

Growth Hormone Benefits Children With 18q Deletions

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Most individuals with constitutional deletions of chromosome 18q have developmental delays, dysmyelination of the brain, and growth failure due to growth hormone deficiency. We monitored the effects of growth hormone treatment by evaluating 23 individuals for changes in growth, nonverbal intelligence quotient (nIQ), and quantitative brain MRI changes. Over an average of 37 months, the treated group of 13 children had an average nIQ increase of 17 points, an increase in height standard deviation score of 1.7, and significant change in T1 relaxation times in the caudate and frontal white matter. Cognitive changes of this magnitude are clinically significant and are anticipated to have an effect on the long-term outcomes for the treated individuals.

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KEY WORDS: 18q-; growth hormone treatment; cognitive development; myelination; chromosome abnormalities

INTRODUCTION

We are performing comprehensive and longitudinal evaluations of individuals with deletions of chromosome 18q. The frequency of this deletion is estimated to be 1/40,000 births [Cody et al., 1997a]. Affected individuals were referred to us after a diagnostic chromosome analysis detected the loss of material from the long arm of chromosome 18. The clinical presentation varies greatly from individual to individual, as does the amount of the chromosome loss (hemizygosity). We have reported previously on both the clinical spectrum [Gay et al., 1997; Cody et al., 1999] and the genotypic variability [Cody et al., 1997a; Brkanac et al., 1998]. Some of the more common characteristics of individuals with 18q deletions are short stature, dysmyelination of the brain, developmental delay, delayed expressive language, flat midface, hearing

impairment, proximally placed thumbs, and atretic or stenotic ear canals.

We reported previously on the use of MRI T1 relaxometry to quantify the subjective observations by other investigators of brain hypomyelination in this population [Miller et al., 1990; Rodichok and Miller, 1992; Ono et al., 1994]. The developmental process of myelination in individuals with deletions of 18q does not progress at a normal rate and never achieves normal adult levels [Gay et al., 1997]. These abnormal T1 values have been correlated with changes in the water-pool fractions of different cellular compartments in the brain, demonstrating that the observed changes in T1 values correlate with changes in myelin content [Lancaster et al., 2003]. This allows us to use T1 relaxometry to measure changes in myelination over time in study participants.

The dysmyelination phenotype was hypothesized to be due to hemizygosity of the gene for myelin basic protein (*MBP*); a major protein component of the CNS myelin sheath [Miller et al., 1990]. We defined a 2 Mb region of 18q that included the *MBP* gene and was hemizygous in all the participants who had the dysmyelination phenotype [Gay et al., 1997]. In fact, all of the individuals who were hemizygous for this region had the dysmyelination phenotype. Since this gene is near the telomere on 18q, it is hemizygous in all the study participants reported here.

Most individuals with 18q deletions have some degree of growth failure, and 68% qualify for growth hormone (GH) replacement therapy using standard assessment criteria [Hale et al., 2000]. A critical region of 18q that was hemizygous in all the study participants with growth hormone deficiency was also identified [Cody et al., 1997b]. This region is almost identical to the dysmyelination critical region, making the gene or genes responsible for these two phenotypes either the same gene or two tightly linked genes. We have not yet been able to unlink these two phenotypes by identifying an individual with an 18q deletion who has dysmyelination and unequivocally normal growth.

In addition to the fact that the gene(s) for these two phenotypes are tightly linked, the biology of the two phenotypes may be linked as well. Fetal rat brain cell aggregates increase production of myelin basic protein when growth hormone is added to the culture medium [Almazan et al., 1985]. However, growth hormone deficiency alone, at least in the growth hormone deficient little mouse, does not cause dysmyelination [Lehman et al., 1999]. Conversely, dysmyelination, as demonstrated in the heterozygous shiverer mouse with one functional copy of the *Mbp* gene, does not result in growth hormone deficiency [Lehman, 1999]. Moreover, in the

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Received 12 September 2004; Accepted 24 May 2005

DOI 10.1002/ajmg.a.30848

mouse one functional copy of Mbp is sufficient for normal myelination [Popko et al., 1987]. We therefore hypothesized that dysmyelination might result from the combination of MBP hemizygosity and GH deficiency or at least be exacerbated in children with 18q- who were also growth hormone deficient.

The fact that central myelination is predominantly a postnatal event provides a potential opportunity to normalize the process through intervention with growth hormone replacement therapy. We set out to determine if growth hormone treatment could affect the process of myelination as measured by T1 relaxometry. Regardless of any correlation between myelination and growth hormone therapy, we still wanted to know if changes in brain microstructure correlated with changes in nonverbal IQ.

METHODS

Participant Recruitment Information

Families were referred for participation in this study by The Chromosome 18 Registry and Research Society or through the family's private physician. The Institutional Review Boards of the University of Texas Health Science Center at San Antonio and the Audie L. Murphy Veterans Administration Hospital approved the study and the informed consent process was documented. Each participant was referred after a cytogenetic diagnosis detected a deletion of the long arm of chromosome 18. Chromosome analyses were repeated by our laboratory in

those cases in which we received incomplete or low quality karyotypes.

Twenty-three study participants were evaluated at the General Clinical Research Center located at Audie L. Murphy Veterans Administration Hospital, San Antonio, Texas. The presence of a chromosome 18q deletion in each participant was confirmed using PCR based polymorphic marker analysis [Cody et al., 1997a] (see Fig. 1). Potential participants with more complex chromosome rearrangements were excluded from this study.

Evaluation of Growth Failure and Growth Hormone Deficiency

Participants were evaluated for growth failure and possible GH therapy using standard clinical protocols. All participants were euthyroid during the period of the study. While many of the children had multiple medical problems, none had cyanotic heart disease or malnutrition that potentially could hinder growth and development.

For children over 3 years of age, height was determined using a wall-mounted stadiometer, and height standardized scores in standard deviation (SD) units was calculated by a computer program designed for this purpose (Growth Base III, Eli Lilly and Company). For children under 3 years of age, length was determined using a board-mounted stadiometer. Height SD was calculated based on normative data [Hall et al., 1989] as described previously [Cody et al., 1999]. The growth

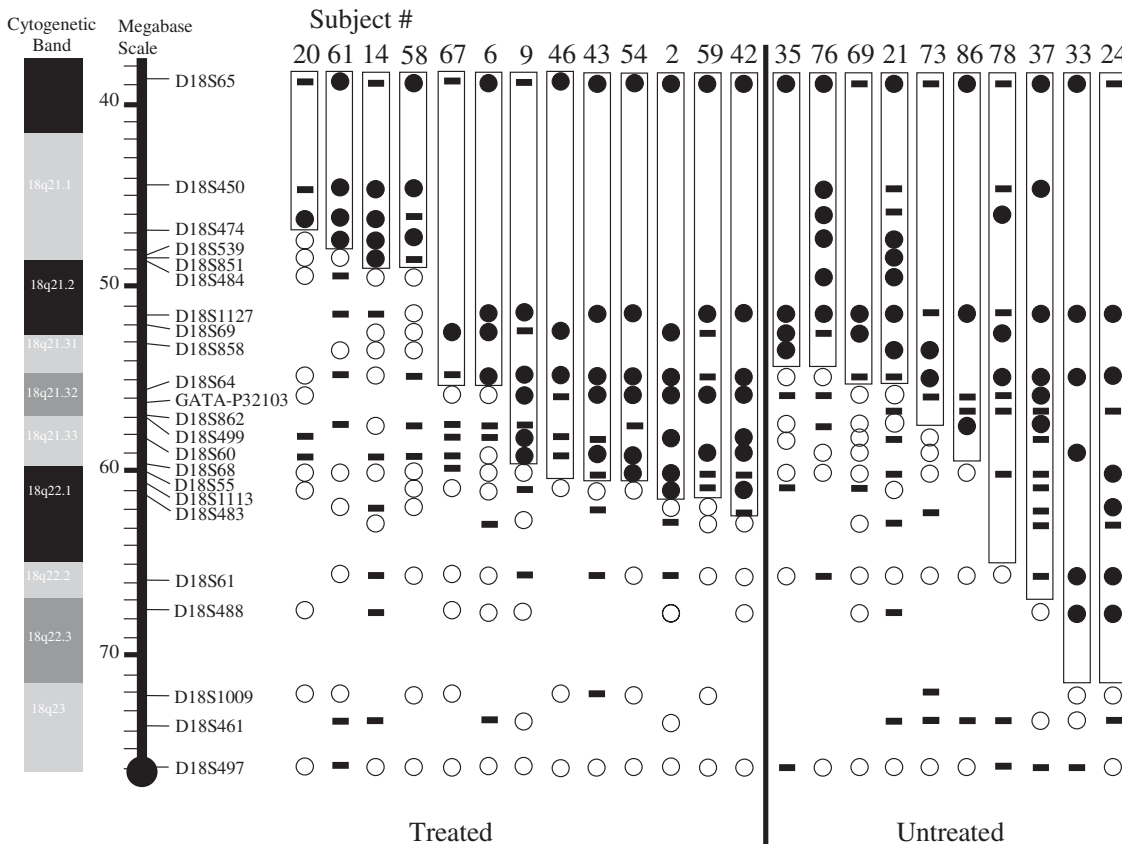


Fig. 1. Molecular analysis of the extent of the deletion for each subject. The data for subject is shown in a column under each subject number. The cytogenetic band designation and the megabase scale for the distal half of chromosome 18q are shown on the left. The PCR-based polymorphic markers used in the analysis were placed on the megabase scale using data from the May 2004 genome sequence data freeze from the UC Santa Cruz Genome Browser (<http://genome.ucsc.edu>). ○, single alleles; ●, two alleles; ■, results were uninformative. The vertical rectangles show the maximum amount of chromosome present in two copies in each individual participant.

velocity was determined by subtracting a height (length) obtained at least 3 months prior to the visit from the height (length) obtained at the time of visit. The growth velocities obtained at our center were consistent with previous velocities from three or more determinations in the historical height record. For children over 3 years of age, growth velocity SD was calculated by Growth Base III (Eli Lilly and Company). For children under 3 years of age, growth velocity SD was calculated in a manner similar to that described for calculating height SD in this age group [Cody et al., 1999]. Throughout the tables and figures, height SD and growth velocity SD are presented compared to chronological age. An X-ray of the left hand and wrist for bone age (BA) determination using the standard method was obtained at the time of initial visit to our Center [Greulich and Pyle, 1959]. A BA that was >2 SD below the mean for age was considered delayed. All children were evaluated using a standard protocol that included the measurement of insulin-like growth factor-1 (IGF-1), insulin-like growth factor binding protein 3 (IGFBP-3), l-thyroxine and thyrotropin. IGF-1 SD and IGFBP-3 SD were calculated using an algorithm and normative data for these assays provided by Genentech, Inc. (South San Francisco, CA). Bone age data are normalized to chronological age in all tables and figures [Frindik et al., 1999]. For purposes of GH provocative testing, children were admitted to the General Clinical Research Center on the evening preceding the evaluations. Following pretreatment of the site with an anesthetic cream (EMLA Cream, Astra Pharmaceuticals), a peripheral intermittent intravenous device was placed in an arm vein at least 30 min prior to the start of the test. Patency was maintained by saline flush every 4 hr. The tests were begun between 8 and 9 a.m. following an overnight fast. GH release was evaluated using arginine hydrochloride and/or clonidine as the provocative agent. After collection, blood for GH analysis was centrifuged within 10 min and the serum and/or plasma was transferred to

clean tubes, labeled and frozen at -20°C until assayed. The Nichols Institute (San Juan Capistrano, CA) performed all hormonal assays. GH was measured by polyclonal radioimmunoassay.

The decisions to treat or not to treat with GH were based on an algorithm that included anthropometric, radiographic, and biochemical measures. For purposes of the algorithm, heights with standardized scores in SD units <-2 SD, growth velocities <-1 SD, bone age <75% of chronological age, insulin-like growth factor-I (IGF-1) <-1 SD, insulin-like growth factor binding protein 3 (IGFBP3) <-1 SD, and peak GH <10 ng/ml were considered abnormal. To qualify for treatment each child had to have at least four abnormal values. The decision to treat was subject to review and approval by the individual participant's medical insurance carriers, since they paid the costs of treatment. Thirteen of the 23 participants qualified for growth hormone therapy using these criteria (Table I). At the start of growth hormone treatment, the mean age of the treated group was 35.3 months and the mean age of the untreated group was 46 months. Individuals were treated with the standard dose of GH of 0.3 mg/kg/wk.

MRI Imagine Acquisition Protocol

All images are acquired on a large-bore 1.9 Tesla clinical MRI system (Elscent, Haifa, Israel/GE medical systems). Three spin-echo images are acquired, a T1-weighted image (TE/TR = 20/500 msec) and dual echo Proton Density (PD)-weighted/T2-weighted images (TE1/TE2/TR = 20/80/3,400 msec). First-order flow compensation is applied prior to the second echo, and presaturation of a slab inferior to the scan volume is used to minimize arterial inflow artifacts. Twenty-two axial images are acquired in a 256 x 256 array (1 mm pixel spacing) with 5 mm slice thickness and a 1 mm gap (132 mm span). Acquisition times are ~20 min, mostly due to the long TR

TABLE I. Initial Growth Parameters of the Individual Participants

| Treatment | Age (months) | Bone age (as % CA) | Height SD | Growth velocity SD | IGF1 SD | IGFBP3 SD | CMAX GH | AMAX GH | |
|-----------|--------------|--------------------|-------------|--------------------|-------------|-------------|-------------|------------|------------|
| None | 92 | 91 | -1.2 | -0.8 | -1.56 | -0.79 | 9.9 | ND | |
| | 42 | 86 | -1.96 | -1.6 | 0.62 | -0.18 | 8.1 | 14.0 | |
| | 75 | 100 | -2.04 | -2.1 | -2.29 | -2.24 | 23.0 | 18.1 | |
| | 75 | 100 | -2.04 | -1.3 | -2.78 | -0.47 | 22.2 | ND | |
| | 41 | 102 | -2.39 | 0.2 | -0.72 | 0.08 | 14.4 | 4.8 | |
| | 11 | 109 | 0.7 | -1.2 | 2.2 | -1.3 | ND | 12.8 | |
| | 14 | 64 | -3.4 | -1.8 | -3.2 | -3.5 | ND | 11.2 | |
| | 22 | 73 | -2.74 | -1.4 | -1.9 | -0.1 | 17.7 | 6.9 | |
| | 71 | 72 | -2.2 | -2.0 | -1.3 | -0.2 | 24.2 | 14.0 | |
| | 17 | 88 | 0.33 | -0.5 | -1.2 | -2.0 | 9.7 | 1.1 | |
| | Mean (SD) | 46.0 (30.0) | 88.5 (14.9) | -1.69 (1.3) | -1.25 (0.7) | -1.21 (1.6) | -1.07 (1.2) | 16.2 (6.5) | 10.4 (5.6) |
| | GH | 13 | 70 | -3.11 | 0.6 | -0.52 | -1.51 | 12.0 | 2.5 |
| | | 35 | 26 | -3.58 | -1.4 | -1.52 | -1.51 | 4.7 | ND |
| | | 33 | 82 | -2.16 | -2.4 | 0.48 | -0.05 | 8.9 | ND |
| 81 | | ND | -4.79 | -1.2 | -2.78 | -0.96 | 7.3 | ND | |
| 35 | | 69 | -2.37 | -2.6 | 0.21 | -0.96 | 8.9 | 5.1 | |
| 20 | | 50 | -2.21 | 1.7 | -2.31 | -0.05 | 3.2 | 3.5 | |
| 31 | | 61 | -4.11 | -2.4 | -0.72 | -1.80 | 7.9 | 6.8 | |
| 56 | | 64 | -2.05 | -2.9 | -1.41 | -0.47 | 8.0 | 2.9 | |
| 46 | | 39 | -1.66 | -1.2 | -1.3 | -2.8 | 5.7 | 5.6 | |
| 64 | | 75 | -2.59 | -2.2 | 0 | -1.0 | 8.7 | 5 | |
| 5 | | 10 | -4.2 | ND | 0 | -3.1 | ND | 3.0 | |
| 9 | | 67 | -2.6 | ND | -3.2 | -3.8 | ND | 12.8 | |
| 31 | | 58 | -3.3 | -2.5 | 0.6 | -1.5 | 1.7 | 5.1 | |
| Mean (SD) | | 35.3 (22.0) | 55.9 (21.2) | -2.98 (1.0) | -1.5 (1.4) | -1.0 (1.2) | -1.5 (1.1) | 7.0 (2.9) | 5.2 (3.0) |
| P* | 0.402 | 0.001 | 0.020 | 0.230 | 0.555 | 0.352 | 0.002 | 0.062 | |

SD, standardized scores in standard deviation units; CMAX, maximum stimulated GH response to clonidine; AMAX, maximum stimulated GH response to arginine; ND, not done.

*P-values based on a Wilcoxon rank-sum test.

(3,400 msec) in the dual echo acquisition. This long TR was needed for PD-weighting in younger subjects, with longer T1s. Subjects are immobilized to minimize movement between scanning sequences. This three-image protocol has been used extensively in our lab to create T1, T2 images in both children with 18q- and typically developing children across the age span of interest [Gay et al., 1997; Lancaster et al., 2003].

MRI Relaxation Times

MRI relaxometry was used to monitor GH mediated effects in several specific regions in the brain (frontal white matter, insular gray matter, and caudate). Both T1 and T2 relaxation times shorten progressively during normal childhood development. Additionally, relaxation times have been shown to be shorter [Gay et al., 1997; Lancaster et al., 2003] in typically developing children compared to children with 18q-. In order to determine if children with 18q- treated with GH experienced any changes beyond that expected due to normal development we performed age correction modeling (this is similar to the strategy that is used when heights on a cohort of children of various ages and gender are converted to a standardized score or z-score). A power model was used to estimate T1 in frontal white matter (FWM), the caudate, and insula at the median age of the group (48 months) for each subject.

MRI relaxometry calculates T1 (spin-lattice) and T2 (spin-spin) relaxation times from MR images. T1 varies by tissue with short values in fat (<500 msec) and long values in CSF (>2,000 msec), with white matter (WM) and gray matter (GM) taking on intermediate values. While T1 is affected by many tissue qualities it is generally shorter in tissues with larger molecules (i.e., fat) and longer in tissues with smaller molecules (CSF). T1 and T2 tend to rise and fall together, but T2 is more affected by changes in the extracellular water fraction in biological tissues. Together these relaxometry parameters provide measurements to track changes in the brain with age and tissue development. T1 images are calculated using a two-point ratio method with in-house software. This method has been used successfully in our lab for many years [Gay et al., 1997; Lancaster et al., 2003], and estimated T1 values for frontal white matter in adults are consistent with values commonly reported. Regions of interest (ROIs) based on preliminary data were defined as FWM, the caudate (deep gray matter), and insular gray matter and mean T1 and T2 values for each region were calculated for each subject. However, the value of this methodology is that all images are stored electronically and any area of the brain can be selected for specific investigation.

Neuropsychological Evaluations

Participants were also evaluated to determine if there were changes in their cognitive ability. The instruments used to measure nonverbal abilities differed depending on the age of the child. The Bayley Scales of Infant Development: 2nd Edition was used for children ages 1-42 months [Bayley, 1993]. The mental scale portion of the Bayley Scales was used for those children who were moderately to severely retarded (IQ less than 50). This strategy permits the child's language development, fine motor skills, reasoning and memory to be assessed. The Differential Abilities Scales (DAS) was used for children who were over 42 months. For children above age 6, the Wechsler Intelligence Scale for Children (WISC-III) was used. Given that a large proportion of children with 18q deletions have hearing impairments, the nonverbal IQ was used rather than the Full Scale IQ. Since the degree of functional hearing impairment is difficult to determine in young developmentally delayed children, we chose to use tests

that did not rely on an ability to hear, thereby removing hearing impairment as a potential variable [Sattler, 1992; Kamphaus, 1993].

Statistical Analysis

The Bayesian Hierarchical Model [Gelman et al., 2000] was used and assumes that given the treatment status, age, and the relationship between the dependent variable (either standardized height or performance IQ) and elapsed treatment time, the measurement error of the dependent variable is exchangeable for any subjects. It also assumes that there is a linear relationship between the dependent variables and elapsed treatment time. Without effective treatment we assume that there is no change in standardized height scores or performance IQ. This is the linear relationship with a slope of zero. Finally, it assumes that the subjects in the same treatment group have the same underlying relationship between elapsed treatment time and the dependent variable, with individual variation due to unobserved, uncontrolled covariates. These assumptions appear valid given the observational nature of the study and the similarities between subjects in the same treatment group.

To estimate the effect of growth hormone on the dependent variables we used the Markov Chain Monte Carlo (MCMC) methods. The software WinBUGS was used to perform Gibbs Sampling for the parameters of the model. This technique consists of sequentially sampling from the full conditional distributions of the parameters in the model in order to form a Markov Chain. The Markov Chain then converges to its stationary distribution, which is the joint posterior of the unknown parameters, using the knowledge of the observed data.

Five Markov Chains were started and run independently. The Gelman-Rubin (G-R) Statistic is often used to gauge convergence when there are multiple chains. This statistic converges to 1 and values less than 1.2 are considered to show that the chains have converged to the stationary distribution. The five chains we ran were observed to have G-R statistics converging to 1 by 250 iterations. Twenty thousand iterations were run for each chain and the first 10,000 were discarded to ensure convergence.

The slopes of the linear relationship between the dependent variables and elapsed time were calculated from the 50,000 samples from the converged Markov Chains. These samples give us estimates of the posterior distributions of the four parameters of interest given the observed data.

RESULTS

The results of the initial growth and endocrine evaluations are shown in Table I. The treated group and untreated group were different from each other with regard to growth parameters at the beginning of the study because the treatment group met the clinical criteria for treatment and the untreated group did not. Because it is unethical to withhold an approved treatment, we were unable to have a treatment and control group with equivalent growth and growth hormone parameters. Therefore, we had a treatment group of children who qualified for therapy and a control group of children who did not. Our results were therefore measured by comparing the pre and post study measures of each group. Height z scores are standardized and children typically track beyond age 3. A child's ability as measured by traditional IQ tests generally remains within the confidence interval for the scores obtained particularly for those children who score in the retarded range. Therefore, it is anticipated that the untreated group of children will have no changes and that the treated children will experience changes in standardized measures during the study interval.

The mean age of the treated children at the initial visit was 35 months and was 73 months at the follow-up visit (a change of 38 months). The mean age of the untreated children at initial visit was 46 months and was 85 months at the follow-up visit (a change of 39 months).

Figure 2 illustrates the longitudinal height standardized scores (z-scores) for the individual children. A child that is growing at a normal rate will have a z-score that does not change over time and would therefore appear as a horizontal line on Figure 2. At baseline, the untreated group had an average z-score for height of -1.7 , with an observed change of -0.27 over the observation period. The average z-score for the GH treated group was -2.9 at baseline, with an average change of $+1.8$ over the observation period.

The age-normalized results of the MRI relaxometry studies were compared across four groups: the untreated group of children with 18q-, the pre treatment group, the post treatment group, and unaffected control children. These studies were performed on 12 of the 13 GH treated children with 18q- (one of the treated children could not be scanned due to dental implants). Our hypothesis was that the MRI results of the treated children would be closer to that of the unaffected control children than to that of the untreated children with 18q-. An analysis of variance across these three groups, controlling for age, showed a significant group effect in T1 relaxation times for frontal white matter ($P = 0.008$), and for caudate ($P = 0.008$).

The results for measures of FWM showed that the untreated children with 18q- had an unadjusted mean T1 relaxation time of 1024.0 msec. The mean relaxation time of the unaffected control children was 816.4 msec, making these two groups significantly different, with an age-adjusted mean difference of 218.0 msec (95% CI: 64.9, 371.0). Therefore, in FWM the children with 18q- were significantly dysmyelinated in comparison with the unaffected control children.

The effect of GH treatment in FWM was to make the T1 relaxation times of the treated children approach those of the unaffected control group. The children with 18q- who were treated with GH had an age-adjusted difference in mean T1 relaxation time of -95.8 msec (95% CI: -234.0 , 42.6) less than that for the untreated children. This was a trend toward normalcy but was not statistically significant.

For the caudate, there was only a small age-adjusted difference (-24 msec) between the untreated children with

18q- and the unaffected children. Therefore, the effect of having an 18q deletion was not as great in this region of the brain. However, the effect of GH treatment was to make T1 relaxation times significantly shorter for the treated children than for either the untreated children with 18q- (age-adjusted $M = -193$ msec, 95% CI: -332.9 , -52.8), or the unaffected control children (age-adjusted $M = -218$ msec, 95% CI: -356.0 , -79.4). In the caudate, growth hormone had a significant effect away from normalcy.

If the analysis of the effect of treatment is performed using only data from those children who have both pre and post treatment scans ($N = 7$), there is a significant treatment effect in the FWM (age-adjusted $M = -244$ msec., 95% CI: -440.3 , -47.7) and in caudate (age-adjusted $M = -243$, 95% CI: -385.9 , -63.7). The same pre- and post-analysis performed on the untreated children on whom we have two scans showed no apparent difference in T1 relaxation times. Therefore in this small subset of patients, GH treatment had a significant effect in both FWM and the caudate.

The T1 relaxation time in the insula was shortened by treatment but the change did not reach statistical significance ($P > 0.065$). These data suggest that caudate T1 was significantly reduced by GH treatment with a smaller reduction in FWM. The trend was for T1 to become shorter following GH treatment in each of the areas evaluated. The shortening of T1 in the two gray matter areas was not toward normalcy as it was in FWM.

Changes in nonverbal IQ were monitored and are presented in Figure 3. Only a subset ($N = 20$) of the participants could be evaluated in this way. Three children could not be evaluated reliably. One child was autistic and was unable to cooperate for any of the testing, another child was blind, deaf and significantly developmentally delayed and the third was under 1 year of age. These three children were included in the other evaluations in this study because we wished to evaluate their growth and MRI changes in response to GH treatment.

The untreated group had nonverbal IQ changes ranging from -19 to $+7$ with a mean change of -3.0 points. The treated group had IQ changes ranging from -1 to $+41$ points with a mean increase of $+16.8$ IQ points on the nonverbal scale. The children in the treated group appeared to be in one of two categories, responders or non-responders. The non-responders all had nonverbal IQ scores at or below the lower limit of the

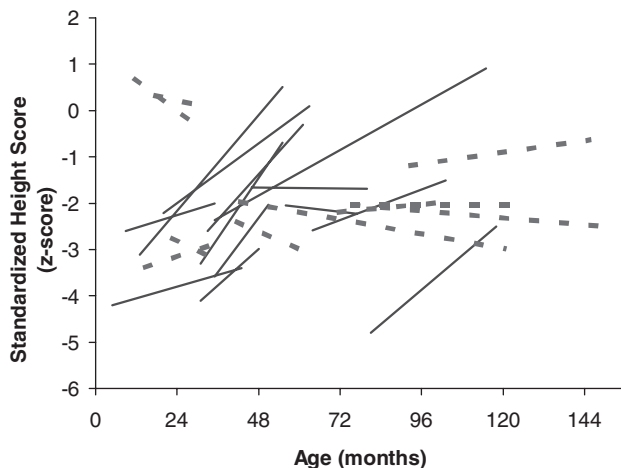


Fig. 2. Longitudinal changes in height standardized scores (z-scores) with and without growth hormone (GH) treatment. The solid lines connect the longitudinal height standard deviation scores of the GH treated individuals. The dashed lines connect the longitudinal data of the untreated individuals.

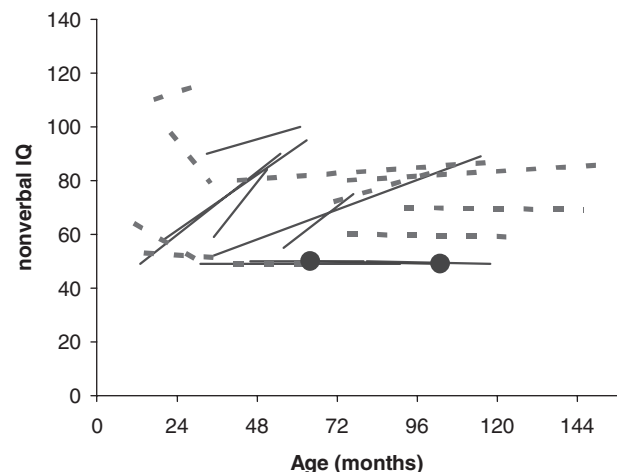


Fig. 3. Longitudinal changes in IQ with and without GH treatment. The solid lines connect the longitudinal IQ scores of the GH treated individuals. The dashed lines connect the longitudinal data of the untreated individuals.

test (nonverbal IQ ≤ 50) at both the pre-treatment and post-treatment evaluations. Therefore any change in nonverbal IQ over the treatment period could not be assessed. The children who began the study with a nonverbal IQ greater than 50 had nonverbal IQ increases of between 10 and 41 points, with a mean increase of 28.3 points.

We have shown that the extent of the deletion correlated with the Vineland Adaptive Scores [Semrud-Clikeman et al., 2005]. We wanted to determine if the response to GH also correlated with the extent of the deletion. Figure 1 illustrates the molecular analysis results of the extent of each participant's deletion of chromosome 18. Subjects number 20, 46 and 67 were not able to undergo neuropsychological testing as explained previously. The non-responders in the treated group are subjects number 61, 14, 58, and 54, three of whom have very large deletions. Subject 54 has autistic behaviors which interfered with his ability to comply with testing procedures. Whether the extent of the deletion prevents cognitive improvements or the limits of neuropsychological testing for severely impaired children hinder the ability to detect improvement remains to be determined.

Separate Bayesian hierarchical linear models [Gelman et al., 2000] were fit for the treated and untreated samples to estimate the distributions of the population relationship between time elapsed on treatment and dependent variables given the observed data (Table II). The results demonstrate that there was no significant change in the population z-scored height for untreated patients; estimated decrease of 0.11 standard deviations per year untreated (95% PI: $-0.64, 0.43$). There was a significant increase in the population standardized height of GH treated patients; estimated increase of 0.57 standard deviations per year of GH treatment (95% PI: $0.15, 0.99$).

Similarly, there was no significant change in the population nonverbal IQ score for untreated patients; estimated increase of 0.5 IQ points per year untreated (95% PI: $-2.37, 3.30$). There was a significant increase in the population nonverbal IQ score of GH treated patients; estimated increase of 5.5 IQ points per year of treatment (95% PI: $1.71, 9.86$).

DISCUSSION

The results presented here demonstrate that GH therapy in individuals with 18q deletions increases linear growth in most of the children, improves nonverbal IQ in the majority of participants, and causes a change in the T1 relaxation times in specific areas of the brain. This is the first study to show changes in response to GH treatment in children with 18q-.

Since GH treatment is a replacement therapy, it might be anticipated that the children who do not qualify for treatment would not need or benefit from GH. If this were the case, we might expect that IQ scores of the untreated children at the beginning of the study would be comparable to the treated children after replacement therapy. However, the nonverbal IQ scores of the untreated children did not differ significantly from the scores of the treated children before treatment. One possible explanation is that the children who did not qualify for GH therapy were not truly GH sufficient. This is consistent with the hypothesis that individuals with 18q- have hypo-

thalamic, as opposed to pituitary, dysfunction. We have preliminary data to support the hypotheses of hypothalamic dysfunction [D.E. Hale, unpublished observations] but will need to perform further studies. All of the participants in this study are hemizygous for the growth hormone deficiency critical region [Cody et al., 1997b] therefore each could potentially be growth hormone deficient.

The results of the present study are limited by the fact that subjects were not assigned randomly to treatment versus control groups. In the present study, those individuals who met standard clinical criteria for GH replacement therapy received GH and those who did not qualify did not. We felt it would be unethical to design a clinical trial in which an approved treatment was withheld from some of those who would normally qualify for treatment. Future work will involve monitoring the effects of GH treatment by randomizing into treatment and control groups only those children who do not meet standard clinical criteria for GH treatment.

The functional significance, if any, of the CNS changes visualized by MRI are not known. Individuals with a deletion of another CNS myelin gene, the proteolipid protein 1 gene, have the mildest form of Pelizaeus-Merzbacher Disease with motor and cognitive delays especially with regard to expressive language [Cailloux et al., 2000]. However, this gene is at Xq22, therefore, males have no functional copies of the gene, a situation not completely comparable to the loss of one allele on an autosome.

The practical consequences of changes in nonverbal IQ of this magnitude are well delineated. The average population score for IQ is 100 with scores ≤ 70 being defined as mental retardation. The nonverbal IQ scores are normalized so that for a typically developing child, a score is likely to range within the confidence interval of ± 7 with repetitive testing. All but one of the children who began this study with a score in the measurable range (i.e., >50) of the IQ test had a score at the end of the study that was >70 (i.e., within normal range). This change in ability would be expected to have a significant impact on their potential as adults.

Of the children who exhibited essentially no nonverbal IQ increase while on growth hormone therapy, three had the largest deletions in the entire cohort of study participants. This raises the possibility that there is a region that when hemizygous negates any intellectual benefits from growth hormone therapy. However, both pre and post nonverbal IQ measures were below the floor of the test, and therefore any change would be impossible to detect. The fourth child had a smaller deletion that the other three, but severe autism rendered him untestable.

The mechanisms by which GH affects cognition and myelination are still unknown, including whether they are independent outcomes or have a cause and effect relationship. The effects of GH therapy on cognition were reviewed by Sartorio et al. [1996]. Whether it is GH or one of the mediators of GH action that is responsible for improved cognition is not yet clear. GH does cross the blood-brain barrier by way of a receptor-mediated mechanism [Johansson et al., 1995]. In addition, Johansson et al. have shown that GH treatment affects neurotransmitter levels in cerebrospinal fluid significantly. They demonstrated that GH treatment causes the

TABLE II. Posterior Probability Intervals for the Yearly Change in Nonverbal IQ and Standardized Height Scores

| | Nonverbal IQ | | | Height (z-score) | | |
|-----------|--------------|--------------------------|--------|------------------|--------------------------|--------|
| | Estimate | 95% probability interval | | Estimate | 95% probability interval | |
| Untreated | 0.5255 | -2.3652 | 3.3000 | -0.1071 | -0.6413 | 0.4256 |
| Treated | 5.5140 | 1.7124 | 9.8604 | 0.5659 | 0.1459 | 0.9895 |

levels of the dopamine metabolite, homovanillic acid, and the neurotransmitter, vasoactive intestinal peptide, to drop and the β -endorphin immunoreactivity to increase. Alternatively, IGF1, a mediator of GH action throughout much of the body, also crosses the blood-brain barrier and could be the mediator of GH dependent effects in the brain. Other unknown mechanisms may also explain this effect.

Of the known genes involved in short stature caused by GH deficiency, none has been localized to chromosome 18q. We have identified a 2 Mb critical region of the chromosome that is hemizygous in all of the individuals with 18q deletions and GH deficiency [Cody et al., 1997b]. Isolated GH deficiency has not been identified as a cause of intellectual disability, although this area has only been investigated in a very limited way. Deficiency of IGF1 due to GH receptor deficiency does not appear to interfere with brain development or the achievement of normal intelligence [Kranzler et al., 1998]. We hypothesize therefore that the intellectual disability in individuals with 18q deletions is due to the synergistic effects of the loss of one or more genes on 18q in combination with GH deficiency. Treatment with GH may upregulate these hemizygous genes resulting in improved cognition.

These data raise numerous intriguing questions. They emphasize the need for continued monitoring of the study participants to determine the extent and the permanence of the cognitive improvement. Further research into the mechanisms by which GH therapy improves cognition in these children may lead to other innovative treatments for individuals with cognitive impairment. The relationship between T1 changes, myelination and cognition also needs to be explored. Additionally, the cognitive and brain maturation effects of GH on children with idiopathic GH deficiency need to be carefully examined.

ACKNOWLEDGMENTS

This work was supported in part by the National Center for Research Resources grant MO1-RR-01346 for the Frederic C. Bartter General Clinical Research Center. Major support for this work came from the MacDonald family, Microsoft Corp., and The Chromosome 18 Registry and Research Society. The authors wish to thank the families who participated in this study for their enthusiasm and support. We would also like to thank Julia A. Norton, Ph.D., for her advice on the statistical analysis.

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