J Hum Genet (1999) 44:26–29

ORIGINAL ARTICLE

Hiroshi Miyake · Kanji Nagashima · Kazumichi Onigata
Tomohisa Nagashima · Yoko Takano
Akihiro Morikawa

Allelic variations of the D2 dopamine receptor gene in children with idiopathic short stature

Received: July 13, 1998 / Accepted: September 9, 1998

Abstract The D2 dopamine receptor (DRD2) plays a major role in growth hormone (GH) secretion. Recent reports indicate that Taq I A *DRD2* gene alleles (A1 and A2) are related to the function of DRD2. Idiopathic short stature (ISS) is defined as short stature without accompanying malnutrition, chronic disease, and endocrinological disorders. However, some reports suggest that ISS is associated with a mild disturbance of GH secretion. In this study, we examined the notion that allelic variants of the DRD2 are associated with ISS. We studied 55 children with ISS aged 8.4 (SD 2.9) years; (group I) and 104 age-matched children of normal stature (group II). Informed consent was obtained from each child's parent or guardian. Genomic DNAs were extracted from peripheral mononuclear cells and amplified by polymerase chain reaction (PCR). The PCR products were digested by Taq1 and resolved by electrophoresis. The frequency of the A1 allele was significantly higher in group I (0.42) than in group II (0.26). The insulin-like growth factor (IGF)-I ratio (the ratio of the individual level to the normal mean value according to age at our laboratory center) was significantly lower in group I than in group II. When group I was subdivided into group A (with the A1 allele) and group B (with only the A2 allele), group A had a significantly lower peak GH response to the *l*-dopa test, lower levels of IGF-I, and retarded bone maturation. These findings indicate that polymorphism of the *DRD2* gene may be one genetic factor that affects body height in childhood, acting through the hypothalamus (GH-releasing hormone) — pituitary (GH) — IGF-I axis.

Key words *Dopamine receptor* gene · Polymorphism · Short stature · Growth hormone

H. Miyake (✉) · K. Nagashima · K. Onigata · T. Nagashima · Y. Takano · A. Morikawa
Department of Pediatrics, Gunma University School of Medicine, 3-39-15 Showa-machi, Maebashi, Gunma 371, Japan
Tel. +81-27-220-8205; Fax +81-27-380-1262

Introduction

Levels of insulin-like growth factor-I (IGF-I) are similar in identical twins, levels of thyroid hormones are similar in families with resistance to thyroid hormones, and the 17-hydroxy-progesterone response to adrenocorticotropic hormone (ACTH) is similar in siblings (Weiss et al. 1993; Kao et al. 1994; Azziz et al. 1994). These findings suggest that the blood levels of several hormones are genetically determined. IGF-I is important for increasing body height in children and it is produced in a growth hormone (GH)-dependent manner under ordinary nutritional conditions. The production and secretion of GH from the pituitary gland is regulated mainly by two hypothalamic factors: GH-releasing hormone (GH-RH) and somatostatin. The secretion of hypothalamic factors is regulated by neurons in the thalamus or brain cortex, with dopamine being an important regulatory neurotransmitter of GH-RH release. Acute administration of *l*-dopa is generally used in testing GH secretion.

It has been reported that long-term administration of dopamine enhanced growth height in children with idiopathic short stature (ISS) (Huseman 1985). This finding suggests that there may be a dysfunction of the dopamine pathway in ISS. Dopamine bioactivity is expressed by binding between ligands and dopamine receptors. Several dopamine receptors have been identified, with the D2 dopamine receptor (DRD2) playing a role in GH secretion. Previous studies have described the Taq I A *DRD2* gene alleles (A1 and A2) (Grandy et al. 1989; Blum et al. 1990; Grandy et al. 1993). In individuals with the A1 allele, the binding activity of DRD2 and the mean relative glucose metabolic rate are significantly reduced (Noble et al. 1991, Noble et al. 1997, Thompson et al. 1997). A decrease in the hormone binding capacity is expressed as a minor dysfunction of the hormone action. Therefore, we considered that individuals with the A1 allele may have dysfunctional GH release. In this study we examined the notion that allelic variants of DRD2 are associated with ISS.

Subjects and methods

We studied 159 unrelated Japanese children; 55 children with ISS (aged 8.4 (SD 2.9) years; group I) and 104 age-matched children of normal stature (group II). None of the controls had any chronic organic diseases. Genomic DNAs of the controls were obtained from peripheral blood samples given for medical check-up or for testing in acute inflammatory disease such as bronchitis. The diagnosis of ISS depended on the following criteria: (1) body height SD score below −2.0SD, (2) peak GH level higher than 10ng/ml in at least one of two GH stimulating tests (insulin and arginine test), and (3) no distinct organic disorders. Informed consent was obtained from each child's parent or guardian.

Genomic DNAs were extracted from peripheral blood by a standard method and amplified by polymerase chain reaction (PCR) to determine the polymorphic Taq1 A site of DRD2. The primers used had the following sequences: 5′-CCGTCGACCCTTCCTGAGTGTCATCA-3′ and 5′-CCGTCGACGGCTGGCCAAGTTGTCTA-3′, and a 310-bp fragment was amplified (Noble 1994). The amplification was carried out in a 100-μl reaction mixture, using 1μg genomic DNA, 2.5μl AmpliTaq DNA polymerase, and the recommended buffer provided by the manufacturer (Perkin Elmer, Branchburg, New Jersey, USA). After initial denaturation at 94°C for 5min, the DNA was amplified by 35 cycles of denaturation at 94°C for 60s, annealing at 58°C for 60s, and extension at 72°C for 60s, using a thermocycler (Astec, Tokyo, Japan). A final extension at 72°C for 5min was added after the last cycle. The 310-bp PCR products (10μl) were digested by a Taq1 restriction enzyme (Takara Shuzo, Kyoto, Japan) at 65°C for 3h. The digested products were resolved by electrophoresis in a 4% agarose gel containing ethidium bromide. The A1/A2 genotype is revealed by 310-, 180- and 130-bp fragments. The A2/A2 genotype is indicated by two fragments of 180- and 130-bp, and the A1/A1 genotype is represented by the uncleaved 310-bp fragment (Noble et al. 1994).

Blood IGF-I, GH, thyroid-stimulating hormone (TSH), and free thyroxine (T4) levels were determined using commercially available kits — Somatomedin-C RIA (Chiba Corning, Tokyo, Japan), Ab beads h-GH-Eiken (Eiken, Tokyo, Japan), Spak S-TSH (Daiichi Radioisotope, Tokyo, Japan), and Graozyme-N-Free T4-M (Wako Jyunyaku, Tokyo, Japan), respectively. Blood IGF-I levels were expressed as the ratio of the individual value to the normal mean value according to age at our laboratory center.

The *l*-dopa test was performed in the children with ISS. At 0900h on the morning after an overnight fast, the antecubital vein was cannulated. *l*-Dopa was administered orally at a dose of 10mg/kg body weight at 0min, and blood was sampled every 30min for 2h. Other GH provocation tests were performed using insulin (0.1U/kg) or arginine (0.5g/kg). Body height was measured with a stadiometer with a direct read-off counter. Bone maturation was determined using the TW2 method (TW2 is not an abbreviation) (Tanner et al. 1983).

The χ^2 test was used to assess significant differences in the frequencies of the A1 and A2 allele between the two groups. The clinical characteristics and blood hormone levels in each group were compared using Student's *t*-test. A level of $P < 0.05$ was considered statistically significant.

Results

The clinical characteristics of each group are presented in Table 1. Age and serum thyroid hormone levels did not differ significantly among the groups. The IGF-I ratio was significantly lower in group I than in group II.

The allelic frequency in each group is presented in Table 2. The A1 allele frequency was significantly higher in group I (0.42) than in Group II (0.26) ($P < 0.01$). The observed genotype frequencies in groups I and II did not deviate from those predicted by Hardy-Weinberg's law.

In accordance with a report that individuals with the A1 allele had reduced DRD2 binding activity (Noble 1991), we subdivided group I into group A (with the A1 allele) and group B (with only the A2 allele). The clinical characteristics of each of these groups are shown in Table 3. There were no significant differences in age, height, or thyroid hormone levels. However, group A had a lower peak GH response to the *l*-dopa test than group B ($P < 0.05$). Group A also had a significantly lower IGF-I ratio ($P < 0.01$) and slightly retarded bone maturation, determined as the ratio of bone age, defined as TW2 method, to chronological age ($P < 0.01$).

Table 1 Clinical characteristics and blood levels of hormones in subjects in groups I and II

	Group I (children with ISS)	Group II (age-matched controls)	*P*
n	55	104	
Age (years)	8.4 ± 2.9	8.0 ± 2.0	NS
Sex (male/female)	33/22	56/48	NS
Height, SD	−2.72 ± 0.31	−0.01 ± 0.95	<0.01
IGF-I (ratio)	0.65 ± 0.20	0.95 ± 0.23	<0.01
TSH (μU/ml)	2.2 ± 1.0	2.5 ± 1.2	NS
Free T4 (ng/dl)	1.1 ± 0.2	1.2 ± 0.2	NS

Data values are means ± SD
ISS, Idiopathic short stature; IGF-I, insulion-like growth hormone-I; TSH, thyroid-stimulating hormone; T4, thyroxine; NS, not significant

Table 2 Dopamine D2 receptor genotypes and allelic frequency in subjects in groups I and II

		No. with genotype			Allelic frequency	
Group	*n*	A1/A1	A1/A2	A2/A2	A1	A2
I	55	11	24	20	0.42	0.58
II	104	8	39	57	0.26	0.74

A1 frequency was higher in group I than in group II ($P < 0.01$)

Table 3 Clinical characteristics and blood levels of hormones in subjects with ISS in groups A and B[a]

	Group A	Group B	*P*
n	35	20	
Age (years)	8.3 ± 0.6	8.5 ± 0.7	NS
Sex (male/female)	21/14	12/8	NS
Height, SD	−2.80 ± 0.13	−2.70 ± 0.12	NS
BA/CA	0.60 ± 0.03	0.74 ± 0.02	<0.01
IGF-I (ratio)	0.59 ± 0.17	0.75 ± 0.21	<0.01
TSH (mU/ml)	2.1 ± 0.2	2.5 ± 0.3	NS
Free T4 (ng/dl)	1.1 ± 0.2	1.2 ± 0.1	NS
l-dopa test			
Baseline GH (ng/ml)	1.4 ± 0.5	1.6 ± 0.6	NS
Peak GH (ng/ml)	12.2 ± 1.8	17.2 ± 1.9	<0.05

Data values are means ± SE
BA, Bone age (see text for definition); CA, chronological age; GH, growth hormone
[a]For details of groups A and B, see text

Discussion

In this study, the frequency of the A1 allele of the *DRD2* gene was significantly higher in children with ISS than in the controls. In addition, the ISS group with the A1 allele had a lower GH response to the *l*-dopa test, lower levels of IGF-I, and slightly retarded bone maturation compared with these parameters in the ISS group with only the A2 allele. These findings indicate that polymorphism of the *DRD2* gene may be one genetic factor that affects body height in childhood, acting through the hypothalamus (GH-RH) — pituitary (GH) — IGF-I axis.

The reported A1 allele frequency of the *DRD2* gene in Japanese controls differs with reports (Arinami et al. 1993; Kono et al. 1997), although that in our study is lower than that reported by others. The reason for this discrepancy is not clear, but it may be explained by differences in the characteristics of control subjects. The reports cited above disregarded the height of the subjects and we selected only children with normal stature as controls.

The Taq I allele of the *DRD2* gene varies in the 3′-side portion outside the last exon (Grandy et al. 1989). Although the reason for the relation between polymorphism in this portion and some phenotypes remains unclear, there are some possible explanations. First, this polymorphism is biologically relevant to short stature. Second, the polymorphism is in linkage disequilibrium with biologically relevant variability elsewhere in the *DRD2* gene. Third, the polymorphism is in disequilibrium with genetic variability in an adjacent gene. The position of polymorphism in the 3′ end of the last exon of *DRD2* suggests that if the polymorphism is biologically relevant, the most likely mode of action would be through modulation of transcription or splicing.

The presence of the A1 allele of the *DRD2* gene has been reported to be closely linked to neuropsychiatric diseases such as Tourette's syndrome, attention deficit hyperactivity disorder, and alcoholism (Blum et al. 1990; Blum et al. 1991; Comings et al. 1991; Berman and Noble 1995). An association between aloholism and the A1 allele of the *DRD2* gene remains controversial, however. Some researchers did not find a significantly higher frequency of subjects with the A1 allele in alcoholic groups (Bolos et al. 1990; Gelernter et al. 1991). Of interest, administration of the *DRD2* agonist, bromocriptine, alleviated alcoholism to a greater extent in patients with the A1 allele than in patients with only the A2 allele (Lawford et al. 1995). As mentioned in our Introduction, some reports indicate that individuals with the A1 allele have a low binding capacity for dopamine and reduced dopaminergic function. These reports support the notion that the A1 allele of *DRD2* affects the action of dopamine in the central nevous system. In the endocrine system, dopamine stimulates GH secretion through GH-releasing hormone by binding to *DRD2*. Dopamine administration alone or in combination with exogenous human GH increases the height of some children with intranterine growth retardation caused by reduced pituitary function (Huseman, 1985). Therefore, it is suggested that endogenous central dopaminergic dysfunction affects body height through decreased GH secretion.

ISS is considered to be a condition with a heterogeneous pathogenesis. Most individuals with ISS present with mild or moderately short stature. Bercu and Diamond (1986) reported that ISS was associated with a mild GH deficiency, which presents with normal secretion in response to provocative stimuli, decreased nocturnal GH secretion, and low blood levels of IGF-I. In addition to low IGF-I levels, retarded bone maturation is an important marker of GH deficiency. In the present study, the children with ISS and the A1 allele had retarded bone maturation and low levels of blood IGF-I. In addition, these children had a slightly lower GH response to the *l*-dopa test than the subjects with only the A2 allele. These clinical findings are in agreement with the characteristics of mild GH deficiency. Therefore, our data suggest that individuals with the A1 allele may have a mild dysfunction of GH secretion associated with defects in the dopamine pathway.

Many complex interrelated factors may be involved in the genetic determination of body height. We consider that *DRD2* gene polymorphism may be one of these factors. Our study was done in a rather limited number of subjects. Therefore, to confirm our speculation, independent studies of much larger groups of subjects and other ethnic groups are needed.

References

Arinami T, Itokawa M, Komiyama T, Mitsushio H, Mori H, Mifune H, Hamaguchi H, Toru M (1993) Association between severity of alcoholism and the A1 allele of the dopamine D2 receptor gene TaqI A RFLP in Japanese. Biol Psychiatry 33: 108–114

Azziz R, Dewailly D, Owerbach D (1994) Nonclassic adrenal hyperplasia: Current concepts. J Clin Endocrinol Metab 78: 810–815

Bercu BB, Diamond FB Jr (1986) Growth hormone neurosecretory dysfunction. Clin Endocrinol Metab 15: 537–590

Berman SM, Noble EP (1995) Reduced visuospatial performance in children with the D2 dopamine receptor A1 allele. Behav Genet 25: 45–48

Blum K, Noble EP, Sheridan PJ, Montgomery A, Ritchie T, Jagadeeswaran P, Nogami H, Briggs AH, Cohn JB (1990) Allelic

association of human dopamine D2 receptor gene in alcoholism. JAMA 263: 2055–2060

Blum K, Noble EP, Sheridan PJ, Finley O, Montgomery A, Ritchie T, Ozkaragoz T, Robert JF, Sadalak F, Sheffild D, Dahlmann T, Halbardier S, Nogami H (1991) Association of the A1 allele of the D2 dopamine receptor gene with severe alcoholism. Alcohol 8: 409–416

Bolos AM, Dean M, Lucas-Derse S, Ramsburg M, Brown GL, Goldman D (1990) Population and pedigree studies reveal a lack of association between the dopamine D2 receptor gene and alcholism. JAMA 264: 3156–3160

Comings DE, Comings BG, Muhleman D, Dietz G, Shahbahrami B, Tast D, Knell E, Kocsis P, Baumgarten R, Kovnes BW, Levy DL, Smith M, Borison RL, Evans DD, Klein DN, MacMurray J, Tosk JM, Sverd J, Gysin R, Flanagan SD (1991) The dopamine D2 receptor locus as a modifying gene in neuropsychiatric disorders. JAMA 266: 1793–1800

Grandy DK, Litt M, Allen L, Bunzow JR, Marchionni M, Makam H, Reed L, Magenis RE, Civelli O (1989) The human dopamine D2 receptor gene is located on chromosome 11 at q22–q23 and identifies a Taq I RFLP. Am J Hum Genet 45: 778–785

Grandy DK, Zhang Y, Civelli O (1993) PCR detection of the TaqI RFLP at the DRD2 locus. Hum Mol Gene 2: 2197

Gelernter J, O'Malley S, Risch N, Kranzler HR, Krystal J, Merikongas K, Kenndy JL, Kidd KK (1991) No association between an allele at the D2 dopamine receptor gene (DRD2) and alcholism. JAMA 266: 1801–1807.

Huseman CA (1985) Growth enhancement by dopaminergic therapy in children with intrauterine growth retardation. J Clin Endocrinol Metab 61: 514–519

Kao PC, Matheny AP, Lang CA (1994) Insulin-like growth factor-I comparisons in healthy twin children. J Clin Endocrinol Metab 78: 310–312

Kono Y, Yoneda H, Sakai T, Nomura Y, Inayama Y, Koh J, Sakai J, Inada Y, Imamichi H, Asaba H (1997) Association between early-onset aloholism and the dopamine D2 receptor gene. Am J Med Genet 74: 179–182

Lawford BR, Young RM, Rowell JA, Qualichefski J, Fletcher BH, Syndulko K, Ritchie T, Noble EP (1995) Bromocriptine in the treatment of alcoholics with the D2 dopamine receptor A1 allele. Nature Med 1: 337–341

Noble EP, Blum K, Ritchie T, Montgomery A, Sheridan PJ (1991) Allelic association of the D2 dopamine receptor gene with receptor-binding characteristics in alcoholism. Arch Gen Psychiatry 48: 648–654

Noble EP, Berman SM, Ozkaragoz TZ, Ritchie T (1994) Prolonged P300 latency in children with D2 dopamine receptor A1 allele. Am J Hum Genet 54: 658–668

Noble EP, Gottschalk LA, Fallon JH, Ritchie TL, Wu JC (1997) D2 dopamine receptor polymorphism and brain regional glucose metabolism. Am J Med Genet 74: 162–166

Tanner JM, Whitehouse RH, Cameron N, Marshall WA, Healy MJR, Goldstein H (1983) Assessment of skeletal maturity and prediction of adult height (TW2 method), 2nd edn. Academic Press, Tokyo

Thompson J, Thoma N, Singleton A, Piggott M, Lloyd S, Perry EK, Morris CM, Perry RH, Ferrier IN, Court JA (1997) D2 dopamine receptor gene (DRD2) Taq1 A polymorphism: Reduced dopamine D2 receptor binding in the human striatum assiciated with the A1 allele. Pharmacogenetics 7: 479–484

Weiss RE, Marcocci C, Bruno-Bossio G, Refetoff S (1993) Multiple genetic factors in the heterogeneity of thyroid hormone resistance. J Clin Endocrinol Metab 76: 257–259