Analysis of Polymorphisms of the Vitamin D Receptor, Estrogen Receptor, and Collagen Iα1 Genes and Their Relationship With Height in Children With Bone Cancer

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ABSTRACT

Purpose: The authors’ objectives were to compare height at diagnosis of children with bone tumors with that of Spanish reference children; to analyze the frequency of the genotypes for the polymorphisms of the vitamin D receptor (VDR), estrogen receptor (ER), and collagen Iα1 (COLIα1) genes in patients and in healthy controls; and to test the relationship between the genetic markers and height.

Patients and Methods: Height and weight at diagnosis were measured in 58 osteosarcoma and 36 Ewing sarcoma patients and compared with standards published for Spanish reference children according to sex and age. For the molecular analysis, genetic polymorphisms of the VDR (Fok I, Apa I, and TaqI), ER (Pvu II and XbaI), and COLIα1 (Msc I) genes were characterized in 72 osteosarcoma and 53 Ewing sarcomas and in a group of 143 healthy matched children.

Results: Osteosarcoma and Ewing sarcoma patients were significantly taller than Spanish reference children. Osteosarcoma patients showed a significantly higher frequency of the Ff genotype for the Fok I polymorphism (VDR gene) than the control group. The odds ratio for this genotype was 1.78, with an increased relative risk of 78% for heterozygous Ff carriers. Among Ewing sarcoma patients, this same genotype was significantly associated with lower height than homozygotes (FF or ff).

Conclusions: Children with bone cancer are significantly taller than the reference population, which may be influenced by the genotype for the Fok I polymorphism of the VDR gene.

Key Words: osteosarcoma, Ewing sarcoma, childhood, height, genetic markers

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INTRODUCTION

Malignant bone tumors account for 4% to 6% of all cancers that occur during infancy and adolescence in most European countries. The age-standardized rate ranges from 4.5 to 7.5 per million.¹ Within the group of childhood bone tumors, two main histologic subtypes can be distinguished: osteosarcoma, which accounts for 50% of the cases, and Ewing sarcoma (including primitive neuroectodermal tumors of bone), which accounts for 40% to 45% of the cases.²

Since the 1950s, several clinical and epidemiologic studies have suggested a link between the acceleration of skeletal growth that occurs during infancy and adolescence, and the development of bone tumors. First, the rates of incidence and mortality due to these tumors show a progressive increase during the two first decades of life, peaking in adolescence, similar to the growth pattern.¹,² The age at diagnosis of most osteosarcomas overlaps with the pubertal growth spurt and with maximal reconstructive activity within the bone cortex. Second, the age of presentation is sex-dependent: girls are diagnosed at earlier ages, in good agreement with the earlier presentation of the growth spurt and their more advanced bone age, and the incidence is higher among boys, in good correlation with their higher growth rate. Third, the metaphyses of the long bones of the extremities are the most frequent location, and they are also responsible for most longitudinal growth. Once the period of growth is achieved, the incidence of these tumors decreases and their anatomic distribution varies, with the long and flat bones being equally affected. Finally, this theory is supported by the association between these tumors and other diseases in which excessive cellular proliferation occurs (eg, Paget disease, benign fibrous lesions).¹,³,⁴

Fraumeni ⁵ described that osteosarcoma and to a lesser extent Ewing sarcoma patients were significantly taller than patients diagnosed with other tumor types. Therefore, the origin of the bone tumors could be related to the increased susceptibility of the proliferative bone cells to oncogenic agents, mitotic errors, or chromosome aberrations during the growth spurt, thus cooperating in the neoplastic transformation. However, this hypothesis is not fully accepted. Multiple studies have been carried out to support the finding of increased height in bone cancer patients,⁶–⁹ but some of the authors did not find evidence of this association¹⁰–¹³ and others found opposite results.¹⁴

Growth is a complex process, regulated by a net of environmental and genetic factors. It is well known that vitamin D is involved in skeletal metabolism: directly, regulating the proliferation, differentiation, and migration of osteoblasts and chondrocytes in the growth plates of the epiphysis and indirectly, regulating the homeostasis of calcium and phosphorus. In addition, vitamin D deficiency may induce a growth failure and/or bone metabolism alteration that can be evident even in the intrauterine and postnatal periods of life.¹⁵,¹⁶

As a result, during the past few years, investigators have tested the association between the genotype for several polymorphisms within the vitamin D receptor (VDR) gene and growth parameters such as height, weight, and bone mineral density (BMD).¹⁷–¹⁹ Minamitani et al ²⁰ analyzed the relationship between height and the genotype for the Fok I polymorphism of the VDR gene in the healthy Japanese population. They observed that heterozygotes (Ff) were significantly taller than homozygotes (FF or ff), and the frequency of the Ff genotype was significantly reduced among patients with
constitutional short stature. Other studies have focused on the TaqI and Bsm I VDR polymorphisms and their association with height or weight at birth, during the first years of life, and during adolescence.\textsuperscript{17-19} Other genes involved in growth and skeletal maturation are the estrogen receptor (ER) gene, which determines growth during the postnatal and pubertal periods,\textsuperscript{21,22} as suggested by the association between the allelic variants of this gene and height, weight, and the expression of the VDR gene. The collagen I\textsubscript{α1} (COLI\textsubscript{α1}) gene is also considered a good candidate, given that it is the main component of the bone matrix.\textsuperscript{23,24}

Our hypothesis was that children with bone sarcoma would be taller at diagnosis than the reference population of Spanish children, and that this could be influenced by the presence of polymorphisms in genes involved in bone metabolism. Therefore, our objectives were to compare height at diagnosis of osteosarcoma and Ewing sarcoma with that of the reference children according to sex and age; to analyze the frequency of the genotypes for the polymorphisms of the VDR, ER, and COLI\textsubscript{α1} genes in patients and in healthy controls; and to test the relationship between genetic markers and height in our patients.

**PATIENTS AND METHODS**

**Subjects**

We included 72 Spanish children with osteosarcoma and 53 Spanish children with Ewing sarcoma who were treated and followed at the Department of Pediatrics of the University Clinic of Navarra, Spain, from 1984 to 2000 using standard protocols.\textsuperscript{25,26} This investigation was approved by the Ethics Committee of the University Hospital, and informed consent was obtained from all participants or their parents. Patients with a family history of cancer, bone disease, or endocrine alterations were excluded from the analysis.

The mean age of the patients at diagnosis was 14.10 (4.01) years for those with osteosarcoma and 13.30 (4.30) years for those with Ewing sarcoma. Sex distribution was 46% girls and 54% boys for osteosarcoma and 41% girls and 59% boys for Ewing sarcoma.

**Anthropometric Analysis**

Fourteen osteosarcoma and 17 Ewing sarcoma patients were excluded from the anthropometric study because their data were obtained in other hospitals. Height (Harpenden stadiometer, Holtain Ltd., UK), weight (electronic scale), and body mass index (BMI, weight [kg]/height [m\textsuperscript{2}]) were measured at diagnosis for 58 osteosarcoma and 36 Ewing sarcoma patients and retrospectively obtained from the medical records. The data were compared with the standards published for the Spanish children according to age and sex\textsuperscript{27} and expressed as standard deviation scores (SDS). All the patients considered for the analysis had a normal stage of development for their age according to Tanner.\textsuperscript{28}
**Genetic Analysis**

We included 72 osteosarcoma patients and 53 Ewing sarcoma patients from whom DNA was available and a control group of 143 healthy Spanish children (49% girls and 51% boys) with a mean age of 16.60 (6.10) years. The polymorphisms for the restriction endonucleases Fok I, Apa I, and TaqI of the VDR gene, XbaI and Pvu II of the ER gene, and Msc I of the COLα1 gene were characterized by polymerase chain reaction (PCR) amplification and restriction fragment length polymorphism (RFLP) analysis. The alleles for the genetic markers were represented in uppercase or lowercase if the restriction sites were absent or present, respectively.

**Statistical Analysis**

The height, weight, and BMI data of osteosarcoma and Ewing sarcoma patients, expressed as SDS, fulfilled the normality criteria and were described as mean (standard deviation [SD]). Their comparison to the standards published for the Spanish reference children was done by the Student t test. Height (in cm) was described as median (interquartile range [IQR]) given the non-normal distribution of the variable. For the comparison of the genotypes, the Pearson chi-square test was used. The relationship between height-SDS of patients and their genotypes was analyzed by analysis of variance with multiple post hoc comparisons (Student-Newman-Keuls). Differences were considered statistically significant if their associated probability was P ≤ 0.05. The statistical analysis was performed using the Statistical Package for the Social Sciences program, version 10.0 (SPSS, Chicago, IL).

**RESULTS**

The mean age of the patients at diagnosis was 14.10 (4.01) years for those with osteosarcoma and 13.30 (4.30) years for those with Ewing sarcomas. At that time, all the patients had a normal Tanner development staging for their age. The age at diagnosis of most osteosarcoma and Ewing sarcoma patients coincided with the acceleration of the pubertal growth spurt reported for the standard Spanish reference children and was similar in boys and girls (Fig. 1). The age of peak growth velocity in Spanish reference children is 12 years for girls and 14 years for boys, and the age at diagnosis for our patients was 13.81 (4.31) and 14.11 (3.95) for boys and girls with osteosarcoma and 12.36 (3.94) and 13.93 (4.97) for boys and girls with Ewing sarcoma.

The location of the primary tumors was femur (41.7%), tibia (40.3%), humerus (5.6%), and fibula (5.6%) for osteosarcomas and femur (13.7%), tibia (21.6%), flat bones (25.5%), and extraosseous (21.6%) for Ewing sarcomas. We did not detect any differences in the age of presentation between the tumors in the humerus with respect to those of the femur or tibia. The distribution of the tumors within the long bones, concerning their distal or proximal portions, was as follows: distal metaphysis of the femur (87.5% for osteosarcoma, 60% for Ewing sarcoma), proximal tibia (68.2% and 55.6%), and proximal humerus (100% and 66.7%).
Both osteosarcoma (P < 0.001) and Ewing sarcoma (P = 0.013) patients were significantly taller at diagnosis than the Spanish reference children (Table 1). If we considered both sexes independently, the differences remained except for boys with Ewing sarcoma. With regard to weight, only osteosarcoma patients were significantly heavier than the reference children. The BMI did not differ significantly between either of the tumor types and the reference population.

The allele frequencies for all the polymorphisms analyzed were in Hardy-Weinberg equilibrium in the control population. The distribution of the genotypes for the Fok I marker of the VDR gene was significantly different in osteosarcoma patients compared with healthy controls (Table 2), with the Ff genotype being overrepresented among patients with osteosarcoma (P = 0.048) but not among those with Ewing sarcoma. The odds ratio for this genotype was 1.78 (1.00–3.16), with an increased relative risk of 78% for heterozygous Ff carriers. We did not detect any other outstanding association between any of the polymorphic markers analyzed and the development of osteosarcoma or Ewing sarcoma.

Height and weight at diagnosis among Ewing sarcoma patients were significantly different depending on the genotype for the Fok I marker of the VDR gene in such a way that Ff heterozygotes were statistically shorter and thinner at diagnosis than any of the homozygotes (FF and ff) (P = 0.013) (Fig. 2). This difference was also evident if both sexes were considered independently. We did not detect any association between height and weight at diagnosis of bone sarcoma and the genotype for the Apa I and TaqI polymorphisms of the VDR gene or for those of the ER and COLIA1 genes. We observed a highly significant association (P < 0.001) between the alleles A for Apa I and t for TaqI markers of the VDR gene and between alleles P-X and p-x for Pvu II and XbaI markers of the ER gene, which confirms the presence of a strong linkage disequilibrium between these markers.

DISCUSSION

The relationship between the development of bone tumors during infancy and growth acceleration continues to be controversial. The fact that the incidence of bone sarcomas peaks during childhood and adolescence suggests that the etiologic mechanism of these tumors must be present during this period. The difference in presentation between boys and girls and the location of these tumors support the hypothesis that the bone growth rate that is characteristic of this age may be involved. Although Ewing sarcoma is of neural origin and therefore presents several clinical differences compared with osteosarcoma, this fact does not seem to influence its epidemiologic characteristics.

Since Fraumeni demonstrated that patients with osteosarcoma and Ewing sarcoma were taller than other cancer patients, and that this could be related to the increase in the growth rate, multiple papers have been published, some supporting and others disagreeing with this hypothesis. Our data, along with those of others, confirm the preferential localization of these bone tumors for the long bones of the extremities, at the metaphyseal portions of the most rapidly growing bones in adolescents, as well as the peak of presentation during adolescence, overlapping with the growth spurt.
Other studies \(^6-^8\) that show an association between the development of bone tumors and high stature are based on the measurement of height at the time of diagnosis, but lack any previous data on growth before that time. Growth is not a linear process but rather is characterized by frequent, intense, and short spurts or increases in its velocity that seem to be determinant of final height.\(^3^3\) A short-term evaluation of growth previous to the diagnosis would possibly yield more precise information.

In this sense, Gelberg et al\(^9\) described the height of bone cancer patients 1 year before diagnosis; they observed that at that time they were already taller than the control group regardless of the stage and age of pubertal development and height at birth. Given the retrospective nature of our study design, we lacked data concerning previous anthropometric measurements or parental height. Other authors have not shown discrepancies in height or in the growth rate during the 3 years before diagnosis of this kind of tumor.\(^1^0-^1^3\)

Our Ewing sarcoma patients did not show significant differences in terms of weight at diagnosis, even though they were taller than the healthy controls. This could be related to the constitutional syndrome that these patients show at diagnosis and that is due to the prolonged period from tumor development to diagnosis if the location of the primary tumor is either central or extraosseous. On the contrary, the peripheral location of most primary osteosarcomas allows a rapid diagnosis.

All of the investigations noted were retrospective and have several limitations.\(^8,^9,^1^1-^1^3\) First, the collection of data was usually done using medical or school records, which introduces differences in measurement techniques, mistakes in data, and even “decreases” in height, or using questionnaires to parents, in which lack of memory may influence the results. Second, the reference population considered was not always sex- and age-matched to the patient group. Third, the interindividual variability at the initiation of the growth spurt and its duration and intensity makes the interpretation of the results difficult. Finally, the contradictory results may be explained by differences in sample size or the statistical analysis performed.

We detected a significantly increased frequency of the heterozygous \(F_f\) genotype for the Fok I polymorphism of the VDR gene among our osteosarcoma patients that had not been previously described in the Spanish population.\(^3^4\) Given that these patients are significantly taller than the reference population and that this genotype has been associated with taller height,\(^2^0\) one would expect that \(F_f\) osteosarcoma patients would be taller than \(FF\) and \(ff\) patients, but this association could not be confirmed in our series. The reason could be that all osteosarcoma patients are taller than the reference population, irrespective of their genotype, which may interfere with the detection of differences among genotypes that have been described in the healthy population with height within the normal range. Further, Ewing sarcoma patients who carried the \(F_f\) genotype had a significantly lower height than homozygous \(FF\) or \(ff\) patients. Nevertheless, the limited number of patients analyzed in this study does not allow us to draw definitive conclusions regarding this apparent contradiction. On the other hand, we observed that homozygous \(ff\) patients with osteosarcoma and Ewing sarcoma tended to have the highest height, which has also been described by Minamitani et al.\(^2^0\) Nevertheless, the fact that the \(F_f\) genotype was overrepresented in this group of significantly tall patients and was associated with an odds ratio of 1.78 suggests a putative involvement of the Fok I genotype in growth.
We did not detect any outstanding association between height at diagnosis and the genotype for the TaqI polymorphism of the VDR gene or the genotype for the ER and COLIα1 genes. Nevertheless, the findings reported by other authors seem contradictory and do not follow a common pattern.\textsuperscript{16–19,21–24} The differences in these results may be due to different reasons: there may be environmental factors that interact with the VDR locus accounting for functional differences among different genotypes, as has been described to happen with the effect of vitamin D and bone response and calcium resorption.\textsuperscript{35,36} The polymorphisms of the VDR gene may be linked to other genes that are also involved in growth and skeletal metabolism, in such a way that the degree of linkage could vary among different populations and provoke the appearance of different phenotypes.\textsuperscript{17,18} Finally, the association between alleles A (Apa I) and t (TaqI) of the VDR gene and alleles P-X andp-x for polymorphisms Pvu II and XbaI of the ER gene found in our series confirms the strong linkage disequilibrium that has already been reported.\textsuperscript{22,37}

In conclusion, children with osteosarcoma and those with Ewing sarcoma are significantly taller at diagnosis than a reference population. It seems that the Fok I polymorphism of the VDR gene may be involved in growth and final height in this group of children. To date, we lack longitudinal analysis of individual growth, height, and pubertal development of these patients, as well as the genes involved in the skeletal metabolism of children with bone cancer.

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\begin{center}
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\end{center}

**REFERENCES**

Figure 1. Comparison of the growth velocity curves according to Spanish reference children\textsuperscript{27} with the histograms of age at diagnosis in patients with osteosarcoma and Ewing sarcoma.
Figure 2. Relationship between height at diagnosis\textsuperscript{27} and the genotype for the Fok I polymorphism of the VDR gene in osteosarcoma and Ewing sarcoma patients.
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<td><strong>P Value</strong></td>
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<td></td>
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<td><strong>Males</strong></td>
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<td><strong>(n = 31)</strong></td>
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<tr>
<td></td>
<td><strong>Age (years)</strong></td>
<td>13.81 (4.31)†</td>
<td>14.11 (3.95)</td>
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<tr>
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<td><strong>Height (cm)</strong></td>
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<td>160.37 (19.22)</td>
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<td>0.08 (0.96)</td>
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*One-sample student t test.
†Mean (SD).
‡Mean (IQR).
§One-sample student t test (P < 0.01).
‖One-sample student t test (P < 0.05).

SDS, standard deviation score; BMI, body mass index.
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