Original

Vitamin D receptor gene polymorphisms in persons with Down's syndrome

Sara Panizo-Garcia1, Eva Parisi-Capdevila[1], José Manuel Valdivielso-Revilla1, Lluís Rosselló-Aubach[2], Elvira Fernández-Giraldez[3]
[1] Laboratori de Genètica Humana del Departament de Ciències Mèdiques Bàsiques de la Universitat de Lleida.
[2] Servei de Reumatologia de l'Hospital de Santa Maria de Lleida.
[3] Servei de Nefrologia de l'Hospital Arnau de Vilanova de Lleida.

Correspondence to:
Dr. Lluís Roselló-Aubach.
Servei de Reumatologia. Hospital de Santa Maria de Lleida.
Rovira Roure, 44.
25198 Lleida (Spain).
E-mail: lrosello@gss.scs.es

Article received: 22-Feb-06

Abstract

Background: Down syndrome (DS) is a genetic alteration caused by having three copies of chromosome 21. Variations in the presence of alleles in the vitamin D receptor (VDR) gene have been linked to a variety in the phenotype and also considered a risk factor in some populations. In the present paper, we analyze whether a variation of the BsmI polymorphism in the VDR gene is overexpressed in patients with DS and whether it is related to any phenotype of the patients.

Patients and methods: We studied the BsmI polymorphism of the vitamin D receptor in DNA from peripheral blood of 85 patients with DS and 122 controls. The detection of each phenotype is performed by amplification of the DNA sequences of intron 8 of the VDR gene by polymerase chain reaction (PCR). We analyzed the differences in distribution of the alleles in patients with DS and the correlation of the genotype to different anthropometric (age, height, body mass index) and biochemical parameters (calcium, vitamin D,{PTH hormone, bone mass).

Results: The analysis of the distribution of the BsmI polymorphism showed a higher frequency of the B allele in the DS patients with respect to controls. In the same group of patients, regression analysis showed no link with any biochemical parameter. However, the homozygous genotype bb is more frequently found in taller individuals (p = 0.04) and the BB in older individuals (p = 0.03).

Conclusions: The B allele of the BsmI polymorphism of the VDR gene is more frequent in people with DS. The genotypes bb and BB are more frequent in taller and longer-living DS patients respectively. This result points out the possibility that VDR genotype could influence these two phenotypic characteristics of DS patients.

Keywords: Vitamin D receptor gene. BsmI polymorphism. Down syndrome. Vitamin D.

Introduction

Down syndrome (DS) is a genetic alteration caused by having three copies of chromosome 21. With an approximate incidence of 1 case per 1,000 births, DS is the most frequent congenital chromosomal disorder and the chief cause of delayed intellectual development in the human species (1). In 95% of cases there is non-disjunction of chromosome 21; 4% are translocations and 1% are mosaic cases (2). Chromosome 21 has been entirely sequenced and is estimated to contain about 360

genes. Triplication of the DS critical region (DSCR) is thought to be responsible for the many traits specific to this syndrome. However, not all of the complex DS phenotypes have been successfully correlated with a gene-dosing effect in this area of the extra chromosome 21 (3). A preceding study noted that individuals with DS had a high prevalence of vitamin D deficiency and reduced bone mineral density (4). Vitamin D is part of the steroid and thyroid hormone superfamily which performs a broad variety of biological functions, such as calcium homeostasis, cell proliferation, or cell differentiation in many target organs. Most of its action is exerted by controlling the transcription of target genes through activation of the vitamin D nuclear receptor (VDR). Vitamin D receptors have been isolated in a number of different sites, such as the parathyroid gland, the pancreas, hematopoietic cells, skin keratinocytes, endothelial cells, vascular smooth muscle cells, and the reproductive organs. Variations in VDR alleles have been associated with a large variety of phenotypes including, among others, different circulating levels of 1,25-(OH)2D3 (5), variations in bone mineral density (BMD) (6), some types of neoplasms (7), blood pressure disorders (8) or even height, muscle strength, and body weight (9,10). Restriction enzymes BsmI, Apa, Taq (in terminal 3 between exons 8 and 9) and Fok (in exon 2) have been used to identify 4 types of polymorphisms related to the VDR gene, but the first is the one most widely used, and the one presented in this paper. Its aim is to analyze whether some VDR genotypes are more prevalent among people with DS than among the general population, and the role this might play in different DS phenotypes.

Materials and methods

1. Patients

The present study comprised 85 people with DS and 122 healthy subjects, all Caucasian. Peripheral blood samples were obtained from all subjects. The subjects with DS were employees at four workshops for people with intellectual disabilities in the province of Lleida. They all had a similar daily schedule, worked in a seated position, and were classified as having moderate or borderline intellectual disability, but no severe mental retardation. Subjects were excluded if they had a history of bone, endocrine or liver disease that might alter bone metabolism, chronic renal failure, alcohol abuse, or treatment with steroids, bisphosphonates, calcium, vitamin D or hormone replacement therapy. The control group subjects were recruited on a random basis and were enrolled in other epidemiological studies carried out at the research laboratory at the Arnau de Vilanova-UDL University Hospital (Lleida, Spain).

Informed consent was obtained from the parents or guardians of all the selected subjects. All the healthy subjects were volunteers.

2. Genotyping

DNA was extracted from peripheral blood white cells using the Aqua Pure Genomic DNA kit (Bio-Rad). This DNA sample was amplified by polymerase chain reaction (PCR) using 5'-AGTGTGCAGGCGATCGTAG-3' as the forward primer and 5'-ATAGGCAGAACCATCTCTCAG-3' as the reverse primer (Proligo) with a Gene Amp PCR System 2700 (Applied Biosystems) for 35 cycles at 95ºC for 15 seconds, 64ºC for 30 seconds, and 72ºC for one minute.

The genetic locus of the BsmI polymorphism of the VDR gene was determined by single-strand conformation polymorphism analysis (SSCP) which separates strands in an acrylamide gel according to their conformation, which depends on the number of bases and on their nature (Fig. 1).

A polymorphism may also be determined by targeting the PCR product with a specific restriction enzyme, BsmI in this case. The digested sample is run through agarose gel stained with ethidium bromide. This technique is more reliable but also more expensive, so it was used only to confirm some of the results obtained by SSCP (Fig. 2).

3. Parameters

Bone mineral density was determined at the calcaneum with a portable Lunar dual photon densitometer (DPX-L) (Lunar Corporation, 313 W. Beltine Hwy., Madison, WI 53713). Z score and T score were determined to assess status as normal, osteopenic or osteoporotic according to WHO criteria, differentiating patients by weight, height and age.

Calcium and alkaline phosphatase (AP) levels were measured with a Hitachi 747 biochemical autoanalyzer (Roche Diagnostic, Mannheim, Germany). Serum 25-hydroxyvitamin D3 was determined with a chemoluminescent immunoassay (Dia Sorin Inc, Stillwater, MN 55082, USA) using a mean reference value (VR) for Europe of 21.6 ng/mL and PTHi (intact PTH) (Immulite Intact PTH,

Diagnostic Products Corporation, Los Angeles, CA, USA) with a reference range of 10-72 pg/mL.

Weight and size were measured with subjects barefoot wearing minimal clothing.

Body mass index (BMI) was calculated using the formula: BMI = weight/height2 (Kg/m2).

4. Statistical analysis

The χ^2 test was performed to compare the frequency distribution of each polymorphism among subjects with and without Down syndrome. Multivariate analysis was used to identify statistically significant factors in relation to polymorphisms in subjects with DS.

The statistical program used was SPSS 13.00 for Windows (SPSS, Inc.), with a required p-value of 0.05 or lower.

Results

A comparison of genotype distribution for the BsmI polymorphism gives almost-significant differences (p = 0.062) between the control group and the group with DS. Among the individuals with DS, there was a higher rate of cases with the bb genotype and a lower rate with the BB type (Table 1). An analysis of allele distribution shows significant differences (p = 0.015), with overrepresentation of the B allele among patients with DS (Table 2).

Table 3 gives the anthropometric data analyzed for the group with DS (sex, age, size, BMI), biochemical parameters (calcium, vitamin D, intact paratohormone), and bone densitometry (T-score).

Multivariate analysis of the different variables shows that size and age are significant among the subjects with DS. Subjects with the bb genotype were taller in this group (p = 0.04) (Fig. 3), and subjects with the BB genotype were older than any of the other genotypes (p = 0.03) (Fig. 4).

Table 4 compares anthropometric, biochemical and densitometric data according to bb, Bb and BB genotype for the group with DS. No statistically significant differences can be observed among the biochemical variables or in terms of presence or absence of osteoporosis relating to VDR alleles.

Discussion

The VDR gene is located in chromosome 12(q12-q14) on the genetic map. DNA was extracted from peripheral monocytes, and allele polymorphisms were assessed using the restriction endonuclease BsmI after specific PCR amplification. Three genotypes were found for the VDR gene: homozygotic BB and bb and heterozygotic Bb. In 1992, Morrison et al. (5) were the first to link VDR polymorphisms to osteocalcin concentration levels and osteoporosis. VDR plays an important role in calcium homeostasis by binding $1\alpha,25(OH)_2D_3$ and effecting its nuclear translocation, and therefore influences both bone resorption and calcium absorption. A recent meta-analysis by Thakkinstain

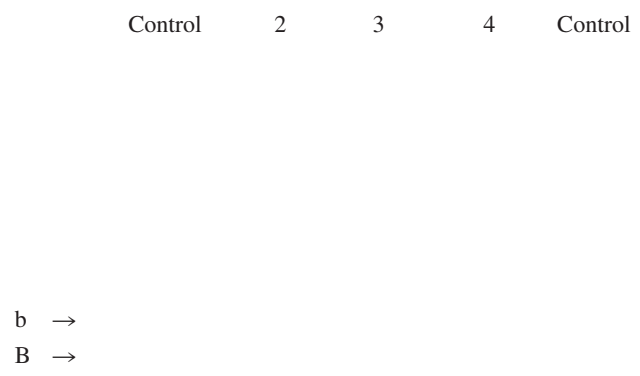

Figure 1. SSCP detection of the *Bsm*I polymorphism.
Line 2 represents homozygotic bb, line 3 heterozygotic Bb, and line 4 homozygotic BB.

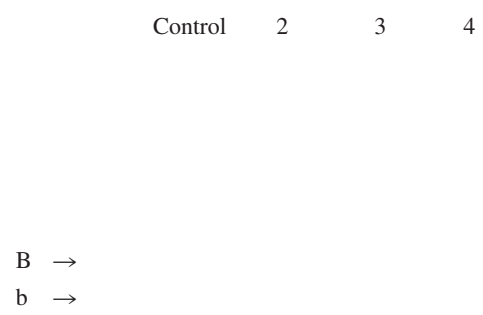

Figure 2. PCR-RFLP detection of the *Bsm*I polymorphism.
The upper band represents the B allele, the lower band the b allele. Line 2 is homozygotic BB, line 3 heterozygotic Bb, and line 4 homozygotic bb.

et al. (6) identified 61 studies which focused on *BsmI*. The overall conclusion was that genotype BB had a well-founded role in lowering spine BMD. VDR alleles have also been linked to other human diseases and anthropometric measurements such as weight, height or muscle development (7-10).

This is the first study we are aware of which looks at VDR polymorphisms together with DS. DS is a genetic disorder caused by the presence of three copies of chromosome 21. It is considered the most frequently occurring genetic disorder and the chief cause of delayed intellectual development in the human species. It has many phenotypical manifestations; in addition to delayed intellectual development, these include short height (attributed to hormonal factors and to chronic hypoxia due to obstructive apnea, among others), heart malformations, gastrointestinal disorders, orthopaedic abnormalities, premature aging with a high rate of dementia, and a lower life expectancy than the general population, which is attributed to a number of causes (11-13).

Chromosome 21 has been fully sequenced and is estimated as having 360 genes. Tripling of the DS critical region (21q22.2-q22.3) is considered to be the reason for the many phenotypical traits of this syndrome. However, there are many doubts as to the correlation between the many, complex DS phenotypes and the genetic contribution of an extra chromosome 21. Mouse models show that some features, such as heart disease, cerebellar malformations with reduced granular cell density, or craneofacial dysmorphia, could be related to the gene-dosing effects of the third copy of the critical region in chromosome 21 (14). Another hypothesis, however, is that overexpression of the many tripled genes disrupts overall genetic homeostasis and thus hampers genetic regulation during the developmental period. The distal portion of murine chromosome 16 contains 141 genes orthologous to those in human chromosome 21. This knowledge was used to develop an experimental mouse model with partial trisomy in this region: Ts65Dn. The model shows that several groups of genes play a role in common cell pathways or processes, and also play a role in a single process with a cumulative effect that is the sum of all their individual contributions; the end consequences are significant. With this theory in mind, we analyzed whether any VDR polymorphisms were more frequent among subjects with DS than among the general population, to gauge its influence on different DS phenotypes.

Table 1
Number of subjects with each genotype, for the normal group and the DS group. Percentages shown in parentheses.

	Control	*DS*
BB	19 (15.6%)	21 (24.7%)
Bb	52 (42.6%)	41 (48.2%)
bb	51 (41.8%)	23 (27.1%)
Total	122	85

Table 2
Distribution of alleles (B, b) among the normal population and the DS population. Percentages shown in parentheses.

	Control	*DS*
B	90 (36,9%)	83 (48,8%)
b	154 (63,1%)	87 (51,2%)
Total	244	170

Table 3
Parameters analyzed for the group with Down syndrome*

Down syndrome	
Gender	37 females; 48 males
Age	41.66 ± 1.27
Weight	65.09 ± 1.08
Height	1.52 ± 0.11
Body Mass Index (BMI)	28.13 ± 0.49
Intact paratohormone	39.65 ± 2.17
Vitamin D	30.41 ± 2.30
Calcium	94.64 ± 0.84
Bone mineral density	-1.44 ± 0.14

*Data shown as average ± of standard error.

Table 4
A comparison of anthropometric and biochemical data and BMD according to genotype.

Down syndrome			
	BB	**Bb**	**bb**
Gender	12 females, 9 males	15 females, 26 males	10 females, 13 males
Age	46.10 ± 2.02	39.98 ± 1.87	40.61 ± 2.61
Weight	63.07 ± 2.18	64.89 ± 1.40	67.28 ± 2.40
Height	1.50 ± 0.01	1.51 ± 0.02	1.57 ± 0.02
Body Mass Index (BMI)	28.09 ± 1.06	28.56 ± 0.75	27.37 ± 0.77
Intact paratohormone	37.29 ± 3.16	42.67 ± 3.90	36.41 ± 2.76
Vitamin D	28.22 ± 4.39	27.28 ± 2.68	37.54 ± 5.71
Calcium	94.68 ± 0.98	95.99 ± 0.90	92.21 ± 2.49
Bone mineral density	-1.58±0.27	-1.50±0.22	-1.21 ± 0.26

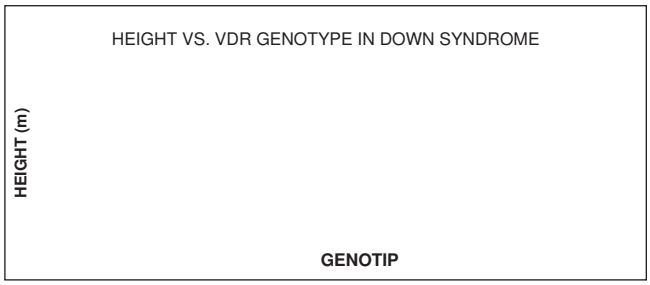

Figure 3. Height in relation to BB, Bb or bb genotype in subjects with DS. Individuals with the bb genotype are taller than those with Bb and BB genotypes, with Bb also taller than BB. Chart shows mean ± standard error.

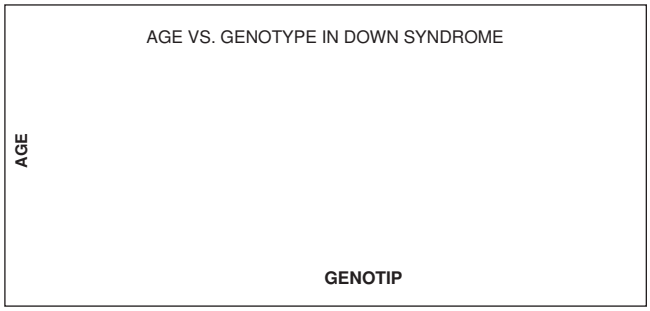

Figure 4. Age in relation to BB, Bb or bb genotype in subjects with Down syndrome. Individuals with the BB genotype had a higher age average than the two other genotypes (Bb and bb). Chart shows mean ± standard error.

VDR polymorphisms were studied in peripheral blood DNA of 85 subjects with DS and 122 control subjects without DS. Each genotype was assessed using PCR amplification of the segments containing BsmIpolymorphisms at intron 8. Genotype distribution analysis shows that subjects with DS have a higher percentage of allele B whereas allele b is predominant among the control group ($p = 0.015$). A greater prevalence of osteoporosis has been described for DS and linked, among other factors, to low vitamin D levels (4, 15). However, a comparison of BMD, vitamin D, calcium, alkaline phosphatase and PTHi levels against VDR polymorphisms grouped as "favorable" (bb) or "unfavorable" (BB and Bb) failed to find a significant statistical relationship to either. We were nevertheless surprised to discover a higher rate of homozygotic bb genotypes among the taller subjects with DS, and a higher rate of homozygotic BB types among older subjects. This had not been previously described, and given that Bb is the most frequent genotype in this population segment, it may be acting as an "unfavorable" factor which would explain short height and lower survival.

Conclusions

Allele B of the BsmI polymorphism at intron 8 of the vitamin D receptor gene is more prevalent among subjects with DS. Genotypes bb and BB are more frequent, respectively, among taller and longer-lived individuals with DS. This suggests that they might play a role in the phenotypical characteristics of DS. In all likelihood, the inclusion of other genes as well as the interaction of genes with each other and with environmental factors may provide an answer that will determine whether these genetic studies are a useful preventive or prognostic tool for Down syndrome.

Acknowledgements

We would like to thank the management of the ACUDAM, ASPROS, APROMI and ASPAMIS centres, the people with DS who took part in the study, and the medical staff, physical therapists, counsellors and other staff members, for enabling us to carry out this research. Thanks to FAES laboratory for its invaluable help.

References

1. Rehder H, Fritz B. Genetic causes of mental retardation. Wien Med Wochenschr 2005; 155: 258-67.
2. Antonarakis SE. 10 years of Genomics, chromosome 21, and Down syndrome. Genomics 1998; 51: 1-16.
3. Olson LE, Richtsmeier JT, Leszl J, Reeves RH. A chromosome 21 critical region does not cause specific Down syndrome phenotypes. Science 2004; 306: 687-90.
4. Roselló Ll, Torres R, Boronat T, Lobet R, Puerto E. Osteoporosis prevelence in a Down syndrome population, mesauring different parameters. DS-SD International Medical Review on Down Syndrome 2004; 8: 18-22.
5. Morrison NA, Yeoman R, Kelly PJ, Eisman JA. Contribution of trans-acting factor alleles to normal physiological variability: vitamin D receptor gene polymorphisms and circulating osteocalcin. PNAS 1992; 89: 6665-9.
6. Thakkinstian A, D'Este C, Eisman J, Nguyen T, Attia J. Meta-Analysis of molecular association studies: Vitamin D receptor gene polymorphisms and BMD as a case study. JBMD 2004; 19: 419-28.
7. Matusiak D, Murillo G, Carroll RE, Mehta RG, Benya RV. Expression of vitamin D receptor and 25.hydroxyvitamin D3-1 alpha-hydroxylase in normal and malignant human colon. Cancer

Epidemiology Biomarkers and Prevention 2005; 14: 2370-6.
8. Muray S, Parisi E, Cardús A, Craver L, Marco MP, Fernández E. Influencia del polimorfismo del gen receptor de la vitamina D y de la 25-hidroxivitamina D en la tensión arterial de individuos sanos. Nefrología 2003; 23 Supl 2: 32-6.
9. Xiong DH, Xu FH, Liu PY, Shen H, Long JR, Elze L, Recker RR, Deng HW. Vitamin D receptor gene polymorphisms are linked to and associated with adult height. J Med Genet 2005; 42: 228-34.
10. Grundberg E, Brändström H, Ribom EL, Ljunggren Ö, Mallmin H, Kindmark A. Genetic variation in the human vitamin D receptor is associated with muscle strength, fat mass and body weight in Swedish women. Eur J Endocrinology 2004; 150: 323-8.
11. Zigman WB, Jenkins EC, Tycko B, Schupf N, Silverman W. Mortality is associated with apolipoprotein E epsilon4 in nondemented adults with Down syndrome. Nuerosci Lett; 390: 93-7.
12. Day SM, Strauss DJ, Shavelles RM, Reynolds RJ. Mortality and causes of death in persons with Down syndrome in California. Dev Med Child Neurol. 2005; 47: 171-6.
13. Hill DA, Gridley G, Cnattingius S, Mellemkjaer L, Linet M, Adami HO, Olsen JH, Nyren O, Fraumeni JF Jr. Mortality and cancer incidence among individuals with Down syndrome. Arch intern Med 2003; 163: 705-11.
14. Vacik T, Ort M, Gregorová S, Strnad P, Blatny R, Conte N, Bradley A, Bures J, Forejt J. Segmental trisomy of chromosome 17: A mouse model of human aneuploidy syndromes. PNAS 2005; 102: 4500-5.
15. Angelopoulou N, Matziari C, Tsimaras V, Sakadamis A, Souftas V, Mandroukas K. Bone mineral density and muscle strength in young men with mental retardation (with and without Down syndrome). Calcified Tissue International 2000; 66: 176-80.