# ORIGINAL ARTICLE

# Does the dopamine transporter protein allele predict growth hormone testing results or response to growth hormone therapy?

Maala Daniel · Lucy D. Mastrandrea · Robbert J. Salis · Richard Erbe · Teresa Quattrin

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**Abstract** Animal studies have shown dopamine transporter protein (DAT1) knock out mice are growth retarded and hyperactive. DAT1 has been researched in several human psychiatric studies with varying results regarding phenotype and DAT1 alleles. However, the relationship between DAT1 and short stature in humans has not been explored. Buccal swabs were collected from patients receiving growth hormone (GH) therapy and were genotyped for variable number tandem repeat (VNTR) by polymerase chain reaction. Forty subjects were included; twenty-three patients had the 10/10 DAT1 genotype and thirteen had the 9/10 genotype. Fifteen of the patients with the 10/10 genotype tested GH deficient. Seven patients with the 9/10 genotype tested GH sufficient. The linear growth rate during the first year of GH therapy was equivalent in both genotypes. In conclusion, polymorphisms in the DAT1 40 base pair (bp) VNTR genotype do not predict GH deficiency or response to GH therapy in short children.

 $\begin{tabular}{ll} \textbf{Keywords} & Growth hormone \cdot Dopamine \cdot \\ Dopamine transporter protein \cdot Growth hormone therapy \cdot \\ Short stature \\ \end{tabular}$ 

M. Daniel · L. D. Mastrandrea · R. J. Salis · R. Erbe · T. Quattrin

Department of Pediatrics, School of Medicine and Biomedical Sciences, University at Buffalo, Buffalo, NY, USA

L. D. Mastrandrea (⋈)

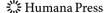
Division of Pediatric Endocrinology, Women and Children's Hospital of Buffalo, 219 Bryant Street, Buffalo, NY 14222, USA e-mail: ldm@buffalo.edu

### Introduction

Growth hormone (GH)-releasing hormone (GHRH) and somatostatin are the classic regulators of GH secretion. However, multiple hormones, neurotransmitters, and neuropeptides are believed to be involved in the regulation of GH secretion. This involvement is thought to be either at the level of the pituitary or at the level of the hypothalamus interacting with GHRH or somatostatin [1]. The role of the dopaminergic system in controlling GH release remains controversial. Animal data suggest dopamine may act as a secretagogue at the level of the hypothalamus for both GHRH and somatostatin [1]. In the rat, dopamine, acting through its receptor in the pituitary gland, stimulates basal GH release, provided all other neural inhibitory inputs to the pituitary are removed [2]. Male mice with absent dopamine 2 receptors have decreased serum GH levels, impaired basal and GHRH-stimulated GH release, and slow linear growth compared to control animals [3].

In humans, stimulatory and inhibitory effects of dopamine on GH release have also been described. In critically ill infants and children, dopamine has been reported to alter secretion of GH [4]. Alternatively, in normal men, dopamine inhibits hypothalamic somatostatin secretion, increasing the stimulatory effect of GHRH on GH release [5]

Levels of dopamine in the brain are highly regulated. One mechanism for terminating dopamine signaling is via reuptake into the presynaptic neurons. The *SLC6A3* gene encodes the dopamine transporter protein 1 (DAT1), which is selectively expressed in all dopaminergic neurons. DAT1 transports dopamine back into presynaptic neurons. Thus, DAT1 represents the primary mechanism by which dopamine is cleared from synapses, therefore playing a role in the regulation of dopamine signaling [6].



362 Endocr (2010) 37:361–364

Variations in the *SLC6A3* gene could impact the regulation of dopamine signaling. The *SLC6A3* sequence contains a variable number tandem repeat (VNTR), a 40 base pair sequence that is repeated 3–11 times. The most prevalent VNTRs are the 10 and 9 repeat alleles [6]. These VNTR polymorphisms in *SLC6A3* have been associated with various psychiatric phenotypes [7]. The VNTR polymorphism in the 3' untranslated region may affect mRNA localization, transcript stability, or protein synthesis regulation [6]. The exact physiologic consequences of the different VNTR sequences remain unknown [8].

Abnormalities in the control of dopamine reuptake have effects on GH secretion. While human studies relevant to *DAT1* alleles and growth are lacking, animal studies have demonstrated that DAT1 knockout mice are smaller than controls. In mice, deletion of the *Slc6a3* gene results in decreased GHRH expression and content of GH within pituitary somatotropes [9]. Mice homozygous for the deletion of the *Slc6a3* gene have considerable postnatal growth-retardation, as measured by suboptimal growth rates, poor weight gain, and decreased long bone length [9]. In addition, the animals demonstrate hyperactivity [10].

DAT1 alleles and their role in the dopaminergic system have been researched extensively in psychiatric disorders and in association with responses to stimulant and anti-depressant medications. DAT1 alleles have been studied with mixed results in attention deficit-hyperactivity disorder (ADHD), schizophrenia, Tourette's disorder, and depression. To our knowledge, no study has addressed the relationship between DAT1 alleles and short stature in humans.

Given the role that the dopaminergic system plays in modulating GH secretion, the idea that polymorphisms in the *DAT1* alleles correlate with growth parameters is an important question. The aims of this study were to evaluate whether there is an association between *DAT1* alleles and GH stimulation test results and whether an association between *DAT1* alleles and response to GH therapy in short children exists.

#### Results

The study included forty subjects on GH therapy. Thirty-five (87.5%) males and five females participated in the study. Thirty-seven were classified as non-Hispanic white (NHW). Twenty-three (57.5%) patients were GH deficient based on provocative testing. Mean height at start of therapy was -2.6 height standard deviation units (HSDU) below the mean for GH deficient subjects and -2.8 HSDU below the mean for GH sufficient subjects. Mean annualized growth rate for the first year of therapy for both GH deficient and GH sufficient participants was 10 cm per year.

Analysis of VNTR polymorphisms in the *SLC6A3* gene demonstrated that twenty-three patients (57.5%) had the 10/10 allele, and thirteen patients (32.5%) had the 9/10 allele (Table 1). The other genotypes represented <5% of the total cohort tested. The 10/10 and 9/10 genotypes had similar HSDU prior to GH treatment. Annualized growth rates for the group with the 10/10 allele (10.3 cm/year) and was similar to that of the group with the 9/10 allele (9.9 cm/year; Table 1). The distribution of GH deficient versus sufficient testing was two-fold higher in males carrying the 10/10 allele (NS). For the 9/10 genotype, five males and two females tested GH sufficient.

#### Discussion

The dopaminergic system has been implicated as a modulator of GH secretion. The *SLC6A3* gene contributes to the regulation of dopamine availability [8]. This study investigated the possibility of a relationship between growth and polymorphisms in *DAT1* alleles.

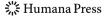
There has been a reported association between ADHD risk and the 10/10 allele [11]. However, results in this area have been conflicting. Variable results have also been published with regards to the response of methylphenidate (MPH) treatment related to *DAT1* alleles. MPH is widely used in the treatment of ADHD due to its role as a DAT1

**Table 1** Study participant characteristics and first year growth rates based on genotype

Genotype	M/F	Age	GHD	GHS	Ht SD @ GH start	BMI SD @ GH start	Age @ GH start	First year growth rate (cm/year)
10/10	23/0	$13.8 \pm 3.5$	15*	8*	$-2.5 \pm 0.7$	$-0.3 \pm 1.0$	$10.3 \pm 3.7$	10.7 ± 2.8*
9/10	9/4	$13.4 \pm 2.8$	7	6	$-2.7 \pm 0.8$	$-0.6 \pm 1.3$	$9.9 \pm 4.1$	$9.6 \pm 2.4*$
9/9	2/0	$14.9 \pm 2.0$	1	1	$-2.05 \pm 0.21$	$0.00 \pm 1.5$	$10.8 \pm 6.1$	$10.4 \pm 0.6$
10/11	0/1	12.9	0	1	-2.1	-0.4	11.9	10.6
9/11	1/0	12.2	0	1	-2.1	-1.9	8.8	8.5
9/9 10/11	2/0 0/1	$14.9 \pm 2.0$ $12.9$	-	1 1 1	$-2.05 \pm 0.21$ -2.1	$0.00 \pm 1.5$ $-0.4$	$10.8 \pm 6.1$ $11.9$	$10.4 \pm 0.6$ $10.6$

<sup>\*</sup> P-value not statistically significant

GHD growth hormone deficient; GHS growth hormone sufficient



Endocr (2010) 37:361–364 363

inhibitor, increasing dopamine levels and signaling through dopaminergic pathways. The National Institute of Mental Health Multimodal Treatment Study of ADHD follow-up showed that children with ADHD on various forms of stimulant medications have mild but statistically significant growth suppression with both height and weight affected compared to untreated children [12]. Thus, there is the possibility that effects on growth are linked to DAT1 activity and expression levels.

In our predominantly NHW male cohort of children receiving GH therapy, the most common DAT1 allele is the 10/10 allele, consistent with the distribution in the general population. Subjects carrying the 10/10 allele were twofold more likely to test GH deficient compared to subjects in the other allele groups, where the likelihood of testing GH deficient or GH sufficient was equal. Although this result is not statistically significant, it may be clinically significant. Future studies could examine this association more closely. Additionally, there were more males in the study, reflecting the fact that the majority of children referred for short stature are males. Interestingly, four of the five females studied had the 9/10 allele. A larger sample size is needed to determine whether the 9/10 allele correlates with short stature in females. However, there was no correlation with GH stimulatory testing results for females carrying the 9/10 allele.

Our data demonstrates that HSDUs were not significantly different at baseline between allele groups. Shorter children were not clustered within a particular allele group. Response to GH therapy, as measured by first year growth rate, was optimal and comparable in all *DAT1* allele groups. In addition, first year annualized growth rates were similar between GH deficient and GH sufficient groups, comparable to results reported for a larger cohort enrolled in the National Cooperative Growth Study [13].

In this dataset of children with short stature treated with GH, VNTR polymorphisms in the *SLC6A3* gene did not predict results of GH testing or dissimilar responses to GH therapy. Additional data could determine if there is a higher frequency of GH deficiency in patients with 10/10 genotype. The relationship between DAT1 and growth needs to be further explored in future studies to investigate if this is a promising alternative or supplemental approach to the evaluation of short stature.

## Methods

Study subjects were recruited from a tertiary care clinic at the Women and Children's Hospital of Buffalo, Division of Pediatric Endocrinology. Inclusion criteria were as follows: male and female subjects aged less than 19 years and receiving GH therapy at the dose of 0.3 mg/kg/week for at

least 12 months. All subjects had GH stimulation testing (two agents) performed prior to initiation of GH therapy. Patients with ADHD, known genetic syndromes, and small for gestational age were excluded. Study participants were defined as GH deficient or GH sufficient based on a peak GH level of 10 ng/ml in response to provocative testing.

The study was approved by the Children and Youth Institutional Review Board of the Women and Children's Hospital of Buffalo. Each subject's guardian signed informed consent and study participants over age 8 years signed assents. Ninety-five percent of eligible subjects agreed to participate.

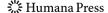
Samples were obtained sequentially using buccal brushes during routine follow-up visits. Brushes were kept at room temperature and analyzed in batches of 10. DNA was isolated using Purgene DNA purification kit following manufacturing instructions (IDT, Inc. Coraville, IA). Total quantity of DNA used was 100 ng. Polymerase chain reaction (PCR) was performed using Hot StartTaq DNA Polymerase (Qiagen, Valencia, CA). Amplification conditions were as follows: initial denaturation at 95° for 10 min, forty cycles of 30 s at 95°, 30 s at 60°, 1 min at 72° followed by 72° for 10 min. PCR primers were (sense) 5'-GGT GTA GGG AAC GGC CTG AG-3' and (antisense) 5'-CTG GAG GTC ACG GCT CAA GG-3'. PCR reactions were resolved on a 6% acrylamide gel. Bands were visualized using ethidium bromide under UV light transillumination. Alleles and genotypes were differentiated by size compared to molecular weight standards. Each PCR run included a negative control (water) and known sequenced positive controls.

Data were expressed as mean  $\pm$  standard deviation (SD). A SPSS 8.0 Database was used, and Mann–Whitney U test was used for statistical analysis.

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364 Endocr (2010) 37:361–364

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