of 34 individuals had deletions that were paternal in origin, while Strathdee et al. (1995) found no parental origin bias in 15 individuals with 18q-. Cody et al. (1997) hypothesized that the prevalence of paternally derived deletions is due to an increased frequency of chromosome breakage in male germ cells. The region of chromosome 18 that can be deleted is thought to contain as many as 350 genes, most of which have not yet been identified (Cody et al., 2000).

**Behavioral Presentation and Cognitive Development in 18q-**

A range of problematic behaviors have been reported in case studies of children diagnosed with 18q-, such as inattention, distractibility, impulsivity, and hyperactivity
(Hansen & Herlin, 1994; Lewkonia, Lin, & Haslam, 1980; Schinzel, 1981; Thompson et al., 1998). Overall, it is estimated that approximately 35% of children with 18q- show symptoms of attention-deficit/hyperactivity disorder, including inattention, impulsivity, disinhibition, distractibility, and hyperactivity. Stimulant medication, such as methylphenidate, helps alleviate symptoms (Oster-Granite et al., 2001). Aggressive behavior has also been reported in children with deletions of chromosome 18q (Bourgeois, Broustet, & Benezech, 1974; Kovnats, & Manorajan, 1991; Lejeine, 1977; Strathdee et al., 1995; Wilson et al., 1979), as well as autistic tendencies (Mahr et al., 1996; Schinzel et al., 1991; Sheshardi et al., 1992; Wilson & Al Saadi, 1989).

Mahr et al. (1996) investigated 27 individuals with 18q- with chromosomal breakpoints ranging from 18q22.3 to 18q21.2. Behavioral problems included inattention, impulsivity, aggression, and temper outbursts. Intelligence ranged from borderline to severely impaired, and moderate to severe deficits were noted across the domains of attention, cognitive flexibility, problem solving, verbal and visual memory, language, motor skills, and visual-spatial ability. Eighty-one percent of the individuals had abnormal MRI findings. No relationship was found between deletion size and phenotype, nor were there any correlations between any measures of cognitive and behavior and MRI results (Mahr et al., 1996). Intelligence was also reported to range from borderline to severely impaired by Schinzel (1984) in children with 18q-, and mental retardation was reported in 87% of the individuals studied by Strathdee et al. (1995).

**Structural Brain Abnormalities and Neuroimaging in 18q-**

Structural brain abnormalities related to myelination have been reported in 18q-, based on neuroimaging studies (Felding et al., 1987; Hardies et al., 2001; Mahr et al., 1996; Miller et al., 1990). Myelin is a substance that forms an insulating sheath around nerve fibers to increase nerve conduction velocity. Myelin is mainly comprised of lipids
and structural proteins and is made by oligodendrocytes, which are support cells, or glial cells, in the central nervous system (Kolb & Whishaw, 1996). The myelination process begins in the primary and sensory motor areas starting with the cranial nerves in the fifth fetal month and occurs most rapidly during the first two to three years of life, although myelination continues through adolescence and into adulthood (Brody et al., 1987; Holland, Haas, Normal, Brant-Zawadzki, & Newton, 1986; Yakolev, & Lecours, 1967). During the first two years, maturation of myelin occurs in the basal ganglia, major white matter tracts, anterior frontal-temporal central white matter, and subcortical association fibers (Kinney et al., 1988).

A disruption in the myelination process results in neural, and therefore behavioral, abnormalities (Kolb & Whishaw, 1996). Myelination can be analyzed using magnetic resonance imaging (MRI) (Barkovich, Kjos, Jackson, & Norman, 1988). White matter development becomes brighter on T1-weighted MRI images and darker on T2-weighted images as the brain matures, indicating shortening of the T1 and T2 relaxation time constants. On T1 and T2 weighted images on MRI scans, relaxation times are longer in the infant brain than in adults, reflecting decreased myelination and high water content of the neonatal brain (Barokovich et al., 1988; Bird et al., 1989). The head of the caudate attains adult relaxation values by 1.5 to two years of age (Holland et al., 1986), with development of the caudate area occurring from approximately age two months to age two to three years (Yakovlev & Lecours, 1967). MRI relaxometry has been used to quantify myelination, showing that changes in T1 and T2 values correlate with changes in myelin content (Holland et al., 1986). Thus, children with abnormal myelination are found to have longer relaxation times than typically developing children.

Abnormal or delayed myelination have been reported in several cases of individuals with 18q deletions, based on MR imaging (Felding et al., 1987; Hardies et al.,
2001; Mahr et al., 1996; Miller et al., 1990; Vogel et al., 1990; Weiss et al., 1991). Gay et al. (1997) reported incomplete myelination in 20 children with short stature, mental retardation, and hypotonia. Evidence for age-related maturation in white matter until age four, with the most pronounced age-related changes between five months and four years of age was also found, suggesting there are factors contributing to myelinogenesis. The myelin content of brain white matter, however, was still reduced at all ages. An investigation of 26 individuals showed evidence of abnormal or delayed myelination, as well as decreased growth, motor impairment, and mental retardation (Strathdee et al., 1995). Lancaster et al. (2005) also reported decreased myelin production, delayed onset of myelination, and reduced rate of myelination in children with 18q- when compared to typically developing children.

Other case studies of structural abnormalities have been reported in 18q-. Ono et al. (1994) observed abnormal myelination in the peripheral white matter but normal corpus callosum in a three-year-old boy with short stature, microcephaly, and delayed motor development. In contrast, Kochunov et al. (2005) found that the corpus callosum was smaller in 12 children with 18q- when compared to same age normal controls. Thinning of the corpus callosum was reported by Bekiesinska-Figatowska and Walecki (2001), in addition to hypoplasia of the anterior pituitary and enlarged ventricles. Ventricular enlargement and reduced white matter was also observed by Vogel et al. (1990), as well as cerebellar hypoplasia.

Differences in the caudate area of the brain in children with 18q- have also been reported. Hypomyelination within the white matter tracks of the basal ganglia, which include the caudate, was observed by Kline et al. (1993). Findings by Cody et al. (2005) indicated that individuals with 18q- have reduced T1 relaxation times in the caudate of the white matter after growth hormone therapy when compared to T1 values before
treatment. Thus, the data suggests that the caudate T1 on MRI was significantly reduced by growth hormone treatment (Cody et al., 2005). Considering T1 relaxation times have been shown to be shorter in typically developing children than in children with 18q- (Gay et al., 1997), and shorter relaxation times reflect greater myelination (Barokovich et al., 1988; Bird et al., 1989), it is possible that an increase in myelination of the caudate accounts for the reduced T1 relaxation times after growth hormone treatment in the study by Cody et al. (2005). Children with isolated growth hormone deficiency, which is commonly detected in children with 18q-, have also been observed to have infarcts of the caudate (Marwaha et al., 1992).

GROWTH HORMONE AND 18Q-

Children with 18q- are short in stature (mean height 2.6 SD below the mean) and have a slow growth velocity (mean velocity 1.89 SD below the mean). Furthermore, insufficient production of growth hormone is a common finding in people with 18q- (Ghidoni et al., 1996; Schwartz & Duck, 1990). Hale et al. (2000) reported that 64% of the children in their sample have a height more than -2 SD below the mean, 68% have a growth velocity more than -1 SD below the mean, and 50% show delayed bone maturation.

Growth hormone secretion from the anterior pituitary gland is regulated primarily by two peptides synthesized in the hypothalamus. Growth hormone-releasing factor (GRF) or somatocrinin is the hormone involved in the stimulation of growth hormone secretion from the pituitary, and somatostatin (SRIF) is responsible for the inhibition of growth hormone secretion from the pituitary gland. Secretion of growth hormone increases during sleep, exercise, and puberty. Factors such as glucose, free fatty acids, clonidine, the amino acid arginine, and the neuropeptide galanin also affect growth hormone secretion (Strobl & Thomas, 1994).
The clinical signs of isolated growth hormone deficiency are growth retardation, with height two standard deviations below average and growth velocity at least one standard deviation below average based on age and gender. The diagnosis is determined by finding a deficit in growth hormone secretion (<10 ng/ml) in response to one or more provocative stimuli, such as clonidine and arginine (Hale et al., 2000). Individuals with idiopathic growth deficiency are below the fifth percentile in height but do not meet the criteria for growth hormone deficiency (Hale et al., 2000).

Children diagnosed with 18q- who are growth hormone deficient have shown improvements in growth with growth hormone therapy (Andler et al., 1992; Schwarz & Duck, 1990). Response to growth hormone replacement therapy is maximal during the first to second year of treatment and is more effective in younger children than adolescents (Strobl & Thomas, 1994). Individuals with mutations or deletions of the growth hormone receptor gene are insensitive to growth hormone and are functionally growth hormone deficient, despite having normal growth hormone concentrations (Rosenfeld et al., 1995).

Growth hormone deficiency is likely affected by deletions within the growth hormone gene cluster (Braga et al., 1986; Goosens et al., 1986) or by mutations in the gene for pituitary transcription factors involved in growth hormone expression (Strobl & Thomas, 1994). Ghidoni et al. (1997) reported five females with 18q- and growth hormone deficiency who showed chromosomal deletions from regions 18q21.3 to 18q23. Cody et al. (1997) identified a 2 Mb critical region of chromosome 18 that is hemizygous, meaning only one copy of the gene is present, in people with growth hormone deficiency. The gene GALNR1 (galanin receptor 1) has been mapped to region 18q23 and is often deleted in patients with 18q- syndrome with growth hormone insufficiency. Its ligand, galanin, enhances growth hormone release mainly via
hypothalamic actions and stimulates basal acetylcholine release in the striatum (Bedecs, Berthold, & Bartfai, 1995).

Hormones such as insulin-like growth factor-I and II (IGF-I, IGF-II) also play a role in regulating growth hormone secretion (Strobl & Thomas, 1994). Growth hormone and IGF-I, a mediator of growth hormone action, are able to cross the blood brain barrier and have a wide distribution of receptors in the brain (Jonannson et al., 1995). IGF-I appears to play a role as a local neuroregulator and brain growth factor (Sara et al., 1982), and has been shown to be involved in the stimulation of nerve and glial cell proliferation and neuronal differentiation (Pahlman, Meyerson, Lindgren, Schalling, & Johansson, 1991). IGF-I is also involved in the regulation of gene expression in oligodendrocytes, which express myelin basic protein prior to myelin formation (De Vellis, 1990). In children with 18q-, 72% of IGF-I values and 83% of the IGF-binding protein-3 values, the major carrier protein for both IGF-I and IGF-II, were below average based on age (Hale et al., 2000).

Considering IGF-I receptors and growth hormone receptors have been found in the caudate nucleus, putamen, cerebral cortex, pituitary, hypothalamus, amygdala, hippocampus, choroid plexus, and cerebellum (Adem et al., 1989; Burman, Hetta & Karlsson, 1993), growth hormone may affect cognitive functions related to these areas, such as attention, memory, and motivation. Growth hormone has been implicated in affecting neurotransmitter activity, considering growth hormone treatment has been found to reduce the cerebrospinal fluid concentration of homovanilic acid, a dopamine metabolite (Burman et al., 1993). Thus, a reduction in growth hormone may alter dopamine turnover, affecting processes such as attention which is affected by dopamine (Deijen et al., 1996; Ernst et al., 1999). Shaywitz et al. (1982) found that in children diagnosed with attention-deficit/ hyperactivity disorder, methylphenidate, a dopamine
reuptake inhibitor, increased plasma growth hormone levels. Furthermore, the concentration of methylphenidate correlated with improvement in a behavior rating scale for attention, hyperactivity, and impulsivity problems.

Although the exact mechanism of how growth hormone may affect cognition is unknown, studies show that growth hormone does impact cognitive functioning. Problems with attention have been reported in children with growth hormone deficiency (Galatzer, Aran, Nagelberg, Rubitzek, & Laron, 1993; Sartorio et al., 1996). Following growth hormone treatment, improvements in attention have been observed (Soares et al., 1999; Van Dan et al., 2000), suggesting that children with growth hormone deficiency and attention deficits can be helped with growth hormone therapy. Johansson et al. (1995) showed that adults with growth hormone deficiency often reported difficulties with concentration and memory, which improved with growth hormone treatment.

A study with males and females diagnosed with growth hormone deficiency and idiopathic short stature showed higher rates of externalizing behaviors on the Child Behavior Checklist (Achenbach, 1990), such as impulsivity, hyperactivity, distractibility, and aggression. Furthermore, they showed greater levels of internalizing behaviors as well as low academic achievement (Stabler et al., 1994). Abbott, Rotnem, Genel, and Cohen (1982) also reported that hypopituitary children tend to show difficulties in modulation of aggression and emotional adjustment but found no improvements in cognitive, academic, and emotional functioning after one year of growth hormone therapy.

Cody et al. (1999) found that 68% of children with 18q- had mental retardation, and 66% of the individuals qualified for growth hormone replacement therapy as a result of growth failure. Individuals treated with growth hormone were compared with untreated subjects for changes in cognitive functioning and height (Cody et al., 2005).
The 18q- participants receiving growth hormone treatment showed an average nonverbal IQ increase of 16.8 points, and the untreated group showed a mean IQ change of -3.0 points. The average height increase for the growth hormone treated group was a z-score of +1.8. The untreated group showed an average z-score change of –0.27 (Cody et al., 2005). Adaptive behavior changes in response to growth hormone treatment were also evaluated in children with 18q-, but no differences in adaptive functioning were found between the treated and untreated groups (More, Semrud-Clikeman, Cody, Hale, & Leach, 2001).

Children under age five with below normal head circumference due to isolated growth hormone deficiency have shown an increase in head circumference with human growth hormone treatment, which may be a reflection of actual brain development (Laron, Roitman, & Kauli, 1979). Furthermore, children receiving human growth hormone therapy showed an increase in social adjustment and IQ, especially before age five, and the increase in IQ paralleled an increase in head circumference (Laron & Galatzer, 1980). The average head circumference change in children with 18q- syndrome treated with growth hormone, however, was not found to be significantly different from the untreated group (Cody et al., 2000).

In children with 18q- who showed evidence of dysmyelination on MRI, preliminary data indicated improvements in T1 values with growth hormone treatment (Hardies et al., 2001). Preliminary findings indicate that individuals with 18q-, who often show evidence of inattention, hyperactivity, and aggressive behavior, were treated with growth hormone therapy and found to have reduced T1 relaxation times in the caudate of the white matter (Cody et al., 2005). Further research is needed to investigate the caudate and attentional factors in this population, as well as the effects growth hormone treatment has on the caudate and attention/ hyperactivity.
ATTENTION DEFICITS AND THE CAUDATE

The striatum is composed of the caudate nucleus and putamen, which are part of a collection of nuclei called the basal ganglia. Several cortical areas send projections to the striatum, which sends further output to the globus pallidus and the substantia nigra in the midbrain, which then project to the thalamus. The thalamic regions then send projections back to the cortical areas (Alexander et al., 1986; Caplan et al., 1990; Heilman et al., 1991). The caudate and putamen also receive the neurotransmitter dopamine from the substantia nigra (see Figure 2), which then supply dopamine to the frontal cortical areas (Gerfen, Keefe, & Steiner, 1996). The putamen receives input from the motor areas and sends projections to the substantia nigra and globus pallidus, and then to the thalamus, thus playing a role in the programming and control of movement (Alexander et al., 1986).

Figure 2. The dopamine system showing projections to the caudate. Adapted from Kandel et al. (1991).
The frontostriatal regions have connections between the caudate nucleus head (Alexander et al., 1987) and the prefrontal areas, mainly the orbitofrontal and dorsolateral areas (Goldman-Rakic, 1987). The head of the caudate nucleus receives projections from the prefrontal cortex (Oberg & Divac, 1977). The dorsolateral circuit, involved in executive function, includes the dorsolateral cortex, caudate nucleus head, internal pallidus, thalamus, and the dorsal cortex of the frontal lobes. The orbitofrontal circuits are formed by two parallel subcircuits. The lateral orbitofrontal circuits send projections to the caudate nucleus, and the medial orbitofrontal circuits send projections to the ventral putamen and accumbens nucleus. The orbitofrontal circuits project to the internal globus pallidus and to the substantia nigra, which then project to the thalamus. Both circuits return to the lateral and medial orbitofrontal cortices and have been associated with behavioral and emotional control. Damage to the caudate or its connections to the prefrontal cortex can result in impulsivity and conduct problems (Alexander et al., 1986).

The third circuit is the anterior cingulate circuit, related to motivational and inhibitory control, which projects connections from the anterior cingulate cortex to the caudate nucleus, putamen, and accumbens nucleus. The circuit continues with projections to the internal globus pallidus and substantia nigra and then to the anterior cingulate cortex (Alexander et al., 1986; Chow & Cummings, 1999). Disruptions of these circuits could result in problems related to self-regulation and cognitive activity (Pineda et al., 2002; Zametkin & Liotta, 1998).

The caudate and frontal areas have been implicated in attention (Posner & Peterson, 1990; Posner & Raichle, 1994), disinhibition (Zametkin & Liotta, 1998), and motor activity (Caplan et al., 1990). Interference with these regions or the dopaminergic system results in impaired attention, impulsivity, hyperactivity, and distractibility seen in individuals with attention disorders (Himelstein et al., 2000). Attention-deficit/
hyperactivity disorder (ADHD) has been regarded as a dopamine-based frontostriatal disorder (Bradshaw & Sheppard, 2000). The findings of Ernst et al. (1999) are suggestive of dopaminergic dysfunction in the midbrain of children, and the striatum and prefrontal cortex are dependent on midbrain dopamine input. Alternatively, the midbrain abnormality could be secondary to dysfunction in regions such as the caudate that regulate midbrain dopaminergic activity.

Individuals with ADHD are hypothesized to have deficits in their ability to inhibit or delay behavioral responses (Barkley, 1997) and have been shown to perform poorly on tasks requiring inhibition of response (Casey, 1997; Chelune, Ferguson, Koon, & Dickey, 1986; Randal et al., 1994). This disinhibition, or the inability to control and direct attention (Loge, Staton, & Beatty, 1990), has been associated with dysfunction of the prefrontal cortex and its connections with the striatum, which is related to motor function (Mesulam, 1990). Thus, dysfunction in the caudate and frontal regions would lead to deficits in the arousal-motor regulatory system, resulting in difficulties with inhibition, impulsive behavior, and sustained attention. Lesions involving the frontostriatal regions in adult and animal studies have resulted in hyperactivity and deficits in the arousal-motor regulatory systems (Caplan et al., 1990; Iversen, 1977; Olmstead & Villablanca, 1980). The caudate has been implicated in problems of sustained attention and inhibition seen in ADHD, a developmental disorder without related lesions (Heilman et al., 1991; Voeller, 1991). Mesulam (1990) noted that major neural connections to the head of the caudate come from the prefrontal cortex, so lesions in the head of the caudate result in symptoms seen in individuals with frontal lobe syndrome, such as problems with attention and inhibition.

Children with ADHD also tend to have a greater degree conduct problems, aggressiveness, impulsivity, and acting out or externalizing behaviors (Barkley, 1990;
Henker & Whalen, 1989). Schachar and Logan (1990) found that the ability to inhibit action is developed by second grade, although the inhibitory control does not improve significantly beyond this age in normal children. Children with attention disorders with hyperactivity showed problems with impulsivity, which improved with methylphenidate treatment. The authors suggested that executive functioning involving selection and execution improve with age, based on their task performance.

The caudate nucleus has been studied in children with ADHD, supporting the frontal-striatal connection in attention disorders. Lou, Henriksen, and Bruhn (1984) reported decreased regional blood flow in the caudate nuclei as well as the frontal lobe region in children with attention disorders. A subsequent study showed children with ADHD to have decreased regional blood flow in the right caudate nuclei region, and individuals with co-occurring neurologic disorder (e.g., mental retardation) showed bilateral hypoperfusion in the caudate-striatal region (Lou et al., 1989). Lower activity in the left caudate has also been observed in adolescents with ADHD during fMRI on the stop task (Rubia et al., 1999). An increase in regional cerebral blood flow in the caudate region and a decrease in blood flow in the motor cortex has been observed following the administration of methylphenidate hydrochloride, which helps attention problems by decreasing dopamine reuptake (Lou et al., 1984; Lou et al., 1989; Lou et al., 1990).

Structural imaging findings have also supported the frontal-striatal hypothesis involving the differences of the caudate in children with attention disorders (Castellanos et al., 1996; Filipek et al., 1997; Mataro et al., 1997). Hynd et al. (1993) reported that the caudate asymmetry (left-greater-than-right-caudate) observed in non-disordered children was reversed in right-handed children with ADHD, due to smaller volumes of the left caudate head. The children with ADHD also scored higher on a measure of externalizing behaviors, including hyperactivity and aggression.
Filipek et al. (1997) found that compared to controls, the volumes of the left caudate and left caudate head in males with ADHD were smaller, resulting in reversed asymmetry from the left-greater-than-right caudate asymmetry noted in controls. Furthermore, children with ADHD who were responders to methylphenidate showed the smallest bilateral caudate volumes and symmetric caudate head volumes when compared to controls and those children who did not respond to medication. Pineda et al. (2002) found no caudate differences between children with ADHD who responded to methylphenidate and control groups.

Castellanos et al. (1994) reported symmetric caudate volumes in males with ADHD, due to smaller volumes of the right caudate head and body, while the healthy controls showed a caudate asymmetry of the right caudate being larger than the left caudate. Decreased caudate asymmetry was also found to be associated with increased perinatal risk (Castellanos et al., 1996). Females with ADHD were found to have smaller caudate volumes, particularly in the left caudate, than healthy females (Castellanos et al., 2001). Aylward et al. (1996) found no caudate asymmetry in boys with ADHD or in controls, but found that the ADHD group had smaller left and total volume of the globus pallidus than controls.

The caudate has been found to be smaller in adolescents born preterm and with attention deficits or low IQ when compared with healthy peers born preterm (Abernethy, Palaniappan, & Cooke, 2002). Healthy females have been shown to have larger caudates than males when adjusting for brain size (Caviness et al., 1996; Filipek et al., 1994; Giedd et al., 1997; Richelme, Kennedy, & Caviness, 1994; Sowell, Trauner, Gamst, & Jernigan, 2002). A study by Castellanos et al. (2002) investigated regional brain volume changes over time and found that caudate nucleus reaches its maximum volume at age
ten, with smaller caudate volumes observed in children with ADHD until midadolescence, when volumetric differences became negligible.

In the right frontal region, individuals with ADHD have been found to have decreased white matter volume (Castellanos et al., 1996; Filipek et al., 1997; Hynd et al., 1990), which has been associated with poor performance on sustained attention tasks (Semrud-Clikeman et al., 2000). Baving et al. (1999) reported reduced right frontal brain activity in boys with ADHD, and girls showed a left frontal deficit. Reduced activation in the frontal region has been observed using fMRI in adolescents with ADHD while doing executive function tasks (Rubia et al., 1999), and adults who had been hyperactive since childhood were found to have reductions in cerebral glucose metabolism in the prefrontal cortex and caudate (Zametkin et al., 1990). Individuals who had greater difficulty on a task of inhibition during fMRI showed greater activity in the ventral caudate nucleus, right prefrontal cortex, and parts of the thalamus, implicating the frontostriatal regions in behavioral inhibition (Casey, Thomas, Davidson, Kunz, & Franzen, 2002).

Few studies have evaluated the relationship between behavioral or neuropsychological measures and imaging findings of the caudate. Casey et al. (1997) found that the volume of the striatum and prefrontal cortex, predominately in the right hemisphere, negatively correlated with performance on response inhibition tasks. Castellanos et al. (2002) reported that caudate and frontal volumes negatively correlated with behavioral ratings of attention and impulsivity. A study by Martaro et al. (1997) found that adolescents with ADHD had a larger right head of caudate than the control group, and that a larger caudate nucleus was associated with worse attentional and behavioral measures in healthy adolescents. In addition, a left greater than right pattern of asymmetry was observed for the control group, with reverse asymmetry for the ADHD group. Semrud-Clikeman et al. (2000) found that the more the asymmetry of the caudate
head was reversed from the control finding (left greater than right), the poorer the performance on measures of disinhibition. Furthermore, the smaller left caudate of the ADHD group was related to higher externalizing behaviors, including hyperactivity, aggression, and conduct problems, on the Child Behavior Checklist (Achenbach, 1990). These findings of a relationship between externalizing behaviors and disinhibition and caudate asymmetry support the theory that frontal-striatal connections are related to problems of disinhibition in ADHD.

**Growth Hormone Effects on the Caudate and Attention in 18q-**

It appears that a relationship among the caudate, attention, externalizing behaviors, and growth hormone may exist in children with 18q-. Individuals with 18q- show evidence of dysmyelination of the caudate (Cody et al., 2005; Kline et al., 1993; Marwaha et al., 1992), and abnormal growth hormone production (Ghidoni et al., 1996; Marwaha et al., 1992). In addition, children with 18q- show attention and externalizing behavior problems seen in children with ADHD (Hansen & Herlin, 1994; Mahr et al., 1996). Children with ADHD have been found to have smaller left caudate head volumes and reversed caudate asymmetry (Filipek et al., 1997; Hynd et al., 1993), which has been related to the severity of externalizing behaviors and disinhibition (Semrud-Clikeman et al., 2000). Thus, children with 18q- are expected to have a higher degree of attention and externalizing problems, and they are not expected to have the typical caudate volumes or asymmetry observed in healthy children.

Preliminary findings provide evidence that children treated with growth hormone show improvements in the caudate of the white matter, based on T1 relaxation times on MRI (Cody et al., 2005). Considering growth hormone receptors have been found in the caudate (Adem et al., 1989; Burman et al., 1993), growth hormone may affect cognitive functions related to the caudate, such as attention and inhibition (Heilman et al., 1991).
Growth hormone also has been implicated in affecting dopamine activity, a neurotransmitter that affects attention and is supplied to the caudate (Deijen et al., 1996; Ernst et al., 1999). Improvements in attention have been observed in children with growth hormone deficiency following growth hormone treatment (Soares et al., 1999; Van Dan et al., 2000). It is therefore expected that children who respond to growth hormone therapy will show improvements in attention and externalizing behaviors, and show greater caudate volumes and more typical caudate asymmetry. However, research is needed to better understand the caudate, attention and externalizing behaviors, and the effects of growth hormone in children with 18q-. 

Chapter 3: Statement of the Problem

Purpose of the Study

Studies investigating 18q- have found that individuals with deletions of the long arm (q) on chromosome 18 have dysmyelination of the brain (Gay et al., 1997; Miller et al., 1990; Vogel et al., 1990), including the caudate (Cody et al., 2005; Kline et al., 1993; Marwaha et al., 1992), and abnormal growth hormone production (Ghidoni et al., 1996; Hale et al., 2000). Symptoms of attention-deficit/ hyperactivity disorder are also common, including inattention and disinhibition, as well as externalizing behaviors such as hyperactivity and conduct problems (Mahr et al., 1996). Children with ADHD have been found to have smaller left caudate volumes and reversed caudate asymmetry (Filipek et al., 1997; Hynd et al., 1993), which was found to be related to the severity of disinhibition and externalizing behaviors (Semrud-Clikeman et al., 2000). Preliminary studies suggest growth hormone treatment in children with 18q- results in increased myelination of the brain and changes in caudate volume, based on T1 values on MRI (Cody et al., 2005; Hardies et al., 2001). Furthermore, individuals with growth hormone deficiency have shown improvements in attention with growth hormone therapy (Soares et al., 1999; Van Dan et al., 2000). Research is needed to evaluate caudate volumes, attention and externalizing behaviors, and the effects of growth hormone on these factors in the 18q- population.

This study will evaluate children with 18q- and healthy controls, and compare children with 18q- before and after growth hormone treatment. The groups will be compared on measures of caudate volume using MRI and on measures of attention and externalizing problems using the Behavior Assessment System for Children (BASC) (Reynolds & Kamphaus, 1998). Results of the study will contribute to the understanding
of development in 18q- and to the effects of growth hormone on brain maturation and attention.

**HYPOTHESES AND RATIONALES**

**Hypothesis 1**

- a. The caudate volume (head and tail) will be smaller before growth hormone treatment in the 18q- group than in the control group.
- b. The caudate volume (head and tail) will be larger in the 18q- group after growth hormone treatment than before treatment.
- c. There will be no difference between the caudate volumes in the 18q- group after growth hormone (GH) treatment and the control group.

**Rationale**

Dysmyelination of the brain is commonly noted in individuals with 18q- (Gay et al., 1997; Hardies et al., 2001; Mahr et al., 1996; Miller et al., 1990; Strathdee et al., 1995; Vogel et al., 1990). Dysmyelination or abnormal myelination of the caudate has also been reported (Cody et al., 2005; Kline et al., 1993; Marwaha et al., 1992). It is therefore expected that individuals with 18q- will show reduced volumes in the caudate head and tail prior to growth hormone treatment compared to controls.

Insufficient production of growth hormone is a common finding in people with 18q-, in addition to short stature, slow growth velocity, and delayed bone maturation (Ghidoni et al., 1996; Hale et al., 2000; Schwartz & Duck, 1990). Preliminary findings provide evidence that children diagnosed with 18q- syndrome with isolated growth hormone deficiency show improvements in myelination, based on MRI T1 values, when treated with growth hormone (Hardies et al., 2001). Changes in the caudate of the white matter have also been found, based on reduced T1 relaxation times on MRI, following
growth hormone therapy (Cody et al., 2005). Growth hormone and insulin-like growth factor-I (IGF-I), a mediator of growth hormone action, have receptors in the caudate (Adem et al., 1989; Burman et al., 1993). IGF-I has been shown to be involved in the stimulation of nerve cell and glial cell proliferation (Pahlman et al., 1991), and in the regulation of gene expression in oligodendrocytes, which express myelin basic protein prior to myelin formation (De Vellis, 1990). Considering IGF-I values are often below normal in children with 18q- (Hale et al., 2000), and growth hormone affects the production of IGF-I, growth hormone treatment may increase myelination of the caudate. It is therefore hypothesized that growth hormone treatment will result in an increase in caudate volume in children with 18q-.

**Hypothesis 2**

a. There will be greater asymmetry of the right and left caudate (left-greater-than-right caudate) in the control group than in the 18q- group before growth hormone treatment.

b. There will be greater asymmetry of the right and left caudate in the 18q- group after growth hormone treatment than before treatment.

c. There will be no difference in caudate symmetry between the 18q- group after growth hormone treatment and the control group.

**Rationale**

Structural imaging findings using MRI have found differences in the caudate of children with attention-deficit/ hyperactivity disorder (ADHD) when compared to controls, particularly related to caudate asymmetry and size of the head of caudate. Caudate asymmetry, with the left being greater than the right caudate, has been reported in non-disordered children (Filipek et al., 1997; Hynd et al., 1993; Mataro et al., 1997; Semrud-Clikeman et al., 2000). In children with ADHD, however, smaller left caudate
head volumes and reversed caudate asymmetry were reported (Filipek et al., 1997; Hynd et al., 1993; Semrud-Clikeman et al., 2000). Symmetric caudate volumes, when including both caudate head and tail, have also been reported in children with ADHD (Castellanos et al., 1994).

It is estimated that approximately 35 percent of children with 18q- show symptoms of attention-deficit/ hyperactivity disorder (Oster-Granite et al., 2001), including inattention, impulsivity, disinhibition, distractibility, and hyperactivity (Hansen & Herlin, 1994; Mahr et al., 1996). Furthermore, children with 18q- show evidence of dysmyelination of the brain (Gay et al., 1997; Miller et al., 1990), including the caudate (Cody et al., 2005; Kline et al., 1993; Marwaha et al., 1992). Because of evidence of ADHD symptoms and dysmyelination in the caudate, it is hypothesized that individuals with 18q- will not show the typical caudate asymmetry (left-greater-than-right) observed in healthy controls.

Following growth hormone treatment, however, it is expected that individuals with 18q- will show greater caudate asymmetry (left-greater-than-right), similar to controls. Children diagnosed with 18q- with growth hormone deficiency show evidence of improvements in myelination and in the caudate based on T1 values on MRI following growth hormone treatment (Cody et al., 2005; Hardies et al., 2001). Growth hormone and insulin-like growth factor-I (IGF-I), a mediator of growth hormone action, have receptors in the caudate (Adem et al., 1989; Burman et al., 1993). IGF-I levels, which tend to be below normal in children with 18q- (Hale et al., 2000), has been shown to be involved in the regulation of gene expression in oligodendrocytes, which express myelin basic protein prior to myelin formation (De Vellis, 1990). Growth hormone treatment may therefore increase myelination of the caudate, so that the caudate volume and asymmetry is similar to that of controls.
Hypothesis 3

a. The BASC externalizing composite and attention problems score will be higher in the 18q- group before growth hormone treatment than in the control group.

b. The BASC externalizing problems composite and attention problems score will be lower in the 18q- group after growth hormone treatment than before treatment.

c. The BASC externalizing problems composite and attention problems score will not differ between the 18q- group after growth hormone treatment and the control group.

Rationale

Approximately 35 percent of children with 18q- show symptoms of attention-deficit/hyperactivity disorder (Oster-Granite et al., 2001), including inattention, impulsivity, disinhibition, distractibility, and hyperactivity (Hansen & Herlin, 1994; Lewkonia et al., 1980; Mahr et al., 1996; Schinzel, 1981). Aggressive behavior has also been reported in children with deletions of 18q (Bourgeois et al., 1974; Mahr et al., 1996; Strathdee et al., 1995; Wilson et al., 1979), and children diagnosed with ADHD show a greater degree of conduct problems, aggressiveness, impulsivity, and acting out or externalizing behaviors (Barkley, 1990; Henker & Whalen, 1989).

Based on these findings, it is expected that the individuals with 18q- will have higher externalizing composite scores, which encompasses the aggression, hyperactivity, and conduct problems scales of the Behavior Assessment System for Children (BASC) Parent Rating Scale (Reynolds & Kamphaus, 1998). The 18q- group is also expected to have higher scores on the attention problems scale of the BASC than controls.

Insufficient production of growth hormone is a common finding in people with 18q- (Ghidoni et al., 1996; Schwartz & Duck, 1990). Due to evidence of growth hormone receptors in the caudate (Adem et al., 1989; Burman et al., 1993), growth hormone may affect cognitive functions related to the caudate, such as attention and inhibition.
Growth hormone also has been implicated in affecting dopamine activity, a neurotransmitter that affects attention and inhibition and is supplied to the caudate (Deijen et al., 1996; Ernst et al., 1999). Improvements in attention have been observed in children with growth hormone deficiency following growth hormone treatment (Soares et al., 1999; Van Dan et al., 2000). It is therefore expected that the BASC externalizing composite score and attention problems scale score will improve with growth hormone treatment in children with 18q-.

**Hypothesis 4**

a. There will be an inverse correlation between the caudate head volume and the BASC externalizing composite and attention score in the 18q- group before treatment.

b. There will be no correlation between the caudate tail volume and the BASC externalizing composite and attention score in the 18q- group before treatment.

c. There will be an inverse correlation between the caudate head volume and the BASC externalizing composite and attention score in the 18q- group after treatment.

d. There will be no correlation between caudate tail volume and the BASC externalizing composite and attention score in the 18q- group after treatment.

e. There will be an inverse correlation between caudate asymmetry and BASC externalizing composite and attention score in the 18q- group before treatment.

f. There will be an inverse correlation between caudate volume asymmetry and the BASC externalizing composite and attention score in the 18q- group after treatment.

**Rationale**

The caudate has been studied in relation to symptoms observed in attention-deficit/hyperactivity disorder (ADHD), including inattention, disinhibition, impulsivity, and hyperactivity (Heilman et al., 1991; Voeller, 1991). The caudate nucleus head has
connections with the prefrontal cortex, an area associated with attention and inhibition (Mesulam, 1990; Oberg & Divac, 1977). Furthermore, the caudate receives dopamine, a neurotransmitter that affects attention, from the substantia nigra and subsequently supplies dopamine to the prefrontal cortex (Gerfen et al., 1996). Thus, disruption to the caudate or its connections to the prefrontal cortex or dopaminergic system results in inattention, impulsivity, hyperactivity and conduct problems (Alexander et al., 1986).

The caudate has been studied in children with ADHD, showing smaller caudate volumes and differences in caudate asymmetry compared to healthy children (Castellanos et al., 1994; Filipek et al., 1997; Hynd et al., 1993; Mataro et al., 1997; Semrud-Clikeman et al., 2000). In relation to behavioral and measures of inhibition, the volume of the caudate has been found to be negatively correlated with behavioral ratings of attention and impulsivity (Castellanos et al., 2002). The caudate and prefrontal cortex volumes have also been negatively correlated with performance on response inhibition tasks (Casey et al., 1997). Semrud-Clikeman et al. (2000) found that the smaller left caudate head was inversely related to externalizing behaviors, which includes hyperactivity, aggression, and conduct problems. They further reported that the greater the asymmetry of the caudate head was reversed from the control finding (left-greater-than-right), the poorer the performance on measures of disinhibition.

Considering it is expected that children with 18q- will not show the typical caudate asymmetry and volumes observed in healthy children, it is hypothesized that there will be an inverse relationship between caudate asymmetry and ratings on the attention and externalizing behavior scales of the BASC. Following growth hormone treatment, it is expected that the caudate volume will increase and the caudate will reflect the typical asymmetry observed in healthy children, and the attention problems score and externalizing behaviors composite will decrease.
The majority of the studies looking at the caudate and ADHD have found differences in the caudate head, but not the tail, when comparing children with ADHD to healthy controls (Filipek et al., 1997; Hynd et al., 1993; Martoro et al., 1997; Semrud-Clikeman et al., 2000). The caudate head has connections to the prefrontal cortex and the dopaminergic system, which are involved in attention and inhibition, and disruptions to these areas results in symptoms observed in ADHD (Alexander et al., 1986). Thus, abnormalities of the caudate head, such as hypomyelination, could result in behaviors observed in children with ADHD and 18q-, including inattention, disinhibition, hyperactivity, and conduct problems such as aggressive behavior (Mahr et al., 1996; Strathdee et al., 1995). It is therefore expected that there will be an inverse relationship between the caudate head, but not the tail, and ratings of attention and externalizing behaviors in children with 18q- both pre and post growth hormone treatment.

**Exploratory Hypotheses**

The relationship among age, caudate development, and attention has not been investigated in the 18q- population. It is hypothesized that there will be an inverse correlation between age and caudate volume and asymmetry in the post hormone treatment group. No correlation is expected between age and caudate volume and asymmetry in the 18q- group before growth hormone treatment.

It is also expected that externalizing problems will decrease with age with the 18q- participants, and there will be no correlation between age and attention problems. An analysis of the right and left caudate volume and attention and externalizing problems is expected to show decreasing behavioral issues with increasing left caudate volume in controls and 18q- post growth hormone participants. The pre growth hormone 18q- participants are expected to show smaller left caudate volumes with greater attention and externalizing problems.
Gender differences among caudate volumes, inattention, and externalizing problems will also be evaluated as an exploratory analysis, due to the small sample size. It is hypothesized that female control participants and post growth hormone 18q- participants will show larger caudate volumes than males. No difference between males and females is expected in pre growth hormone 18q- participants. Males with 18q- are expected to show greater externalizing problems than females. No gender differences in attention are expected.

**Rationale**

Age-related decreased in caudate volume and asymmetry of the left and right caudate have been reported in healthy adults (Krishnan et al., 1990) and in typically developing children, primarily after age ten (Castellanos et al., 2002). However, the decrease in caudate volume observed in typically developing children is not seen in the caudate of children with ADHD (Castellanos et al., 1996; Filipek et al., 1997). Thus, the pre growth hormone 18q- participants, who are reported to have symptoms of ADHD (Mahr et al., 1996), are not expected to show a decrease in caudate volume with age. Those children with 18q- post growth hormone treatment are expected to show a decrease in caudate volume and symmetry of the left and right caudate with age, similar to controls. It is also expected that externalizing problems, but not inattention, will decrease with age in 18q- participants. Overt signs of externalizing behaviors (i.e., hyperactivity and impulsivity) decline with age, while inattentive symptoms remain stable from ages eight to 15 in individuals with ADHD (Hart et al., 1995).

In children with ADHD, smaller left caudate head volumes and reversed caudate asymmetry have been reported (Filipek et al., 1997; Hynd et al., 1993; Semrud-Clikeman et al., 2000). Symmetric caudate volumes, when including both caudate head and tail, have also been reported in children with ADHD (Castellanos et al., 1994). Left greater
than right caudate asymmetry has been reported in non-disordered children (Filipek et al., 1997; Hynd et al., 1993; Mataro et al., 1997; Semrud-Clikeman et al., 2000). Because pre-growth hormone 18q- participants are reported to have attention problems and externalizing problems (Mahr et al., 1996), it is expected that decreased left caudate volume will be related to greater attention and externalizing problems. Post growth hormone participants are expected to resemble controls, with no attention problems and larger left caudate volumes.

Typically developing females have been reported to have greater caudate volumes than males (Geidd et al., 1996), but no gender differences were reported with the caudate in ADHD (Castellanos et al., 2002). Considering children with 18q- show symptoms of ADHD (Mahr et al., 1996), no gender differences in caudate volume are expected in the 18q- participants before treatment. Participants with 18q- after treatment are not only expected to reflect the caudate volumes seen in controls and show a decrease in ADHD symptoms, but they are also expected to reflect gender differences seen in controls, with females showing greater caudate volumes than males. Males with 18q- are expected to show greater externalizing problems, but not attention problems, than females. Males with ADHD generally present with greater hyperactivity and impulsivity compared to females with ADHD (Staller & Farone, 2006).


Chapter 4: Methods

Participants

Thirteen participants between the ages of two and 15 were recruited from an ongoing study of individuals with chromosome 18 abnormalities at the University of Texas Health and Science Center in San Antonio. Twelve volunteer (no disorder) control participants were recruited from the community of San Antonio, Texas. The Institutional Review Boards of the University of Texas Health Science Center at San Antonio, University of Texas at Austin, and the research and development committee of the Audie L. Murphy Veterans Administration Hospital approved the ongoing study.

Participants in the 18q- group were referred to the study from the Chromosome 18 Registry and Research Society or through their private physician after a cytogenetic analysis detected a deletion of the long arm of chromosome 18 (18q). They were evaluated at the General Clinical Research Center located at Audie L. Murphy Veterans Administration Hospital in San Antonio, Texas, to confirm the presence of a chromosome 18q deletion and to determine if the child had a growth hormone deficiency. Participants who were included in the study had breakpoints ranging from 18q21 to 18q23. Individuals with more complex chromosome arrangements were excluded. Participants with untreated thyroid dysfunction were also excluded from the study.

Control participants were recruited locally or were siblings of the 18q- participants. To qualify for the control group, participants were required to have normal prenatal, developmental, and medical histories. Children with a history of psychopathology or neurological pathology, including organic brain disorders, seizures, or head injury, were excluded from the study. A developmental history by the study coordinator was taken to determine their eligibility for the study. Participants in the
control group were required to have average or better intellectual ability, with an IQ of at least 85.

**PROCEDURE**

The participants and their families were provided transportation and a schedule of procedures, which was arranged by the project’s Research Coordinator. Participants generally stayed for four days at accommodations near the research facilities. During this time a general research protocol was completed, including genetic analysis, Magnetic Resonance Imaging (MRI), endocrine screening to determine GH deficiency, audiology exam, and psychological testing. Participants in the control group were evaluated one time using MRI and psychological testing. The handedness of the participants was not included as a variable of interest in this study due to insufficient data for all participants.

After participants and their parents were explained the parameters of the study, they signed an informed consent form to participate in the study. It was explained that they could withdraw from the study at any time. As part of this ongoing study of 18q-, chromosome and growth hormone analyses were conducted by the research team at the General Clinical Research Center located at Audie L. Murphy Veterans Administration Hospital in San Antonio, Texas. Magnetic Resonance Imaging scans were conducted at the Research Imaging Center (RIC) in San Antonio. The parents of the participants were asked to complete the BASC PRS during their scheduled visit. The analysis of the MRI scans was completed by this author who was blind as to whether the participant was receiving growth hormone treatment. Parents were provided with written feedback regarding individual assessment results.
**Genotypic Analysis**

The presence of a chromosome 18q deletion in each participant was confirmed using polymerase chain reaction (PCR)-based polymorphic marker analysis (Cody et al., 1997; Weber & May, 1989). To obtain DNA for analysis, blood samples from participants were obtained in acid citrate dextrose (ACD) tubes, and the DNA was extracted from the peripheral blood leukocytes (Bell, Karam & Rutter, 1981). The DNA was analyzed using PCR-based markers (Gyapay et al., 1994). A marker for the myelin basic protein (MBP) gene was also used in the analysis (Polymeropoulos et al., 1992), which has been mapped to chromosome 18q23 (LeBeau et al., 1993).

The molecular analysis was conducted using PCR by end-labeling one primer of the set at the 5’ end with γ 32P-dATP (Cody et al., 1997). PCR was carried out in a total reaction volume of 10 µl containing 50 ng of genomic DNA, 50 ng of each primer, 200 µM dNTPs, 0.5 U Taq polymerase, and 1.5 mM of MgCl2. PCR amplification consisted of 30 cycles of 1 minute at 94°C, then 1 minute at the optimal annealing temperature, and 1 minute elongation at 72°C. PCR products were separated on a seven percent polyacrylamide gel run at 65 watts for four to six hours. To visualize the PCR products, autoradiography was performed using Kodak XAR-5 film.

**Growth Hormone Treatment**

The growth hormone levels of individuals with deletions of chromosome 18q were evaluated by a board certified endocrinologist to determine if the child qualified for growth hormone treatment. Those children with 18q- who qualified for growth hormone treatment were included in the study. Individuals with hypothyroidism, that is those participants with low circulating T4 levels and/or high thyroid stimulating hormone levels, were excluded from the study because these results were consistent with thyroid dysfunction (Hale et al., 2000).
The diagnosis of growth hormone insufficiency was determined by a number of factors: Current height, growth history, growth velocity, bone maturation, insulin-like growth factor-1 (IGF-1), insulin-like growth factor binding protein 3 (IGFBP-3), 1-thyroxine and thyrotropin (Hale et al., 2000). Growth hormone release was evaluated using arginine hydrochloride and/or clonidine as the provocative agent. To obtain blood for the growth hormone analysis, an intravenous device was placed in an arm vein following an overnight fast. The blood for the growth hormone analysis was centrifuged within ten minutes and the serum and/or plasma was transferred to tubes and frozen at -20 degrees Celsius until hormonal assays were performed. Growth hormone was measured by polyclonal radioimmunoassay (Nichols Institute Diagnostics, San Juan Capistrano, CA).

Participant height, length, and head circumference was measured, with SD calculated using the computer program Growth Base III (Eli Lilly and Company) or based on normative data (Hall, Froster-Iskenius, & Allanson, 1989). Growth velocity was found by calculating the difference between height or length measurements obtained at the time of the visit and at least three months prior to the visit. Bone age was obtained from X-ray films of the left hand and wrist. Delayed bone age was < 2 SD below the mean for age. Individuals were treated with the dose of 0.3 mg/kg/wk of growth hormone if they had at least four of the following abnormal values: Height < -2 SD, growth velocities < -1 SD, bone age < 75 % of chronological age, IGF-1 < -1 SD, IGFBP < -1 SD and peak GH < 10ng / ml.

**Magnetic Resonance Imaging**

Magnetic Resonance Imaging (MRI) was used to investigate growth hormone (GH) effects on the caudate. Volumetric analysis of the caudate measurements were
calculated before the initiation of growth hormone treatment and repeated after the participant had been on growth hormone for at least one year.

**Imaging Protocol**

Magnetic resonance images were acquired using a 1.9 Tesla clinical MRI system (Elscint, Haifa, Israel / GE medical systems). Three spin-echo images were acquired: Axial T1 (spin-lattice) weighted image with TE / TR (echo time / repetition time) equaling 20 / 500 msec and dual-echo proton density (PD) – weighted / T2 (spin-spin) weighted images (TE1 / TE2 / TR = 20 / 80 / 3400 msec). Twenty-two axial images were acquired in a 256 x 256 array (1 mm pixel spacing) with 5 mm slice thickness and a 1 mm gap (132 mm span). The image acquisition time was approximately 20 minutes. Participants unable to lie still were sedated with chloral hydrate, 50 - 100 mg / kg of body weight, to minimize movement between scanning sequences and were monitored by a nurse anesthetist (Gay et al., 1997; Herndon et al., 1996; Lancaster et al., 2001).

**Image Analysis**

Raw imaging data was converted to quantitative T1 and T2 image maps using Magnetic Resonance Parametric Analyzer (MRPA) software at the Research Imaging Center, University of Texas Health Science Center in San Antonio (Downs et al., 1992; Herndon, 1996). This process was conducted by a research technician at the Research Imaging Center. Brain images were reformatted into a spatially normalized scan in Talairach space to account for differences in brain size, orientation, and position (Talairach & Tournoux, 1988). The images were oriented along the anterior-posterior commissure and resliced into 1.0-mm coronal scans to remove differences in head position at the time of image acquisition.
The process of spatial normalization was used to rescale the image to a standard brain size, allowing brain structures and volumes to be compared while controlling for developmental size differences. For example, although the brain volume of a ten-year-old child is typically larger than the brain of a three-year-old child, spatial normalization would adjust for the age difference, allowing the two brains to be compared. Spatial normalization also accounts for differences in brain size due to gender, considering males tend to have larger brain volumes than females (Castellanos et al., 2002; Mitchell et al., 2003).

The images were converted into MINC (Medical Image NetCDF) file format and stored electronically for investigation. The MR images were processed on computer workstations from Sun Microsystems, Inc. (Mountain View, CA). T1 and T2 parametric image maps were transferred to a PC workstation for viewing and analysis. Volumetric analysis of the images were conducted using a PC version of Display software developed at the Montreal Neurological Institute (MacDonald, 1996). Display is a program designed to manipulate three-dimensional images of a brain, allowing the user to conduct anatomic segmentation in the coronal, sagittal, and horizontal planes. To control for potential differences caused by voxel intensity, the normalized image intensity contour algorithms was kept constant across all images. The caudate was manually highlighted using the paint function of Display. Highlighting was completed and checked in all three dimensions, using a paint brush size of 1 voxel (1 millimeter). The volume of the highlighted caudate was calculated by Display. Volumetric analysis was performed blind to subject and control information.

Guidelines for anatomical boundaries of the caudate were obtained from Filipek et al. (1997) and Semrud-Clikeman et al. (2000). Regions were divided as determined by the presence of the corpus callosum. Precallosal (prefrontal) and retrocallosal (posterior
parietal / occipital) regions include slices anterior and posterior to the corpus callosum, respectively. The pericallosal regions included the coronal slices surrounding the corpus callosum and were divided into anterior pericallosal (anterior to the anterior commissure) and posterior pericallosal (posterior to the anterior commissure) regions. The anterior and posterior pericallosal regions were also divided into superior, inferior, and temporal pericallosal regions by lines connecting the sylvian fissure, superior circular insular sulcus, and the superolateral lateral ventricle; and the sylvian fissure, inferior circular insular sulcus and optic tract, amygdala, and hippocampus. The head of the caudate was located within the anterior inferior pericallosal region, and the tail of the caudate was located within the posterior inferior pericallosal region.

**Behavior Assessment System for Children**

Parents of the participants completed the Behavior Assessment System for Children (BASC) Parent Rating Scale (PRS) (Reynolds & Kamphaus, 1998) computer entry form. The BASC is a measure of a child’s adaptive and problem behaviors. The respondent rates the behaviors on a four-point scale of frequency (never, sometimes, often, almost always). Completion of the PRS takes approximately 10 to 20 minutes. The responses are then entered on computer using the BASC Enhanced ASSIST program, which generates profiles, calculates validity indexes, identifies strengths and weaknesses, and computes multirater comparisons. The PRS may be interpreted with reference to national age norms (general, males, or female) or to clinical norms. In the present study the general combined-sex norms will be used so that the level of behaviors occurring in the population could be compared to the study groups. T-scores and percentiles were provided.

The BASC has three forms at three age levels: Preschool (ages 2½ - 5), child (ages 6 - 11), and adolescent (ages 12 - 18). Scale scores combine to form the following
composites: The externalizing problems composite is comprised of aggression, hyperactivity, and conduct problems scales for the child and adolescent forms, and aggression and hyperactivity scales for the preschool form. The internalizing problems composite is comprised of anxiety, depression, and somatization scales. The adaptive skills composite includes adaptability and social skills scales for the preschool form; adaptability, leadership, and social skills scales for the child form; and leadership and social skills scales for the adolescent form. The attention, atypicality, and withdrawal scales are also included on the BASC. The Behavioral Symptoms Index (BSI) is a broad composite composed of the scales and assesses the overall level of problem behaviors. The PRS also includes an F ("fake bad") validity index designed to detect a negative response set based on parent ratings.

In this study the externalizing problems composite (hyperactivity, aggression, and conduct problems scales) and the attention scale were investigated. The hyperactivity scale assesses hyperactivity and impulsivity, which is the tendency to be overly active, rush through work and activities, and act without thinking. Aggression is the tendency to act in a hostile manner that is threatening to others, and conduct problems are the tendency to engage in anti-social and rule-breaking behavior. Attention problems include being easily distracted and unable to concentrate more than momentarily. The attention, hyperactivity, aggression, and conduct problems scales are likely to be elevated in children with ADHD (i.e., T scores of 60 or greater) (Henker & Whalen, 1989; Semrud-Clikeman, 1991). The attention problems scale has moderate loadings on the externalizing problems composite (64 preschool, 71 child, 66 adolescent) on the first unrotated factor in principal-axis analyses using the general norms sample.

The BASC PRS was standardized on 3,483 male and female children (16% African-American, 11% Hispanic, 70% White, and 3% other, defined as Native
Americans, Alaskan Natives, Asians, and Pacific Islanders) to collect data for the general norms. Internal consistency reliabilities of the composite scores range from the middle .80s to low .90s at all three age levels and for both genders in the general population. The internal consistency reliability for the externalizing problems composite ranges from .86 to .89 across age groups, with the hyperactivity scale ranging from .65 to .83, conduct problems scale ranging from .70 to .74, aggression scale from .81 to .83, and attention problems scale from .70 to .82. The test-retest correlations for the externalizing problems composite are .85, .91, and .74 for the three age groups, respectively. The attention problems scale correlations are .88, .92, and .78 for the three age groups, respectively (Reynolds & Kamphaus, 1998).

Correlations between the externalizing composites (ranging from .71 to .84) from the BASC PRS and the Child Behavior Checklist (CBCL; Achenbach, 1991) were reported in convergent validity studies. The correlations ranged from .67 to .78 on the attention problems scales when comparing the BASC PRS to the CBCL. A sample of children diagnosed with Attention-Deficit/Hyperactivity Disorder were found to have a mean T score of 66.9 on the externalizing problems composite, with T = 68 on the hyperactivity scale, T = 63 on the aggression and conduct problems scales, and T = 65.7 on the attention problems scale.

**Differential Abilities Scale**

Participants of the study were given the Differential Abilities Scale (DAS) (Elliott, 1990) to assess cognitive functioning using the Nonverbal Reasoning Composite (NVC) of the DAS. Those participants unable to complete the DAS due to cognitive impairment were administered the Bayley Infant Scales of Infant Development, which was not included in the analysis of intellectual functioning for this study.
The Differential Ability Scales (DAS) is a test for determining the level of intellectual functioning in children ages 2 years, 6 months to 17 years, 11 months. The DAS contains 17 cognitive and 3 achievement subtests in the Preschool and School Age batteries. The School age battery is for children ages 6-0 to 17-11 years of age. The Preschool battery is divided into the lower (ages 2-6 to 3-5 years) and upper (ages 3-6 to 5-11 years) levels according to the child’s age range. There are four core subtests for the lower preschool level, six core subtests for the upper preschool level, and six core subtests for the school age level. There are two to five additional diagnostic subtests that are administered according to the child’s age.

Verbal, Nonverbal, Spatial, General Cognitive Ability, and Special Nonverbal Composite indices can be calculated from the DAS. In this study the Nonverbal Reasoning Composite (NVC) of the DAS was used as the index for intellectual ability instead of the General Cognitive Ability (GCA) score or the Special Nonverbal Reasoning Composite. The NVC has been shown to be positively correlated with the WISC-III PIQ scale (Elliott, 1990). The NVC is also appropriate for use with children who are hearing or language impaired (Kamphaus, 2001), which is a common characteristic in children with 18q-. The NVC mean internal consistency reliabilities ranged from .70-.92 for individual subtests, .88-.94 for composite scores, and .90-.95 for the GCA. The DAS Nonverbal Reasoning Composite reliability is reported to be .89 for preschool aged children and .83 for school-aged children. Construct validity for the DAS Nonverbal Ability Cluster score is .75 when compared to the Wechsler Preschool and Primary Scale of Intelligence-Revised (WPPSI-R, Wechsler, 1989) for the Performance IQ for preschool aged children (3 years, 6 months to 5 years, 11 months) and .78 for the Nonverbal Ability Cluster score when compared with the Performance IQ WISC III for school-aged children (6 years, 0 months to 16 years, 11 months) (Elliott, 1990).
**DATA ANALYSIS**

The controls and pre and post growth hormone treated 18q- participants were compared on measurements of caudate volume and caudate asymmetry. The caudate measurements were compared using repeated measures analysis of variance to determine if there were differences between the caudate volumes of the 18q- group and controls. To examine the relationship between the caudate volumes and BASC scores, Pearson Product-Moment Correlation coefficients were completed using the mean scores from the BASC and caudate measures. The data was analyzed using SPSS 11.5. An alpha of .05 was set for all analyses.

**Reliability**

An intrarater reliability analysis was completed by re-conducting the volumetric analysis of 30 percent of the caudate images. Intraclass correlations were calculated for the following brain regions: Total caudate volumes \( r = .84 \), right caudate volume \( r = .80 \), and left caudate volume \( r = .85 \).

**Power Analysis**

A large effect size was expected in this study, considering it is important clinically to have a large effect size when children are being treated pharmacologically. A power analysis was computed using a t-test for means, using a total sample size of 25 participants with an alpha of .05 and a large effect size (Cohen’s \( d = .8 \)). The calculated statistical power was .50, indicating a 50 percent chance of detecting an effect size of .8. The level of power was affected by the number of participants in this study, which is limited by the rarity of 18q-, estimated to occur in approximately 1 / 40,000 live births (Cody et al., 1997).
Chapter 5: Results

OVERVIEW

Twenty-five individuals participated in the study, comprised of 13 18q- participants (5 males and 8 females) and 12 controls (5 males and 7 females). Table 2 presents the ages of participants. The average number of years between pre and post growth hormone treatment was 1.9 years, ranging from 1.2 years to 5 years. The time period between pre and post treatment scores varied due to difficulty attaining adequate MRI scans over the same time interval. Seven 18q- participants who qualified for the study were rejected due to poor MRI scans. Five 18q- participants treated with growth hormone were not included in the study because they did not receive an MRI scan prior to growth hormone treatment. Control participants were measured one time for the BASC and MRI acquisition as a result of cost constraints. The participants were predominately Caucasian and from middle class socioeconomic backgrounds.

Table 2

<table>
<thead>
<tr>
<th>Age of 18q- Participants and Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pre GH 18q-</td>
</tr>
<tr>
<td>Mean</td>
</tr>
<tr>
<td>Age (years)</td>
</tr>
</tbody>
</table>

INTELLECTUAL FUNCTIONING

The intellectual functioning of the participants was measured using the Nonverbal Reasoning Composite (NVC) of the Differential Ability Scales (DAS) (Elliott, 1990). Results from an ANOVA showed that the pre GH treatment scored significantly lower than controls on the DAS Nonverbal Reasoning Composite \( (F_{1,17} = 20.44, p = .001) \). The
pre GH treatment 18q- group had a mean score of 81.71 \( (SD = 15.4) \), which is in the below average range of intellectual functioning. The control participants scored in the high average range, with a mean NVC score of 113.16 \( (SD = 14.3) \). An ANOVA comparing post-GH treated 18q- participants with controls on the NVC scale revealed a statistically significant difference between the two groups \( (F_{1,18} = 22.44, p = .001) \). Post GH treated 18q- participants had a mean score of 81.25 \( (SD = 15.46) \). There was no significant difference from pre to post treatment for the 18q- participants on the NVC score, based on ANOVA \( (F_{1,12} = .04, p = .83) \). The Nonverbal Reasoning Composite (NVC) scores for the three groups are presented in Table 3.

Table 3

<table>
<thead>
<tr>
<th>Nonverbal Reasoning Composite of Participants</th>
<th>Pre GH 18q-</th>
<th>Post GH 18q-</th>
<th>Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean SD</td>
<td>Mean SD</td>
<td>Mean SD</td>
<td>Mean SD</td>
</tr>
<tr>
<td>Nonverbal Reasoning Composite(^a)</td>
<td>81.71 15.21</td>
<td>81.25 15.46</td>
<td>113.17 14.29</td>
</tr>
</tbody>
</table>

\(^a\)N=7 for NVC analysis

HYPOTHESES RESULTS

Caudate Volume

The caudate volumes of 18q- participants, pre and post growth hormone (GH) treatment, and controls were analyzed. There were no statistically significant differences on caudate volume between the pre GH 18q- participants and controls, based on the results of a MANOVA \( (F_{2,22} = .040, p = .961) \). A small effect size \( (d = .111) \) was calculated using Cohen’s \( d \) (Becker, 2000). Analysis of follow-up between subjects effects revealed no significant differences between the pre treatment 18q-participants and controls on caudate head \( (F_{1,23} = .036, p = .851, d = .075) \) and tail \( (F_{1,23} = .063, p = \)
.805, \( d = .101 \) measurements. The pre and post treated 18q- participants were compared using a repeated measures MANOVA, and results showed no significant effect of time on overall caudate volume \((F_{1,12} = .586, p = .459, d = .721)\), head \((F_{1,12} = 3.644, p = .080, d = .710)\), and tail \((F_{1,12} = 2.719, p = .125, d = .493)\). Comparison of the post treatment 18q-participants and controls using MANOVA showed that the two groups were not significantly different \((F_{2,22} = 1.641, p = .217, d = .745)\). Analysis of follow-up between subjects effects also revealed no significant differences between the two groups on caudate head \((F_{1,23} = 2.398, p = .135, d = .621)\) and tail \((F_{1,23} = 2.343, p = .139, d = .621)\) measurements. The caudate measurements for the 18q- participants and controls are presented in Table 4.

Table 4

<table>
<thead>
<tr>
<th>Caudate Measurements</th>
<th>Pre GH 18q(^a)</th>
<th>Post GH 18q(^a)</th>
<th>Controls(^b)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total Caudate(^c)</td>
<td>9.15 .88</td>
<td>10.15 1.75</td>
<td>9.03 1.21</td>
</tr>
<tr>
<td>Caudate Head(^c)</td>
<td>7.02 .43</td>
<td>7.59 1.06</td>
<td>6.96 .97</td>
</tr>
<tr>
<td>Caudate Tail(^c)</td>
<td>2.13 .72</td>
<td>2.56 1.00</td>
<td>2.07 .51</td>
</tr>
<tr>
<td>Total Asymmetry</td>
<td>.02 .08</td>
<td>.02 .07</td>
<td>-.02 .06</td>
</tr>
<tr>
<td>Head Asymmetry</td>
<td>-.02 .12</td>
<td>-.05 .08</td>
<td>-.04 .08</td>
</tr>
</tbody>
</table>

\(^a\)n = 13. \(^b\)n = 12. \(^c\)cm\(^3\).

Overall, the results show that the 18q- participants before treatment and controls did not differ on caudate volume, which is contrary to the hypothesis that the caudate volume would be smaller in the 18q- group. No significant differences were found between the post 18q- participants and controls, as hypothesized. Contrary to what was
predicted, there was no statistically significant increase in caudate volume with treatment in the 18q- participants. A graph depicting changes in total caudate volume from pre to post treatment for each 18q- participant is presented in Figure 3.

![Graph showing changes in total caudate volume from pre to post treatment for 18q- participants](image)

**Figure 3.** Changes in caudate volume for 18q- participants from pre to post treatment.

A medium effect size was calculated (Becker, 2000) when comparing the 18q-participants pre and post treatment for overall caudate volume ($d = .721$) and the caudate head ($d = .710$). There was also a medium effect size between the post GH 18q-participants and controls for total caudate volume ($d = .745$), head ($d = .621$), and tail ($d = .621$). Based on the calculated effect size, a difference may be present but was not detected by conventional measures of statistical significance, due to a lack of adequate power. A difference may be detected with a larger sample size, but due to the rarity of this disorder, only a small sample size could be obtained for the current study. An MRI scan with the caudate highlighted is displayed in Figure 4.
Caudate Asymmetry

The 18q- participants, pre and post GH treatment, and controls were analyzed separately on caudate symmetry. Comparisons of left-right volumetric symmetry were based on an asymmetry coefficient computed using the formula (left caudate - right caudate) / 0.5 (left caudate + right caudate) (Galburda et al., 1987). One-sample t-tests were conducted to determine if the mean asymmetry differed from zero for the pre and post treatment groups and controls. Results of one-sample t-tests for the total caudate ($t_{12} = .906, p = .383, M = .019$) and head of caudate ($t_{12} = .664, p = .519, M = -.021$) were not significant for the pre GH treated 18q- participants, as hypothesized. These results indicate that the left and right caudate of the pre treated 18q- participants were symmetrical. There was no asymmetry of the total caudate ($t_{11} = -1.425, p = .182, M = -.024$) or caudate head ($t_{11} = -1.844, p = .092, M = -.040$) for the controls, which is contrary to the expected L > R asymmetry. No asymmetry of the total caudate was found for the post GH 18q- participants ($t_{12} = .968, p = .352, M = .018$), which is also contrary
to the hypothesized L > R asymmetry. There was asymmetry (R > L) of the caudate head for the post GH treated 18q- participants ($t_{12} = -2.534, p = .026, M = -.05$), which is reverse of the expected L > R asymmetry. Asymmetry coefficients for the total caudate and caudate head are presented in Table 4.

Differences in caudate asymmetry among the pre and post treatment 18q- participants and controls were also explored. Results of a one-way ANOVA revealed there was not a statistically significant difference between the pre GH treated 18q- participants and controls in total caudate asymmetry ($F_{1,23} = 2.510, p = .127, d = .632$) or caudate head asymmetry ($F_{1,23} = .217, p = .645, d = .195$). A within subjects repeated measures ANOVA showed that there was no significant difference with the 18q- participants from pre to post GH treatment with total caudate asymmetry ($F_{1,12} = .001, p = .980, d = .010$) and caudate head asymmetry ($F_{1,12} = 1.056, p = .324, d = .337$). Results of a one-way ANOVA showed that the post GH treated and control groups did not differ significantly in total caudate asymmetry ($F_{1,23} = 2.791, p = .108, d = .102$) or caudate head asymmetry ($F_{1,23} = .206, p = .654, d = .181$). Although these results are consistent with the hypothesis that the two groups would not differ on asymmetry, the 18q- participants and controls failed to show the expected L > R asymmetry.

Overall, there were no statistically significant differences in caudate asymmetry between the 18q- participants and controls, and there were no significant changes in asymmetry with growth hormone treatment. There was a medium effect size when comparing pre GH treated 18q- participants and controls on total caudate asymmetry ($d = .632$), suggesting a difference may be present but was undetectable with a small sample size.
Attention and Externalizing Problems

The pre GH 18q- participants and controls were compared on the externalizing composite score and the attention problems scale score from the BASC. It was expected that the BASC scores would be higher in the 18q- participants. Results of a MANOVA showed no significant difference between pre GH 18q- participants and controls on BASC scores ($F_{2,22} = .981, p = .268$). Analysis of follow-up between subjects effects showed a significant difference in the attention problems scale ($F_{1,23} = 8.208, p = .009, d = 1.15$), with the 18q- group scoring higher ($M = 57.62$) than the control group ($M = 46.0$). Contrary to predicted differences, no significant difference was found between groups with the externalizing composite score ($F_{1,23} = .148, p = .704, d = .156$). Results show that pre GH treatment 18q- participants have greater attention problems than controls, but they do not differ in the level of externalizing behaviors.

The 18q- participants were compared pre and post GH treatment on the attention problems score and externalizing problems composite from the BASC. It was hypothesized that both scores would decrease with treatment. Results of a repeated measures MANOVA showed no significant effect of time on BASC scores ($F_{1,12} = .442, p = .519$). The BASC externalizing problems composite ($F_{1,12} = 1.064, p = .323, d = .148$) and attention problems score ($F_{1,12} = .155, p = .701, d = .131$) were examined using repeated measures ANOVA, but results were not significant. There was no significant change over time in the BASC externalizing composite and attention problems with controls or with 18q- participants treated with growth hormone.

The post GH treated 18q- participants and controls were also compared on the externalizing problems composite score and attention problems scale scores. Results of a MANOVA showed the main effect for group was close to, but did not reach statistical significance ($F_{2,22} = 3.236, p = .059$). Analysis of follow-up between subjects effects...
revealed a significant difference between the groups on the attention problems scale score ($F_{1,23} = 6.508$, $p = .018$, $d = 1.02$). The post GH 18q- group showed greater attention problems than the control group. As predicted, the post GH treated 18q- group and controls did not significantly differ on the externalizing problems score ($F_{1,23} = .760$, $p = .392$, $d = .351$). Table 5 shows the BASC scores for the 18q- participants and controls.

Table 5

**BASC Scores of the 18q- Participants and Controls**

<table>
<thead>
<tr>
<th>BASC Scales</th>
<th>Pre GH 18q-&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Post GH 18q-&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Controls&lt;sup&gt;b&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Attention Problems</td>
<td>57.62 10.67</td>
<td>56.23 10.47</td>
<td>46.0 9.50</td>
</tr>
<tr>
<td>Externalizing Problems</td>
<td>46.39 9.35</td>
<td>47.69 8.24</td>
<td>45.17 5.95</td>
</tr>
</tbody>
</table>

<sup>a</sup>n = 13.  <sup>b</sup>n = 12.  <sup>c</sup>t-scores.

**Caudate and BASC Scores**

The relations among caudate head and tail volumes and BASC externalizing composite and attention problems scores in 18q- participants were examined using correlation analyses (Pearson Product Moment Correlations). For the pre GH 18q-participants, correlations were found to be nonsignificant, as seen in the correlation matrix in Table 6. There were no significant relations among the caudate head volume and BASC externalizing composite ($r = -.114$, $p = .711$) or attention problems score ($r = -.251$, $p = .408$), which is contrary to the hypothesized inverse correlation. As predicted, there were no significant relations among the caudate tail volume and externalizing composite ($r = -.466$, $p = .109$) or attention problems scores ($r = .422$, $p = .151$) for the 18q- participants pre treatment.
Table 6

*Correlations Among Caudate and BASC Measures for Pre GH 18q- Participants*

<table>
<thead>
<tr>
<th></th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Caudate Head Volume</td>
<td>1.00</td>
<td>.123</td>
<td>-.391</td>
<td>-.251</td>
<td>-.114</td>
</tr>
<tr>
<td>2. Caudate Tail Volume</td>
<td>1.00</td>
<td>.249</td>
<td>.422</td>
<td>-.466</td>
<td></td>
</tr>
<tr>
<td>3. Caudate Asymmetry</td>
<td>1.00</td>
<td>.176</td>
<td>-.124</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4. BASC Attention Problems</td>
<td>1.00</td>
<td>.222</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5. BASC Externalizing Problems</td>
<td>1.00</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Correlation is significant at 0.05 level (2-tailed)*

In the 18q- post growth hormone treatment, it was hypothesized that there would be an inverse correlation among caudate head volume and the BASC externalizing composite and attention problems scores. It was also expected that there would be no correlation among caudate tail volume and the BASC scores. Contrary to predictions, results of the correlation matrix show no correlations that are statistically significant among the caudate head volumes and BASC attention scale and externalizing composite scores. Consistent with the research hypothesis, there were no correlations among the caudate tail volume and BASC scores with the post GH 18q- participants. Results of the correlations among caudate volumes and BASC scores in the post GH 18q- group are presented in Table 7.

The relationship among caudate asymmetry and BASC scores was evaluated using correlation analyses in the 18q- group pre and post growth hormone treatment. It was hypothesized that there would be an inverse correlation among caudate asymmetry and BASC externalizing problems composite and attention problems scores with 18q-
participants pre and post GH treatment. Contrary to what was hypothesized, there were no significant correlations among caudate asymmetry and BASC scores with 18q-participants, pre and post GH treatment. Correlation matrix results are shown in Table 6 for pre GH treatment and Table 7 for post GH treatment.

Table 7

*Correlation is significant at 0.05 level (2-tailed)

**EXPLORATORY HYPOTHESES**

**Age and Caudate Measures**

The relations between age and caudate measures were examined using correlation analyses (PPMC) with the pre and post GH 18q- participants and controls. In the pre GH 18q- group, no significant relations among age and caudate measures (total caudate, head, tail, right, left, and asymmetry) were found. Likewise, there were no significant relations among age and caudate measures with the post GH 18q- participants. Analysis of the control group found no significant relations among age and caudate head volume or
asymmetry. Inverse correlations between age and total caudate volume ($r = -.553, p = .062$), right caudate ($r = -.553, p = .062$), and left caudate volumes ($r = -.530, p = .076$), although of moderate magnitude, were not statistically significant. A significant inverse relation between age and caudate tail volume ($r = -.615, p = .033$) was found, indicating that caudate tail volume decreases with increasing age in controls. The inverse relations between caudate volume and age is an expected finding in controls, but this finding was not present in the 18q- groups (pre or post GH). Results of the correlation matrix are shown in Table 8.

Table 8

<table>
<thead>
<tr>
<th>Caudate Measures</th>
<th>Age of 18q- Pre GH</th>
<th>Age of Controls</th>
<th>Age of 18q- Post GH</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$r$</td>
<td>$p$</td>
<td>$r$</td>
</tr>
<tr>
<td>Total Caudate</td>
<td>-.391</td>
<td>.186</td>
<td>-.553</td>
</tr>
<tr>
<td>Caudate Head</td>
<td>-.430</td>
<td>.142</td>
<td>-.370</td>
</tr>
<tr>
<td>Caudate Tail</td>
<td>-.223</td>
<td>.464</td>
<td>-.615*</td>
</tr>
<tr>
<td>Asymmetry</td>
<td>.189</td>
<td>.536</td>
<td>-.033</td>
</tr>
<tr>
<td>Right Caudate</td>
<td>-.430</td>
<td>.142</td>
<td>-.553</td>
</tr>
<tr>
<td>Left Caudate</td>
<td>-.301</td>
<td>.317</td>
<td>-.530</td>
</tr>
</tbody>
</table>

*Correlation is significant at 0.05 level (2-tailed)

**Age and BASC Scores**

The relations between age and BASC externalizing composite and attention problems scores were investigated using correlation analyses (PPMC) with the pre and post GH 18q- and control groups. In the 18q- pre GH group, no significant relation was
found between age and the BASC attention problems score ($r = .218$, $p = .474$). There was a significant relation between age and the BASC externalizing composite ($r = .584$, $p = .036$), showing that externalizing problems increase with age in pre GH 18q-participants. In the post GH 18q-group, there was a significant relation between age and the BASC attention problems score ($r = .610$, $p = .027$). The attention problems score increased with age in the post GH 18q-group. There was no significant relation between age and the BASC externalizing problems score in the post GH 18q-group ($r = .447$, $p = .125$). In the control group, there were no significant relations among age and BASC attention problems and externalizing composite scores. Overall, there was an increase in externalizing problems with age in the pre GH 18q-group, and attention problems increased with age in the post GH 18q-group. Results of the correlations between age and BASC scores are shown in Table 9.

Table 9

<table>
<thead>
<tr>
<th>BASC Scales</th>
<th>Age of 18q- Pre GH</th>
<th>Age of Controls</th>
<th>Age of 18q- Post GH</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$r$</td>
<td>$p$</td>
<td>$r$</td>
</tr>
<tr>
<td>Externalizing Problems</td>
<td>.584*</td>
<td>.036</td>
<td>-.300</td>
</tr>
<tr>
<td>Attention Problems</td>
<td>.218</td>
<td>.474</td>
<td>-.496</td>
</tr>
</tbody>
</table>

*Correlation is significant at 0.05 level (2-tailed)

Caudate Volume and BASC Scores

The relations among the BASC attention and externalizing problems scores and right and left caudate volumes were examined using correlation analyses (PPMC) for the pre and post GH 18q-groups and controls. No significant relations were found between
the BASC scores and right or left caudate volumes for the pre GH 18q- group, post GH 18q- group, or controls. Results of the correlation analyses are shown below in Table 10.

Table 10

*Correlation is significant at 0.05 level (2-tailed)*

<table>
<thead>
<tr>
<th>Caudate Measures</th>
<th>Attention Problems</th>
<th></th>
<th>Externalizing Problems</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>r</td>
<td>p</td>
<td>r</td>
<td>p</td>
</tr>
<tr>
<td>Right Caudate Pre GH 18q-</td>
<td>.140</td>
<td>.649</td>
<td>-.357</td>
<td>.231</td>
</tr>
<tr>
<td>Left Caudate Pre GH 18q-</td>
<td>.274</td>
<td>.366</td>
<td>-.454</td>
<td>.119</td>
</tr>
<tr>
<td>Right Caudate Post GH 18q-</td>
<td>-.052</td>
<td>.866</td>
<td>.012</td>
<td>.968</td>
</tr>
<tr>
<td>Left Caudate Post GH 18q-</td>
<td>-.105</td>
<td>.734</td>
<td>-.151</td>
<td>.623</td>
</tr>
<tr>
<td>Right Caudate Controls</td>
<td>.377</td>
<td>.226</td>
<td>.532</td>
<td>.075</td>
</tr>
<tr>
<td>Left Caudate Controls</td>
<td>.430</td>
<td>.163</td>
<td>.353</td>
<td>.260</td>
</tr>
</tbody>
</table>

**Gender, Caudate Volume, and BASC Scores**

Male and female participants were compared on total caudate volume. The caudate volumes of 18q- participants (five males and eight females), pre and post growth hormone (GH) treatment, and controls (five males and seven females) were analyzed. Based on results of ANOVAs, there were no gender differences on total caudate volume for pre GH 18q- participants ($F_{1,11} = .049, p = .828, d = .132$), post GH 18q- group ($F_{1,11} = .535, p = .480, d = .426$), and controls ($F_{1,10} = 1.474, p = .253, d = .661$).

The male and female participants were also compared on the attention problems and externalizing problems scores from the BASC. Results of ANOVAs showed no gender differences on attention problems for pre GH 18q- participants ($F_{1,11} = .628, p = .
and post GH 18q- group ($F_{1,11} = 1.851, p = .201, d = .750$). The controls showed a gender difference ($F_{1,10} = 11.083, p = .008, d = 2.0$) on attention problems, with males showing greater attention problems. These results may not be clinically significant considering both males and females scored in the average range on the BASC. No statistically significant gender differences were found on the externalizing problems composite pre treatment ($F_{1,11} = 2.371, p = .152, d = .989$), post treatment ($F_{1,11} = 2.476, p = .144, d = .977$), and with controls ($F_{1,10} = 2.145, p = .174, d = .839$), although large effect sizes were calculated for the three groups. The small sample size in these analyses may have made it difficult to detect significant differences.
Chapter 6: Discussion

Results and Implications of Findings

Caudate Volume in 18q-

The volumetric analysis of the caudate revealed no differences in caudate volumes between controls and 18q- participants prior to growth hormone treatment. These findings are contrary to what was expected based on reports of dysmyelination of the brain in 18q- (Gay et al., 1997; Kline et al., 1993). It was predicted that because of reduced myelin, and with reports of smaller caudate volumes in children with attention disorders (Castellanos et al., 1996; Castellanos et al., 2002), the associated caudate volume would be smaller in 18q- participants. Although the children with 18q- in this study showed greater attention problems than controls, they did not show clinically significant ADHD symptoms. Casey et al. (1997) reported that children diagnosed with ADHD who had milder problems with attention and disinhibition had caudate volumes comparable to controls. Castellanos et al. (2002) found that smaller caudate volumes were correlated with greater symptom severity of inattention and impulsivity in children with ADHD. Similarly, Semrud-Clikeman et al. (2000) found that a smaller left caudate head was inversely related to externalizing behaviors in children with ADHD. The relationship between the severity of attention problems and caudate volume was not investigated in the current study, but it is possible that 18q- participants with the most severe attention problems would show smaller caudate volumes than controls.

No significant change in caudate volumes was found from pre to post growth hormone treatment, which was contrary to what was expected. Preliminary findings identified changes in the caudate following growth hormone treatment, suggesting an increase in myelination of the caudate based on reduced MRI T1 relaxation times (Cody
et al., 2005). A differing factor between the current study and Cody et al. (2005) was the age of participants at the start of treatment. Response to growth hormone therapy is more effective in younger children (Strobl & Thomas, 1994). Considering the average age of initial treatment was 35.3 months in Cody et al. (2005) study, and the age of initial treatment in the current study is 73.2 months, the participants in the Cody et al. (2005) study may have benefited more from treatment.

Without further study investigating the gray / white matter differences before and after GH treatment, it is difficult to rule-out if there was an increase in white matter and a decrease in gray matter with time, making it appear that there was no change in volume from pre to post growth hormone treatment. The current study was unable to provide this data due to limitations of the software utilized by the Research Imaging Center at the University of Texas Health Science Center of San Antonio. It was also possible that the difference between groups could not be detected due to a lack of adequate power. Although it is possible a difference could be detected with a greater sample size, only a small sample size could be obtained for this study due to the rarity of this disorder.

When comparing the caudate volumes between the post growth hormone treated 18q- participants and controls, no significant difference in caudate volumes was found. These results are not surprising given there was no difference in caudate volumes between controls and pre treatment 18q- participants, nor was there any change in volumes with growth hormone treatment. It would therefore be expected that the caudate volumes of controls and post growth hormone 18q- participants would be similar.

**Caudate Asymmetry in 18q-**

Results of the caudate asymmetry analysis with the 18q- participants prior to growth hormone treatment were consistent with predicted findings. It was expected that the 18q- group would not show the L > R asymmetry seen in typically developing
children (Hynd et al., 1994). Instead, the 18q- participants showed symmetric caudate volumes (L = R), similar to children with ADHD in the study by Castellanos et al. (1994). The controls in the current study also showed symmetric caudate volumes (L = R), rather than the expected L > R asymmetry in controls reported by Hynd et al. (1993) and Semrud-Clikeman et al. (2000).

The discrepancy in caudate asymmetry noted between the current study and the aforementioned studies may be related to a number of factors, including age range, gender, and handedness. The current study used younger participants and a broader age range (4 to 14 years), due to the limited number of subjects. The age ranges in the studies by Semrud-Clikeman et al. (2000) and Hynd et al. (1993) were 9 to 18 years and 8 to 13 years, respectively. Semrud-Clikeman et al. (2000) also used only right-handed males, while the current study used both males and females with mixed handedness.

The lack of difference in asymmetry between controls and 18q- participants prior to growth hormone may be related to a lack of adequate power. It is possible that a difference could have been detected with a greater sample size. However, a larger sample size was difficult to attain due to inadequate MRI scans and the limited number of participants who received MRI scans pre and post growth hormone treatment. Specifically, seven 18q- participants who qualified for the study were rejected due to poor MRI scans. Five participants treated with growth hormone were not included in the study because they did not receive an MRI scan prior to growth hormone treatment.

18q- participants post treatment also showed symmetrical (L = R) caudates, suggesting that growth hormone did not have a significant effect on caudate asymmetry. It was expected that with treatment, the caudate would show L > R asymmetry similar to controls, considering there was preliminary evidence of increased myelination with growth hormone (Cody et al., 2005; Hardies et al., 2001). Just as the caudate showed no
volumetric changes with treatment, there was also no change in asymmetry. Furthermore, there was no difference between the post growth hormone 18q- group and controls, which was expected. However, it was predicted that both groups would show L > R asymmetry, but instead the groups showed symmetrical (L = R) caudate volumes.

**Behavior in 18q-**

An analysis of attention and externalizing problems revealed that the 18q-participants prior to growth hormone treatment had greater attention problems, albeit mild, than the typically developing children. The two groups did not differ in the level of externalizing problems. The mild attention problems and lack of externalizing problems in 18q- participants may be misleading, considering a truncated sample was used in this study. Other studies have reported more severe attention problems with hyperactivity and impulsivity in the 18q- population (Mahr et al., 1996; Oster-Granite et al., 2001).

The effect of growth hormone on attention and externalizing problems was investigated, but no significant growth hormone effects were found. The 18q- group continued to show elevated attention problems after treatment, and there was no difference in externalizing problems between controls and 18q- participants post growth hormone treatment. The lack of growth hormone effect is contrary to expectations considering improvements in attention have been reported in children with growth hormone deficiency following growth hormone treatment (Soares et al., 1999; Van Dan et al, 2000). There are studies, however, reporting no improvement in cognitive or behavioral functioning after growth hormone therapy (Abbott et al., 1982; Degerblad et al., 1990; Pavel et al., 2003).
**Age Effects on the Caudate**

The caudate volume in controls was found to decrease with age, which is consistent with a number of studies (Castellanos et al., 1996; Giedd et al., 1996; Thompson et al., 2000). Thompson et al. (2000) reported up to 50 percent tissue loss in the caudate head in typically developing children from age seven to 11. The tissue loss likely reflects pruning, a process that eliminates synapses to reduce the number of connections and decrease structural volume (Giedd et al., 1996). Pruning removes synaptic connections to establish more functional networks of neuronal connections, therefore leading to greater accuracy of information processing and improved performance on cognitive tasks (Sowell et al., 2001).

The 18q- participants, pre and post growth hormone treatment, did not show this age related decrease in caudate volume. These findings are similar to children with ADHD. Enlargement of the caudate in ADHD suggests a deficiency in synaptic pruning, likely resulting in attention problems due to a disruption in the connections from the caudate to the prefrontal cortex (Castellanos et al., 2002). Due to the caudate’s connections with the frontal region, reductions in myelin and synaptic density in the prefrontal cortex may be correlated with changes in the structure of the caudate (Thompson et al., 2000). In children with 18q-, the lack of decrease in caudate volume combined with delayed myelination may result in impaired cognitive functioning, such as reduced speed and accuracy of information processing.

**Age Effects on Behavior**

There was no change in the severity of attention or externalizing problems with age in controls. There was an increase in externalizing problems with age, but not attention problems, in the 18q- pre growth hormone treatment participants. At post treatment, there was an increase in attention problems with age but not externalizing
problems. The average age for pre growth hormone 18q- participants was 6.1 years and 8.0 years for post growth hormone participants. Overt signs of externalizing behaviors (i.e., hyperactivity and impulsivity) decline with age, while inattentive symptoms remain stable from ages eight to 15 (Hart et al., 1995). Wilens, Biederman, and Spencer (2002) reported a greater reduction of hyperactivity and impulsivity when compared with levels of inattention from age six to 16 years. Thus, the externalizing problems may be more evident with the younger age group, while the attention problems become more apparent as the child ages and progresses in school and hyperactivity decreases.

SUMMARY

In summary, results did not support the hypotheses of smaller caudate volume and reduced caudate asymmetry in pre treated 18q- participants when compared to controls. Instead, there were no significant differences in caudate volume and asymmetry among the 18q- participants pre and post treatment and controls. The 18q- participants and controls showed no caudate asymmetry (L = R), which is contrary to the L > R asymmetry expected in controls and post growth hormone participants.

The 18q- participants pre and post treatment showed greater attention problems, although mild, than the control group. There was no difference in externalizing problems among the groups. It was hypothesized that there would be a decrease in behavioral problems with growth hormone treatment, but there was no change in behavioral functioning with treatment. There was also no change in the severity of attention or externalizing problems with age in controls. The 18q- participants pre treatment showed an increase in externalizing problems with age, and at post treatment they showed greater attention problems with increasing age.

Caudate volumes were found to decrease with age in controls, which is an expected finding. However, this age-related decrease was not observed in 18q-
participants. The lack of decrease is likely related to the process of pruning, which removes synaptic connections to establish more functional networks of neuronal connections. A disruption in synaptic elimination and myelination would likely interfere with cognitive functioning, particularly speed and accuracy of information processing in individuals with 18q-.

LIMITATIONS

There are a number of limitations that should be addressed in the context of this study. First of all, the rare occurrence of 18q- limited the number of participants in this study. It is possible that more significant findings could have been found with a larger sample size. The sample size was also limited by the number of viable MRI scans pre and post treatment. The original proposal of the study was to utilize non-growth hormone treated participants, but the number of participants with functional scans was limited to three children.

Results of the present study were also limited by the inability to control random assignment to the 18q- and control groups. Individuals with 18q- who met the criteria for growth hormone replacement therapy were placed in the 18q- group, and those individuals with no disorders were placed in the control group. Although selection bias is a threat to internal validity, it would be unethical to design a random clinical trial where treatment was withheld from some children who qualified for growth hormone therapy. In addition, an issue with control participants was the lack of pre / post testing, which was limited due to financial constraints.

An additional limiting factor of the study is that chromosome deletions differed in size and region in participants with 18q-. It is currently unknown whether different breakpoints contribute to more or less severe problems with cognition, behavior, or myelination. In addition, the time between pre and post testing varied, ranging from 1.2
years to 5 years, due to difficulty obtaining viable MRI scans. The age at which growth hormone treatment was initiated also ranged from 2.5 years to 13.8 years. This inconsistency may have affected the results outcome due to developmental considerations, considering younger children have shown a greater response to growth hormone than older children. A final limitation is that many of the participants in this study were receiving rehabilitative services that may have influenced their cognitive and behavioral functioning.

**Future Directions**

Considering response to growth hormone treatment is more effective in younger children (Strobl & Thomas, 1994), earlier treatment in children with 18q- may have a greater impact on development of the brain and cognitive functioning. Myelination is especially rapid the first two years of life, so children with 18q- may show a greater benefit from growth hormone therapy during this time period.

The relationship between the severity of attention problems and caudate volume was not investigated in the current study, but it would be interesting to investigate a greater sample of 18q- participants with severe attention problems to determine if this group shows smaller caudate volumes than controls. It would also be helpful to investigate the gray/white matter differences before and after GH treatment to evaluate changes in both white and gray matter with time. An analysis of gray and white matter would provide better understanding of the developmental changes of the brain and the effects of growth hormone on neuroanatomy.

Although no significant differences were found between 18q- and control participants in caudate volume and asymmetry, the lack of decrease in caudate volume with increasing age suggests disrupted development of the neural system. This disruption likely involves the white matter connections from the caudate to the prefrontal cortex,
given the dysmyelination of the brain in individuals with 18q-. The attention problems observed in 18q- also point to dysfunction of the frontostriatal circuitry. Deficient maturation of this circuitry could lead to delayed maturation of other areas of the brain, resulting in poor control over other cognitive functions. Overall, the functional implications related to neuroanatomical abnormalities in 18q- require further investigation. Future research analyzing the neuroanatomical correlates, behavior, and cognitive functioning in individuals with 18q- will help researchers, parents, and educators better understand how to best meet the needs of children with 18q-.


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Vita

Susannah Jaeger More was born in Rockford, Illinois on February 1, 1975 to Joan Vandre and Douglas More. She was raised in Sycamore, Illinois. Susannah earned her Bachelor of Science degree in Psychology at the University of Illinois in Champaign-Urbana in May 1997. Susannah attended graduate school at the University of Texas at Austin, where she received her Master of Arts degree in Educational Psychology, with a specialization in School Psychology, in May 2003. Following completion of her doctorate in Educational Psychology in May 2006, Susannah will begin a postdoctoral residency in neuropsychology at the University of Michigan in Ann Arbor.

Permanent address: 2901 Barton Skyway, Apt 1506, Austin, Texas, 78746

This dissertation was typed by the author.