Scanning Microscopy

Volume 4 | Number 3 Article 23

8-22-1990

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Scanning Microscopy, Vol. 4, No. 3, 1990 (Pages 775-780) 0891-7035/90$3.00+.00
Scanning Microscopy International, Chicago (AMF O'Hare), IL 60666 USA

CALCOSPHERITE (CALCIFICATION NODULE) SIZE IN THE SHORT RIB POLYDACTYLY SYNDROMES

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(Received for publication April 11, 1990, and in revised form August 22, 1990)

Abstract

The short rib polydactyly syndromes (SRP) are lethal neonatal skeletal dysplasias with a narrow chest, short limbs, and other abnormalities. Type II (Majewski) short rib polydactyly can be distinguished from the Type I/III (Saldino-Noonan) type on the basis of radiographic and histologic changes. Our previous transmission electron microscopic studies suggested unusual patterns of cartilage calcification in these syndromes. We evaluate calcification in the present study using scanning electron microscopy and quantitative morphometry of calcification regions digested to expose calcospherite nodules (calcification nodules), distinctive morphologic structures which form during cartilage calcification. Mean calcospherite area of the Majewski Type II SRP ($3.5 \pm 0.24 \times 10^{-6}$ mm^2 (3) (mean $\pm$ sem (n)) did not differ from normal control means ($3.1 \pm 0.5 \times 10^{-6}$ mm^2, (3)). The mean area for Type I/III, however, was significantly larger than both the control and Type II means ($8.9 \pm 1.16 \times 10^{-6}$ mm^2 (7), p=.001). This difference in calcospherite size adds a further differentiating feature between these two dysplasias.

KEY WORDS: Mineralization, calcification, short rib polydactyly, skeletal dysplasias, quantitative morphometry, scanning electron microscopy

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Introduction

The short rib polydactyly syndromes (SRP) are lethal neonatal skeletal dysplasias in which the chest is narrowed, limbs shortened and in which other congenital abnormalities may be present (Taybi and Lachman, 1990). The reason these two syndromes were given the same name derives solely from the combination of the features of short ribs and polydactyly, each of which is present in other skeletal dysplasias. The names of these syndromes in no way suggest a common pathogenetic mechanism.

Our previous transmission electron microscopic examinations of growth plate specimens from patients with SRP revealed unusual calcification sites (Gruber et al, 1989). Because of this finding we were especially interested in visualizing and quantitatively measuring calcospherites in SRP. In the present study we apply digestive procedures to growth plate tissues to remove soft and cellular tissue; the exposed calcospherites are then examined with scanning electron microscopy and quantitatively studied with morphometric analysis.

The calcospherite calcification nodule is a distinctive morphologic structure which develops at the loci of calcification in the growth plate. In the past it has been studied under a variety of names ("initial calcification nodules", Bernard, 1972; large aggregates of "crystal ghosts", Bonucci, 1987; "crystal clusters", Ali, 1983 and Bonucci, 1971; "calcospherites", Boyde and Jones, 1983, and Ornoy et al, 1980). As chondro-osseous growth progresses, these sites of calcospherite clusters merge and coalesce forming the individual septum upon which the primary trabeculae form at the growth plate.

Materials and Methods

Specimens were obtained from SRP patients at autopsy and from normal subjects at autopsy from abortuses, stillbirths or individuals who died from non-skeletal-related diseases. This group of normal subjects has been utilized in our previous study of cartilage cell columns (Gruber and Rimoin, 1989). Specimens are part of

the Cedars-Sinai International Skeletal Dysplasia Registry collection.

Tissue specimens at growth plate regions of long bones (femur, radius), iliac crest, and rib (at the costo-chondral junction) were fixed in glutaraldehyde, digested in 5% solutions of NaOCl for one hour at room temperature and rinsed thoroughly with distilled water to remove soft tissues and bleach (Boyde, 1984). Specimens were critical-point dried, coated with gold/palladium and examined with an Hitachi S405 A/2 scanning electron microscope. Original micrographs at a magnification of 1500 x were selected for prints in which clear, distinct calcospherite profiles were imaged. Calcospherite areas were traced onto a Summagraphics Bit Pat One interfaced with an IBM XT computer. Software utilized was "Morphom General" produced by BioMed Stats, Inc. (Tacoma, WA).

Statistical analyses utilized Student's t-test for independent groups (Snedecor and Cochran, 1973). Data are expressed as means $\pm$ s.e.m. (n).

For quantitative study of calcospherites, the following mean numbers of individual nodules were measured: controls, 33 $\pm$ 10 (range 18-51); SRP Type I/III, 39 $\pm$ 6 (range 16-64); SRP Type II, 50 $\pm$ 9 (range 37-66).

Case Summaries

Two types of SRP were studied: SRP syndrome Type I/III (Saldino-Nonan/Verma-Naumoff) and SRP syndrome Type II (Majewski). Clinical and radiologic features of these autosomal recessive syndromes have recently been reviewed by Taybi and Lachman (1990). Types I and III appear to represent one disorder with variable expressivity, Type I being the most severe. Radiographically Type I/III is characterized by shortened horizontal ribs, small iliac bones with flattened acetabular roofs, markedly shortened long tubular bones with irregular metaphyses with spurs, incomplete metacarpal, metatarsal and phalangeal ossification and postaxial polydactyly (Figure 1). Histologic features of I/III include shortened or absent proliferative zone, absence of chondrocyte columns and irregularly dispersed hypertrophic cells with areas of acellular cartilage abutting directly on bony trabeculae.

SRP Type II radiologically exhibits underdeveloped mandible, short horizontally oriented ribs, shortened tubular bones (especially the tibiae) with rounded metaphyseal ends, precocious ossification of the proximal femoral epiphysis and pre and/or post- axial polydactyly (Figure 2). Histologically, the growth plate is short with irregular columnization.

Case summaries of cases 1-10 are outlined in Table 1.

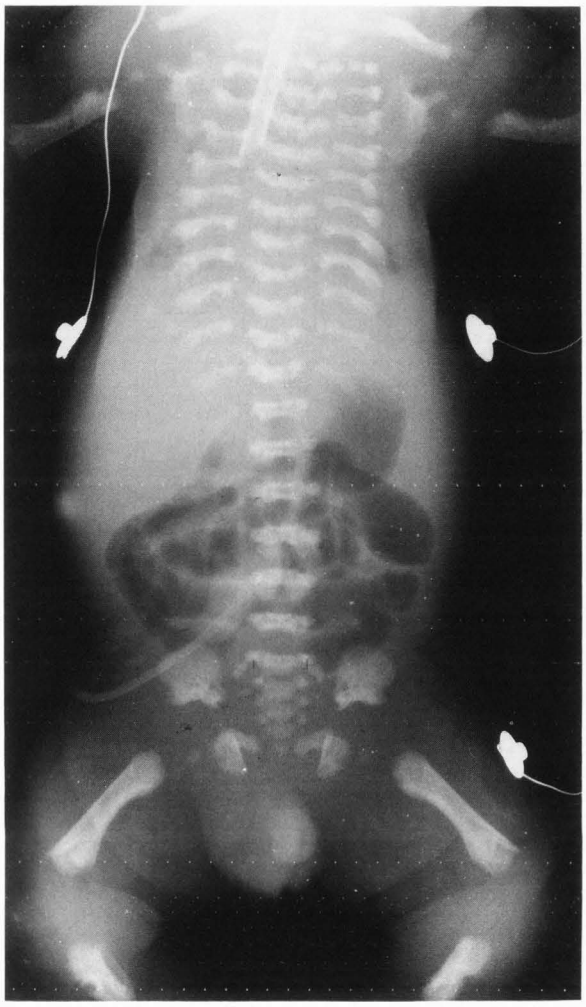

Figure 1. Representative radiograph of the skeletal features of SRP Type I/III. Case #4 is illustrated.

Results

The special digestion techniques used here allow direct visualization of the calcospherite domains in the matrix septal regions surrounding hypertrophic chondrocytes (Figure 3). Calcospherites line elongated cup-shaped depressions (Figure 4). They are closely packed but, with our preparative techniques, appear round and not angled as would be expected in objects which show hexagonal close packing.

Calcospherites from normal subjects had a mean area of 3.07×10^{-6} mm^2 $\pm$ 0.52 (3) (Figures 4 and 5). Mean calcospherite area from patients with Type II SRP did not differ from normal (3.46×10^{-6} mm^2 $\pm$ 0.24 (3)) (Figures 5 and 6).

The mean calcospherite area from seven well-defined cases of SRP Type I/III, however, was significantly larger than both control and Type II SRP means (8.92×10^{-6} mm^2 $\pm$ 1.16, p=.001) (Figures 5 and 7). No overlap was seen

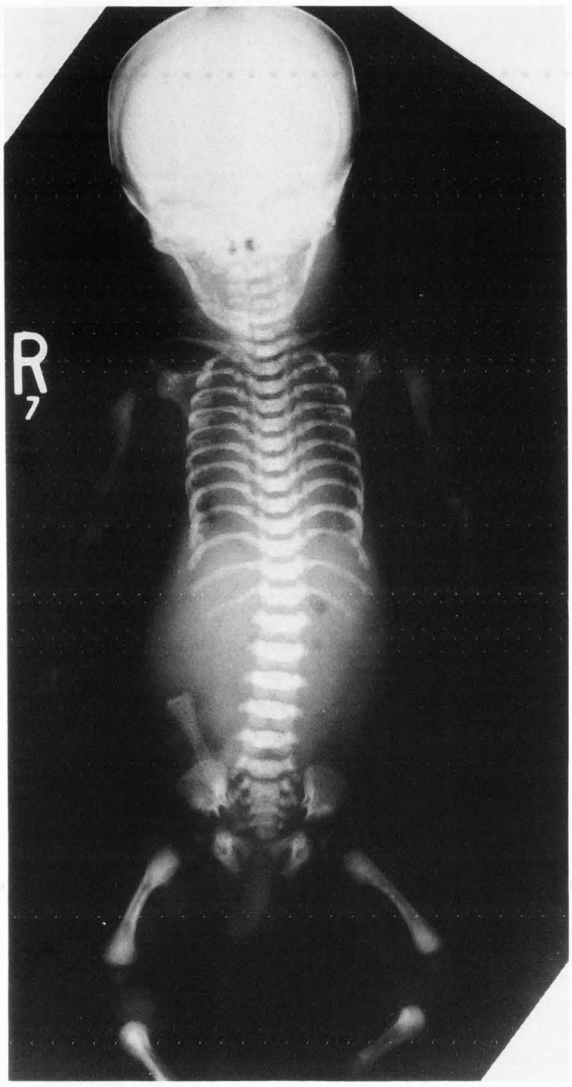

Figure 2. Representative radiograph of the skeletal features of SRP Type II (Majewski). (The structure above the right ilium is an umbilical cord clamp.) Case #8 is illustrated.

for the Type I/III mean values with either control or SRP Type II mean values (Figure 8).

Discussion

Cartilage calcification is an intricate and complex process. The initial changes which occur in matrix near hypertrophic chondrocytes are many; one current model suggests involvement of the C-propeptide of the type II collagen fibril, proteoglycan aggregates and link protein (Poole et al, 1989). The morphologic structure studied here, the calcospherite, is a later

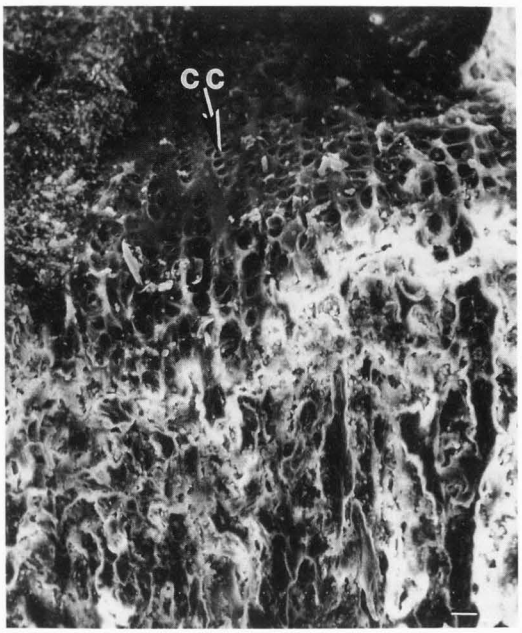

Figure 3. Low magnification scanning electron micrograph shows the chondrocyte columns (cc, arrow), lacunae of hypertrophic chondrocytes, and primary spongiosa in the growth plate of a normal subject (bar = 1 μm).

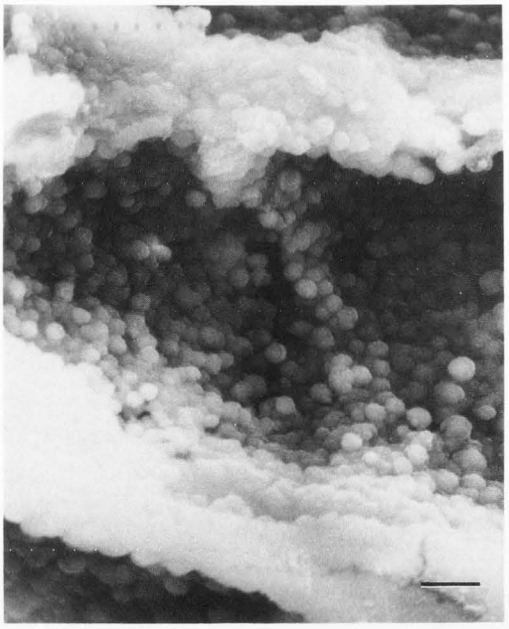

Figure 4. Scanning electron micrograph of calcospherite domain in a normal newborn (bar = 1 μm).

Table 1: Description of SRP Patients

	Age/Sex*	History/Comments
SRP Type I/III:		
Case 1	F, 25 wks	Short limbs detected on ultrasound at 24 weeks. Siblings. Another male sibling reported to have died from "thanatophoric dysplasia". 1 normal sibling.
Case 2	23-24 wks	Short limbs detected on ultrasound at 18 weeks
Case 3	M, newborn	Second affected sibling in 1989 had identical radiology at 21 weeks.
Case 4	M, 28 wks	
Case 5	Baby girl	
Case 6	Baby girl	Edema; situs inversus (except for liver). Ventricular septal defect. Bilateral urinary bladders; 2 spleens
Case 7	M, 30 wks	Partial cleft lip and palate. Malrotation of GI tract, hydrocephalus, absence of external penis
SRP Type II:		
Case 8	Baby boy	Short femora detected on ultrasound at 31 weeks.
Case 9	F, 41 wks	Multiple frenula in lips. 5 spleens. Malrotation of large bowel, polycystic kidneys, bicornate uterus.
Case 10	Baby boy	

* Ages are weeks of gestation

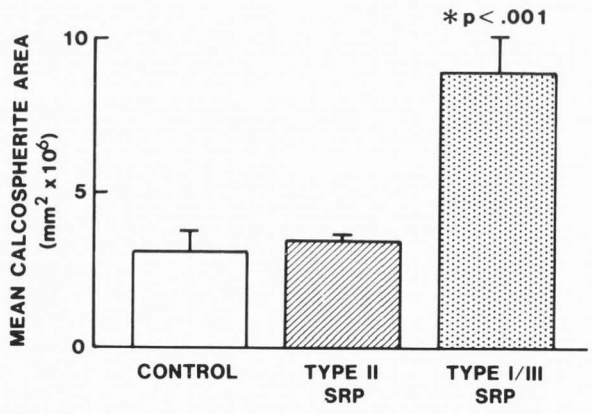

Figure 5. Mean calcospherite size is significantly larger in SRP Type I/III compared to either control subjects or SRP Type II (p=.001). (Open bar, mean of 3 control subjects; hatched bar, mean of 3 SRP Type II patients; solid bar, mean of 7 SRP Type I/III patients.) (Error bars are s.e.m.)

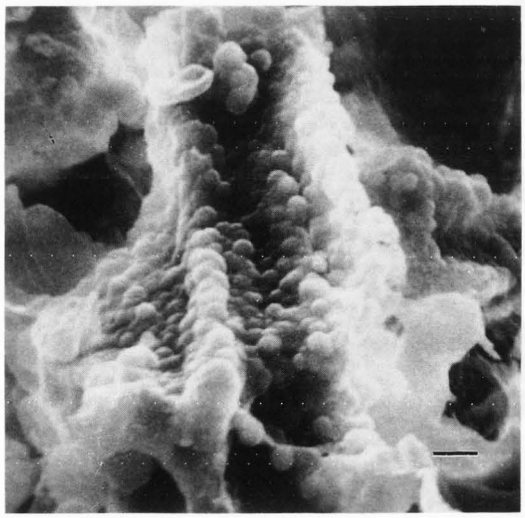

Figure 6. Scanning electron micrograph of calcospherite domain in SRP Type II (bar = 1 μm).

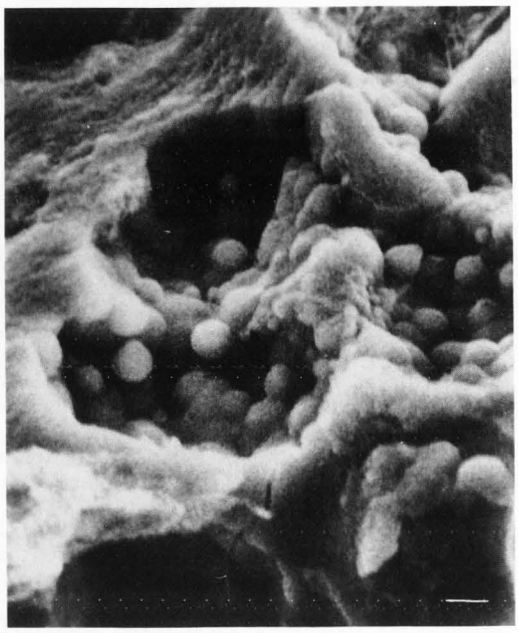

Figure 7. Scanning electron micrograph of calcospherite domain in SRP Type I/III (bar = 1 μm).

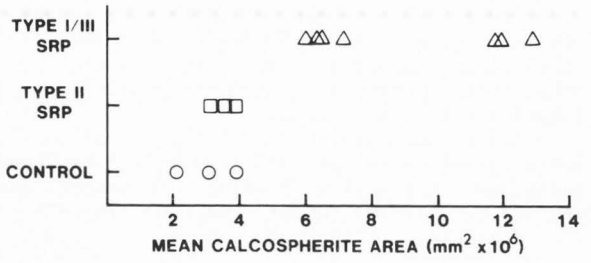

Figure 8. Distribution of mean calcospherite areas in normal subjects (circles), SRP Type II (squares) and SRP Type I/III (triangles).

developmental stage formed when these initial calcification sites have grown in size. Other hypotheses for the calcification process invoke mediation and initiation by matrix vesicles located in the longitudinal septae or origin directly from the 150 nm matrix vesicles (Ali, 1983; Bernard, 1972; Boyde and Jones, 1983; Bonucci, 1971). The SEM methods employed here are well-suited for morphologic studies of calcospherites.

This study has provided new information on the quantitative morphology of calcospherites in the human term infant and in patients with SRP. One form of SRP, Type II, was characterized by calcospherites which were similar in size to those of the normal human. In Type I/III patients, however, calcospherites averaged twice as large as normal. One individual patient had a mean calcospherite size of 12.9×10^{-6} mm^2, more than four times normal.

The cause of these enlarged calcospherites is not known. There are hypothetically at least two possible explanations: 1) matrix alterations may be present which act to offer resistance to, or chemically inhibit, growing calcospherites; 2) there may be alterations in the matrix which result in fewer, more widely spaced initial sites of calcification. A third alternative hypothesis is that these bone dysplasias are due to defective endochondral ossification and the unusual calcospherite nodules are the result, but not the cause, of these changes. The calcospherites which develop may then grow larger in their less crowded domain. Since these disorders are inherited as autosomal recessive traits, an inborn error of metabolism which could result in increased calcospherite size is a likely cause of the bone dysplasia.

Acknowledgements

The authors wish to thank Mrs. L. Nolasco for technical assistance, Mrs. M. Priore for assistance in coordinating the International Skeletal Dysplasia Registry and Ms. G. Reyburn for assistance in typing the manuscript.

This study of SRP was possible due to the generous referral of cases from a number of physicians and investigators: Dr. J. Bass (Torrance, CA); Dr. A. Uri (Philadelphia, PA); Dr. K. Lay (Glendale, CA); L. Suslak and Dr. L-Y Shih (Newark, N.J.); Dr. R. Bachman (Oakland, CA); Dr. D. Flannery (Richmond, VA); Dr. P. Bowen (Alberta, Canada); Dr. J. Mann (San Jose, CA); Dr. L. Karp (Seattle, WA).

This work was supported, in part, by a USPHS-NIH program project grant 5 P01 HD22657-04.

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Discussion with Reviewers

E. Bonucci: Abnormalities of calcospherites might reflect abnormalities of MV [matrix vesicles]. Have you any information about numbers, morphology, and distribution of MV in SRP?

S.C. Marks: Can your data be correlated with anything known about matrix vesicles in the syndrome?

Authors: There are no previously published data on MV abnormalities in SRP. We have begun to collect transmission EM data on this but have not yet examined enough cases to formulate a sound description. This aspect also demands very well-preserved specimens, and not all of our autopsy cases are well suited for such studies.

S.C. Miller: Are there any clues concerning themolecular defect(s) or the gene loci for these disorders?

Authors: Type I/III SRP is quite different from Type II. It was only the shared features of short ribs and polydactyly that first caused them to be combined into one group. Their nomenclature should not suggest they share a common pathogenetic mechanism. To date, there are no molecular data on the etiology of these syndromes.

E. Bonnucci: Because structure and organization of the growth zone appear deeply altered in SRP type I/III, so that acellular cartilage abuts directly on osseous trabeculae, could the enlargement of calcospherites be due simply to the fact that they derive from bone in type I/III and from cartilage in type II?

Authors: The size difference we found cannot be due to differences in origin of the calcospherites since all calcospherites measured were in the calcifying cartilage site.