

Growth Hormone Insufficiency Associated With Haploinsufficiency at 18q23

Jannine D. Cody,¹ Daniel E. Hale,² Zoran Brkanac,¹ Celia I. Kaye,² and Robin J. Leach^{1*}

¹Department of Cellular and Structural Biology, University of Texas Health Science Center at San Antonio, San Antonio

²Department of Pediatrics, University of Texas Health Science Center at San Antonio, San Antonio

Growth hormone insufficiency is a common cause of growth failure in children with the 18q- syndrome. Individuals with this syndrome have a deletion as large as 36 Mb from the long arm of chromosome 18. We have evaluated 33 children with this syndrome for growth hormone production and have identified a region of approximately 2 Mb, which is deleted in every growth hormone insufficient patient. Two genes contained in this region, myelin basic protein, and the galanin receptor, are candidate genes for the growth hormone insufficiency phenotype. Am. J. Med. Genet. 71:420–425, 1997. © 1997 Wiley-Liss, Inc.

KEY WORDS: 18q- syndrome; growth hormone; chromosome 18; haploinsufficiency; galanin receptor; myelin basic protein

INTRODUCTION

The 18q- syndrome is characterized by the loss of chromosomal material from the long arm of chromosome 18. The deletion can be interstitial or terminal and can range from 2–36 Mb. The syndrome comprises short stature, developmental delay, decreased muscle tone, hearing impairment, and midface hypoplasia [Strathdee et al., 1996]. No trait is seen in all cases, presumably reflecting the variability in the size of the deleted region. By correlating genotype with phenotype in a large group of affected children, we may be able to identify genes responsible for specific phenotypic traits.

Children with the 18q- syndrome are very short (mean height 2.6 S.D. below the mean) and have a slow growth velocity (mean velocity 1.89 S.D. below the

mean). Evaluation of growth failure has demonstrated that insufficient production of growth hormone is a common finding in these children [Schwartz and Duck, 1990; Adler et al., 1992; Ghidoni et al., 1996]. The diagnosis of growth hormone insufficiency was made after assessing multiple parameters including stature, growth history, growth velocity, bone maturity, growth hormone associated factors such as insulin-like growth factor 1 (IGF-1) and insulin-like growth factor binding proteins (IGFBP-3), and serum growth hormone levels in response to pharmacological stimuli.

Growth hormone deficiency can be caused by a primary defect in the production of growth hormone or by secondary defects in the signaling for appropriate secretion of growth hormone. Primary defects in growth hormone production can occur due to a deletion of the growth hormone gene [Braga et al., 1986; Goossens et al., 1986], which is on chromosome 17 [Owerbach et al., 1980], or mutations in the gene for the essential pituitary transcription factors such as Pit-1 [Parks et al., 1993], which is on chromosome 3 [Ohta et al., 1992]. Secondary defects in the neurosecretory regulation of growth hormone have also been documented and are considered to be the major cause of growth hormone deficiency [Pintor et al., 1989]. For example, individuals with mutations in the growth hormone releasing hormone receptor (on chromosome 7) have been identified [Wajnrejtch et al., 1994, 1996].

We report here the localization of (a) gene(s) at 18q23 associated with growth hormone insufficiency in individuals with the 18q- syndrome. This gene(s) is associated with this phenotype when present in a single copy, consistent with haploinsufficiency.

MATERIALS AND METHODS

Patient Population

Families of children with the 18q- syndrome were enrolled through their individual physicians or through contact with The Chromosome 18 Registry and Research Society, a support group for families. The study was approved by the Institutional Review Boards of the University of Texas Health Science Center at San Antonio and The Santa Rosa Health Care Corporation. All subjects provided informed consent/assent.

*Correspondence to: Dr. Robin J. Leach, Department of Cellular and Structural Biology, University of Texas Health Science Center at San Antonio, 7703 Floyd Curl Drive, San Antonio, TX 78284. E-mail: leach@uthscsa.edu

Received 30 August 1996; Accepted 12 February 1997

Growth Hormone Evaluation

Growth failure was evaluated in accordance with standard testing procedures [Rosenfeld et al., 1995]. Each subject was evaluated for current height (which was compared to familial heights and growth patterns), growth history, growth velocity, bone age, insulin like growth factor 1 (IGF-1), insulin like growth factor binding protein 3 (IGFBP-3), and growth hormone response to clonidine or arginine. In five individuals the axis was also evaluated using GHRH as the provocative stimulus. Underlying systemic diseases were ruled out by thorough health history, extensive medical record review, physical examination, and general screening laboratory analysis.

Molecular Analysis

DNA was isolated and lymphoblastoid cell lines were established from patients and parents (when available) using the methods described by Cody et al. [1997]. Molecular analysis to confirm the deletions of material from the long arm of chromosome 18 was performed using polymerase chain reaction (PCR)-based microsatellite markers [Dib et al., 1996]. Hybrids were constructed from lymphoblastoid cell lines derived from the two patients who defined the critical region. The methods for hybrid construction are described elsewhere [Cody et al., 1997].

YAC Contig Construction

In order to isolate yeast artificial chromosomes (YACs) spanning the critical region, we first queried the CEPH/G n thon QUICKMAP data base at the "level 7" confidence index. Level 7 includes ALU-PCR hybridization and YAC fingerprinting data as well as STS-content of YACs for polymorphic markers described by Weissenbach et al. [1992]. The STS markers used to identify patient breakpoints were used to identify YACs in this region. The chromosome 18q YACs were individually arrayed and screened with all available chromosome 18 STSs (sequence information obtained from Genome Data Base) by PCR amplification. The PCR data were converted to a STS \times YAC content data table and analyzed with the SEGMAP computer program. G n thon's genetic maps enabled us to orient the resulting YAC contig with respect to the centromere and telomere.

RESULTS

Of 33 children evaluated, 12 (36%) had height and growth velocity above the 5th centile for age, indicating normal growth. Within this group of children, three had normal bone maturity, growth factors, and growth hormone response to pharmacological stimulation, while the remaining nine had an abnormality of one of these parameters. Twenty-one children (64%) exhibited growth failure. Of these children, seven had low growth factors, delayed bone ages, and abnormal response to pharmacological stimuli (growth hormone deficiency). The other 14 children with growth failure had either a growth factor within the normal range or had a normal growth hormone response to stimuli (growth

hormone insufficiency). The children with growth failure (either growth hormone insufficiency or deficiency) are candidates for growth hormone treatment under current guidelines.

Growth hormone response to GHRH was examined in five children. Four of the five individuals gave a more vigorous response to the direct pituitary stimulus, GHRH, than they did to the indirect stimulus, clonidine. This suggests that growth failure results from hypothalamic rather than primary pituitary dysfunction.

In order to determine which region of the long arm of chromosome 18 was deleted in all of the patients with growth hormone insufficiency or deficiency, molecular analysis of the DNA was performed on each of these patients. This analysis utilized the highly polymorphic PCR-based markers from G n thon [Dib et al., 1996]. The results of this analysis are shown in Figure 1. A critical region for growth hormone insufficiency/deficiency is defined by the breakpoints in patients number 1 and 13. This region was deleted in all those patients who were either insufficient or deficient for growth hormone.

The critical region was further refined by the construction of somatic cell hybrids from these two key patients. Hybrid construction allowed us to separate the chromosome 18 with the deletion from the normal chromosome 18. DNA from these hybrids could then be analyzed with the more abundant nonpolymorphic markers, which we have placed in a linear order on a YAC contig, a portion of which is shown in Figure 2. The data in Figure 3 are derived from the two patients whose deletions bracket the growth hormone insufficiency region. Figure 3 illustrates the results from analysis of the genomic DNA using only polymorphic markers compared with the results from the analysis using the somatic cell hybrids. The critical region for growth hormone insufficiency could then be defined as a region of approximately 2 Mb between markers AFM242yf2 and D18S462. A region of this size is estimated to contain approximately 30 genes. Only two genes have been identified in this region: myelin basic protein (MBP) and the galanin receptor (GALNR).

DISCUSSION

We have shown that there is a high incidence of growth failure and abnormal growth hormone production in children with the 18q- syndrome. Although there are case reports of growth hormone deficiency associated with the 18q- syndrome [Schwarz and Duck, 1990; Adler et al., 1992], our group is the first to assess a large number of children. We have also determined that all of our patients who are either growth hormone deficient or insufficient have a common deleted region of approximately 2 Mb at 18q23.

Our group of 18q- patients exhibit a very broad spectrum of growth hormone production, ranging from normal to severely deficient. There are several possible reasons for this variability. The vigorous response to GHRH as the growth hormone provocative agent suggests that there is not a primary pituitary problem with growth hormone production. It is reasonable to

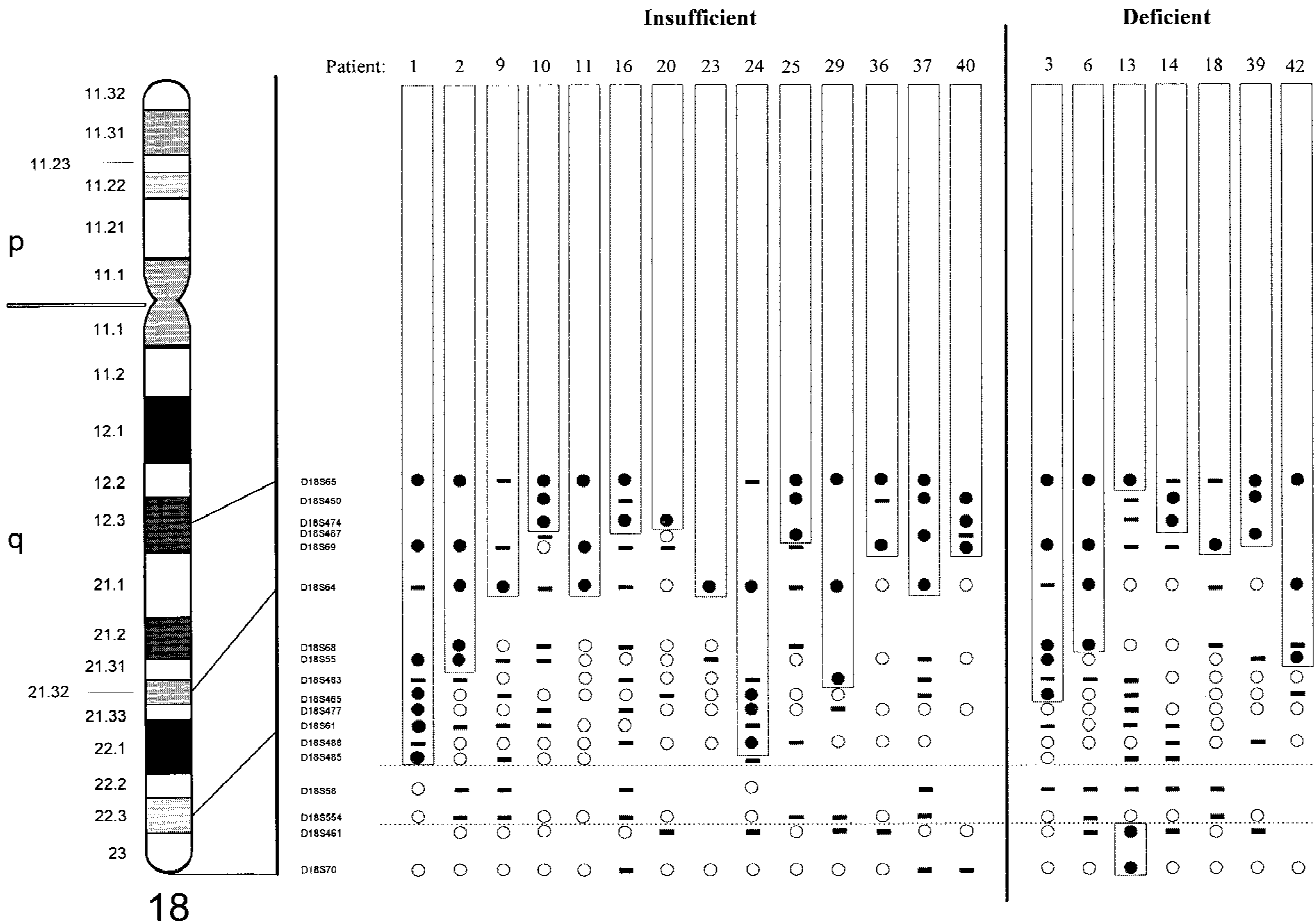


Fig. 1. Molecular analysis of patients with the 18q-syndrome who are either deficient or insufficient for growth hormone production. The black circles indicate those markers for which the patient had two alleles. The open circles are those markers for which the patient had one allele. The dashes are those markers that were uninformative. The rectangles represent the minimum size of the deleted chromosome for each patient. The horizontal dotted line indicates the common critical region lost in all of these patients.

assume that defects in the pathway which are further displaced from the actual production of growth hormone would generate a variable response, given the highly complex regulatory factors involved in the production and secretion of growth hormone. This spectrum from normal growth to growth hormone deficiency in our patients is consistent with a hypothalamic or neurosecretory dysfunction.

We also assessed this group of individuals for growth hormone deficiency based solely on the diagnosis of 18q-syndrome and not on a previous history of growth failure. In most instances, evaluation for growth hormone deficiency is not initiated unless a child presents with short stature and reduced growth velocity. This represents a significant ascertainment bias in the identification of individuals with growth hormone deficiency. Shalet et al. [1979] assessed children for growth hormone deficiency based not on growth history but on previous cranial irradiation for the treatment of leukemia or brain tumors. Such treatment had been previously shown to lead to growth hormone deficiency. The study by Shalet et al. [1979] demonstrated that some children whose growth was normal failed to produce normal levels of growth hormone, suggesting that deficiencies in growth hormone production as determined

by provocative stimuli are not always associated with short stature. Our data confirm this observation.

Our data have also shown that a deletion in the critical region of 18q23 is not sufficient in itself to cause growth insufficiency or deficiency. Two individuals whose deletions include this region had no evidence of growth failure (including their response to provocative stimuli). Furthermore, the defect is probably at the hypothalamic level and the relationship between growth failure and growth hormone production is not always a direct one. It is therefore not surprising that a deletion of a critical region is necessary but not sufficient to cause growth hormone insufficiency in these children.

We have also determined that the severity of growth hormone deficiency does not correlate with the size of the deletion. Individuals with smaller deletions did not in general demonstrate lesser degrees of growth failure than individuals with larger deletions. This finding is consistent with a single gene on chromosome 18q being influential for appropriate growth. Since genotypic data from our investigations demonstrated that the critical region for growth hormone insufficiency is approximately 2 Mb, we propose that there is a single gene or very tightly linked genes responsible for this phenotype.

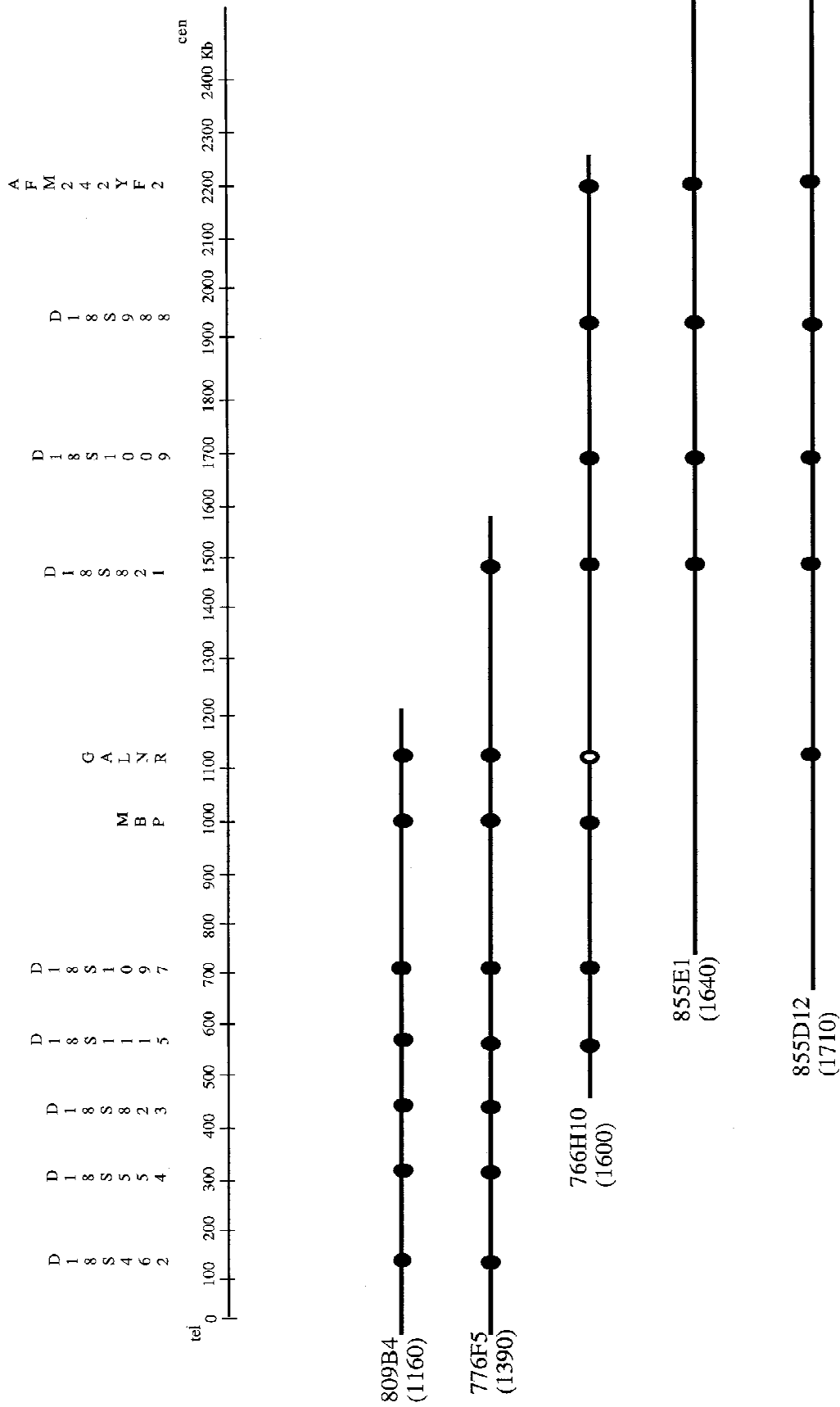


Fig. 2. A diagrammatic representation of a portion of the YAC contig at 18q23. The contig was constructed using the SEGMAP program. Markers with distances in Kb are listed across the top. The bold horizontal lines represent each YAC and indicates with a closed circle the markers present on that YAC. Open circles represent markers not present on the YAC by PCR analysis.

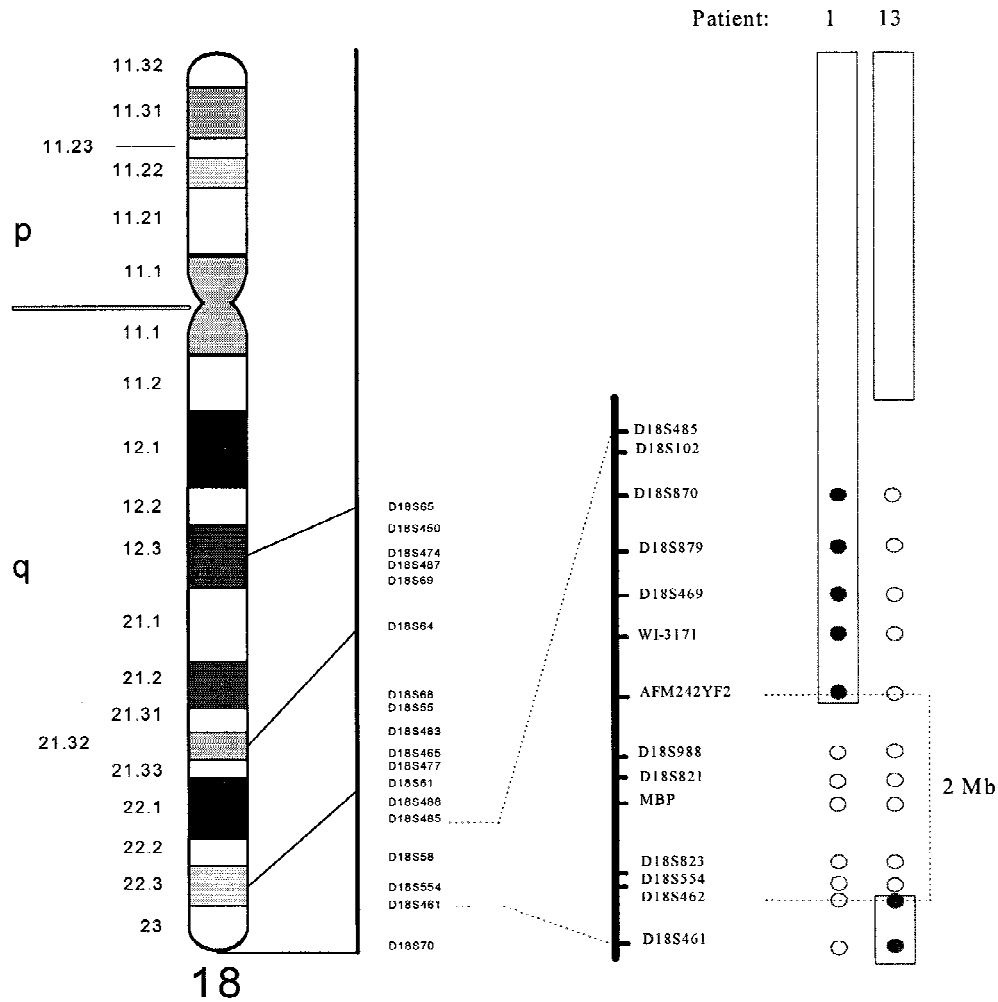


Fig. 3. Molecular analysis of the somatic cell hybrids from the two patients whose deletions bracket the growth hormone insufficiency region. Black circles represent markers present and open circles represent markers absent in those hybrids. The markers used in the analysis are shown in the center of the figure in their order as determined by the YAC contig.

Two genes have been identified in this region of chromosome 18 to date. They are MBP and GALNR. MBP is the major protein component of the myelin sheath in the central nervous system. It facilitates the compaction of the myelin sheath by joining the apposing cytoplasmic faces of the oligodendrocyte cell membrane. The absence of MBP results in reduced CNS myelination and, therefore reduced conduction velocity [Mathieu, 1993].

Miller et al. [1990] first observed that a mother and child, both with the 18q- syndrome, had reduced myelination of the brain. We have studied 20 individuals with the 18q- syndrome and have found reduced brain myelination in *all* individuals who were hemizygous for the MBP gene [Gay et al., in press]. One patient in this study had normal myelination and had an interstitial deletion of 18q which did not include the MBP gene. We observed a 100% correlation between hemizygosity for the MBP gene and reduced CNS myelination.

It is possible that a reduced gene dosage of the MBP gene leads to CNS hypomyelination, resulting in inefficient signal transduction. This signal transduction in-

efficiency in the hypothalamus could result in growth hormone insufficiency, with a spectrum of growth failure as is found in our patients. Arguing against this hypothesis is the fact that this scenario would also result in a reduction of other hypothalamic functions. There is minimal direct evidence for other hypothalamic dysfunction in 18q- syndrome, although this has not been evaluated rigorously.

The other gene in the growth hormone insufficiency region is GALNR. This gene was previously mapped to the telomeric region of 18q by fluorescence in situ hybridization [Nicholl et al., 1995]. Using a YAC contig, we have further mapped GALNR to a region adjacent to MBP. The GALNR is a G_o protein coupled receptor which is coupled to voltage-gated calcium channels. Its ligand, galanin, is expressed in the central and peripheral nervous systems. Galanin has been implicated in a wide range of biological responses including insulin response, intestinal motility, feeding, learning, memory, and growth hormone secretion [Bedecs et al., 1995]. Galanin has in fact been used as a provocative agent for the measurement of growth hormone reserves [Martul et al., 1993; Sartorio et al., 1995].

There is a precedence for G protein coupled receptors to be gene dosage dependent. The homozygous children in one family with a loss of function mutation in the adrenocorticotropic (ACTH) receptor gene displayed isolated glucocorticoid deficiency, while the heterozygous parents and grandmother had subclinical resistance to ACTH [Tsigos et al., 1993]. Therefore, a galanin receptor gene dosage dependent growth hormone response is feasible, especially if this is a rate limiting step, making GALNR an excellent candidate gene for this phenotype. Growth hormone response to galanin stimulation in subjects with the 18q- syndrome will also provide insight into the functioning of GALNR.

Individuals with mutations in the galanin receptor have not yet been identified. However, individuals with mutations in the growth hormone releasing factor receptor (GHRHR) have been reported [Wajnrajch et al., 1996]. GHRHR is a G protein coupled receptor, which is found on the surface of anterior pituitary cells. This receptor is also an important component in the growth hormone signal transduction pathway. However, it is closer to the end product of the pathway than is the galanin receptor. Individuals who are homozygous for a nonsense mutation in the GHRHR gene are abnormally short and are unable to produce growth hormone under standard provocative testing. The growth status of heterozygotes for the GHRHR mutation was not reported; however, it would be of interest to know if they had subtle evidence of growth failure. Such a finding would indicate that the growth hormone pathway is susceptible to a reduced gene dosage of the GHRHR gene, a gene whose product has a similar function to the GALNR.

It is possible that hemizyosity for MBP as well as GALNR might produce a synergistic effect which then results in growth hormone insufficiency. We are unable to address this possibility at this time, since we do not have patients who have a deletion which includes one gene but not the other.

ACKNOWLEDGMENTS

We thank the MacDonald family and Microsoft Corporation for their generous support of this work. We also thank Charles T. Leach for critical review of the manuscript.

REFERENCES

- Adler W, Heuvelod A, Polichronidou T (1992): Endokrinologische Störungen bei Deletionen des Chromosoms 18. *Mschr Kinderheilkd* 140:303-306.
- Bedecs K, Berthold M, Bartfai T (1995): Galanin: 10 years with a neuroendocrine peptide. *Int J Biochem Cell Biol* 27:337-349.
- Braga S, Phillips JA III, Joss E, Schwarz H, Zuppinger K (1986): Familial growth hormone deficiency resulting from a 7.6 kb deletion within the growth hormone gene cluster. *Am J Med Genet* 25:443-452.
- Cody JD, Pierce JF, Brkanac Z, Plaetke R, Ghidoni PD, Kaye CI, Leach RJ (1997): Preferential loss of the paternal alleles in the 18q- syndrome. *Am J Med Genet* 69:280-286.
- Dib C, Faure S, Fizames C, Samson D, Drouot N, Vignal A, Millasseau P, Marc S, Hazan J, Séboun E, Lathrop M, Gyapay G, Morissette J, Weissenbach J (1996): A comprehensive genetic map of the human genome based on 5,264 microsatellites. *Nature* 380:152-154.
- Gay CT, Hardies LJ, Rauch RA, Lancaster JL, Plaetke R, DuPont BR, Cody JD, Herndon RC, Ghidoni PD, Schiff JM, Kaye CI, Leach RJ, Fox PT (1997): Magnetic resonance imaging demonstrates incomplete myelination in the 18q- syndrome: Evidence for myelin basic protein haploinsufficiency. *Am J Med Genet (Neuropsych Genet)* (in press).
- Ghidoni PD, Hale DE, Cody JD, Thompson NM, McClure EB, Gay CT, Danney MM, Leach RJ, Kaye CI (1997): Growth hormone deficiency associated with 18q deletion syndrome. *Am J Med Genet* 69:7-12.
- Goossens M, Brauner R, Czernichow P, Duquesnoy P, Rappaport R (1986): Isolated growth hormone (GH) deficiency type 1A associated with a double deletion in the human GH gene cluster. *J Clin Endocr Metab* 62:712-716.
- Martul P, Pineda J, Pombo M, Penalva A, Bokser L, Dieguez C (1993): New diagnostic tests of growth hormone reserves. *J Pediatr Endocrinol* 6:317-323.
- Matthieu J-M (1993): An introduction to the molecular basis of inherited myelin disease. *J Inher Metab Dis* 16:724-732.
- Miller G, Mowrey PN, Hopper KD, Frankel CA, Ladda RL (1990): Neurologic manifestations in 18q- syndrome. *Am J Med Genet* 37:128-132.
- Nicholl J, Kofler B, Sutherland GR, Shine J, Iimaa TP (1995): Assignment of the gene encoding human galanin receptor (GALNR) to 18q23 by *in situ* hybridization. *Genomics* 30:629-630.
- Ohta K, Nobukuni Y, Mitsubuchi H, Ohta T, Tohma T, Jinno Y, Endo F, Matsuda I (1992): Characterization of the gene encoding human pituitary-specific transcription factor, Pit-1. *Gene* 122:387-388.
- Owerbach D, Rutter WJ, Martial JA, Baxter JD, Shows TB (1980): Genes for growth hormone, chorionic somatomammotropin and growth hormone-like genes on chromosome 17 in humans. *Science* 209:289-292.
- Parks JS, Kinoshita EI, Pfaffla RW (1993): Pit-1 and hypopituitarism. *Trends Endocrinol Metab* 4:81-85.
- Pinter C, Loche S, Puggioni R, Cella SG, Locatelli V, Lampis A, Muller EE (1989): Growth hormone deficiency states: Approach by CNS acting compounds. In Muller EE, Cocchi D, Locatelli V (eds): "Advances in Growth Hormone and Growth Factor Research." Roma-Milano, Italy: Pythagora Press and Berlin-Heidelberg, Germany: Springer-Verlag, pp 375-388.
- Rosenfeld RG, Albertsson-Wikland K, Cassorla F, Frasier SD, Hasegawa Y, Hintz RL, Lafranchi S, Lippe B, Loriaux L, Melmed S, Preece MA, Ranke MB, Reiter EO, Rogol AD, Underwood LE, Werther GA (1995): Diagnostic controversy: The diagnosis of childhood growth hormone deficiency revisited. *J Clin Endocrinol* 80:1532-1540.
- Sartorio A, Conti A, Monzani M, Faglia G (1995): Galanin infusion restores the blunted GH responses to GHRH administration during GH treatment in children with constitutional growth delay. *J Endocrinol Invest* 18:109-112.
- Schwarz HP, Duck SC (1990): Growth hormone deficiency in children with chromosomal abnormalities. *Arch Dis Child* 65:334.
- Shalet SM, Price DA, Beardwell CG, Morris Jones PH, Pearson D (1979): Normal growth despite abnormalities of growth hormone secretion in children treated for acute leukemia. *J Pediatr* 94:719-722.
- Strathdee G, Zackai EH, Shapiro R, Kamholz J, Overhauser J (1995): Analysis of clinical variation seen in patients with 18q terminal deletions. *Am J Med Genet* 59:476-483.
- Tsigos C, Arai K, Hung W, Chrousos GP (1993): Hereditary isolated glucocorticoid deficiency is associated with abnormalities of the adrenocorticotropic receptor gene. *J Clin Invest* 92:2458-2461.
- Wajnrajch MP, Chua SC, Green ED, Leibel RL (1994): Human growth hormone-releasing factor receptor (GHRHR) maps to a YAC at chromosome 7p15. *Mamm Genet* 5:595.
- Wajnrajch MP, Gertner JM, Harbison MD, Chua SC Jr, Leibel RL (1996): Nonsense mutation in the human growth hormone-releasing hormone receptor causes growth failure analogous to the little (lit) mouse. *Nat Genet* 12:88-90.
- Weissenbach J, Gyapay G, Dib C, Vignal A, Morissette J, Millasseau P, Vaysseix G, Lathrop M (1992): A second-generation linkage map of the human genome. *Nature* 359:794-801.