Craniosynostosis in vitamin D-resistant rickets

A mouse model

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Premature fusion of the coronal suture occurred in hypophosphatemic mice by 4 weeks of age. The proportion of the suture obliterated by bone varied among individual animals, but craniosynostosis was present in all animals studied at 4 weeks and older. Fusion of the coronal suture did not occur through 13 weeks of age in any of the normal mice studied. The X-linked hypophosphatemic mouse is an animal model that can be used to study the role of vitamin D-resistant rickets in the development of craniosynostosis, to relate craniosynostosis to the development of associated skull deformities, and to test new treatment procedures.

KEY WORDS: cranial suture, craniosynostosis, craniostenosis, vitamin D-resistant rickets, cranial suture
duces three different genotypes: hemizygous (Hyp/Y) males, heterozygous (Hyp/X) females, and homozygous (Hyp/Hyp) females. Iorio, et al.,$^{27}$ have quantitated the skull deformities in adult mice of the first two of these genotypes using craniometric techniques. The mutant mice have a short, wide, high neurocranium, which suggested an impairment of coronal suture growth; therefore, we compared histologically the postnatal development of the coronal sutural areas of hemizygous hypophosphatemic and normal male C57BL/6J mice. The hemizygous hypophosphatemic genotype was chosen because the mutant gene is consistently expressed as craniofacial anomalies in this genotype in both humans and mice. The term “sutural area,” as used here, includes the two adjacent bone edges as well as the intervening cellular and fibrous connective tissue of the suture per se.$^{41}$

The purposes of this paper are to describe the premature synostosis we found in male X-linked Hyp mice and to suggest some possible ways in which this new animal model can be used to answer questions concerning the development and treatment of craniosynostosis and associated skull deformities.

Materials and Methods

Mice of the C57BL/6J strain* were used. Normal males were bred from normal (+/X) mothers and normal (+/Y) fathers or from heterozygous hypophosphatemic (Hyp/X) mothers and normal (+/Y) fathers. Hemizygous hypophosphatemic males were bred from heterozygous (Hyp/X) females mated to normal (+/Y) males or from homozygous (Hyp/Hyp) females and hemizygous (Hyp/Y) males. The young mice were weighed weekly until sacrifice, tail length was measured, and samples of blood withdrawn.

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*Mice obtained from Jackson Laboratory, Bar Harbor, Maine.
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**Results**

**Basic Structure of Coronal Sutural Area**

The coronal sutural area of both mutant and normal mice was beveled along most of its length, beginning medially at the junction of the sagittal and coronal sutures (Fig. 1). In this part of the sutural area, the tapered anterior edge of the parietal bone overlapped the tapered posterior edge of the frontal bone (Fig. 2). Near its lateral end, the sutural area changed to a simple interdigitating structure with the tip of the parietal bone located between two processes of the frontal bone (Fig. 3). Distinct layers were not observed in the fibrous connective tissue of the developing suture, except in some sections from older animals where the fibrous connective tissue had partially separated from the adjacent bones. In these sections, three layers were formed, a central vascular layer of collagen fibers and fibroblasts bordered on each side by a peripheral layer of coarse Sharpey's fibers, fibroblasts, and osteogenic cells (Fig. 4).

**Hyp Sutural Area**

The sutural areas of normal and Hyp mice began to differ significantly by 3 weeks of age. The frontal and parietal bones of the Hyp mice consisted of a central area of immature bone sandwiched between two layers of mature bone (Fig. 6). The immature bone had an uneven, deeply basophilic, intercellular matrix surrounding numerous irregularly arranged osteocytes. The mature bone had fewer osteocytes and an evenly eosinophilic matrix. In some highly localized regions, thick collagen fiber bundles ran obliquely across the suture from bone to bone (Fig. 5). Fibroblasts were located on the surfaces of these fibers. In the surrounding parts of the suture, the...
collagen fibers and elongated fibroblasts were oriented parallel to the adjacent bone surfaces.

Four-week-old Hyp mice had a well defined synostosis, or bone fusion, of the frontal and parietal bones at the coronal suture (Fig. 6). In consecutive sections, the position of this synostosis within the suture varied. Sometimes it was located ectocranially at the anterior tip of the parietal bone, sometimes nearer the middle of the suture (Fig. 7). The number of consecutive 6-μ sections in which these sites of bone fusion characteristically appeared indicated that they were confined to approximately 80 to 100 μ of the total length of the suture. The bone making up the synostosis per se was of the mature type, with an even eosinophilic matrix and few osteocytes.

In animals 4 weeks and older, the size of these bone bridges, that is, the proportion of the suture obliterated by bone, varied among individual animals. In a few animals, the entire ectocranial half of the suture was obliterated by bone (Fig. 8). In other animals, the site of fusion remained small. These variations in the proportion of the suture obliterated by bone were real, in spite of minor differences in the plane of section among individual heads. No evidence of osteoclastic resorption of the bone bridges was found. The changes observed in the unfused part of the Hyp sutural area between 4 and 13 weeks of age were a thickening of the frontal and parietal bones and increased packing of fibers and connective tissue cells in the suture.

Sutural Area in Normal Mice

Obliquely oriented collagen fiber bundles and connective tissue cells were not present in normal animals except where the fibrous tissue of the suture had partially separated from bone during processing. Instead, collagen fibers and fibroblasts were oriented parallel to the adjacent bone surfaces. Neither synostosis nor any indication of impending synostosis of the coronal suture were observed in any normal mice up to 13 weeks, the oldest age group studied. Changes observed in normal animals with increasing age were thickening of the frontal and parietal bones and increased packing of cells and fibers in the suture.

Discussion

The present study histologically demonstrated premature synostosis of the coronal suture in 4-week-old male X-linked hypophosphatemic mice. This premature fusion of the coronal suture is associated with the development of a short, wide, high neurocranium by 13 weeks of age. The neurocranial width of Hyp animals reported by Iorio, et al., is not statistically different from normal animals of the same age, but this "normal" width occurs in the presence of a statistically significant shortening and increase in height of the neurocranium. Taken together, the relative increase in width, the decrease in length, and the increase in height of the neurocranium constitute oxycephaly.

Pathogenesis and Treatment of These Craniofacial Anomalies

The relationship of premature suture fusion to the development of associated craniofacial deformities, such as oxycephaly, is still unclear. Two different
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FIG. 8. More extensive fusion of the parietal (P) and frontal (F) bones in an 8-week-old Hyp animal. A smaller proportion of the suture remains unobliterated by bone (arrows). E = endocranial space. H & E, × 102.

Schools of thought exist as to the pathogenesis of these associated deformities. One school proposes that premature suture fusion directly causes a deformity of the cranial vault. The other school questions a direct causal relationship between early suture fusion and the subsequent development of a vault deformity. In the Hyp mice, the deformities of the neurocranium are predicted by the observations of Virchow that: 1) growth is inhibited at right angles to the prematurely synostosed suture; and 2) compensatory overgrowth of the cranium occurs at the sites of open sutures to accommodate brain growth. These results support the concept of a direct causal relationship between premature suture fusion and subsequent deformity of the cranial vault. The possibility that rickets is responsible for the cranial vault deformity through some mechanism other than the development of craniosynostosis, such as through a primary deformity of the cranial base, cannot presently be excluded. The Hyp mice can be used to study the relationship between prematurely fused sutures and the development of skull deformities in VDRR.

Confusion concerning the role of premature suture fusion in the development of cranial vault deformities has resulted in different opinions concerning the best treatment of craniosynostosis. Many neurosurgeons advocate an early linear craniectomy to release artificially fused sutures. Other neurosurgeons favor surgery at a young age only in cases where symptoms of neurological or ophthalmological involvement are present. These surgeons question whether early surgery for purely cosmetic reasons is justified, or even very effective. Postoperative studies following neurosurgical procedures for coronal suture synostosis in some instances lend support to the latter position. In a longitudinal cephalometric study, Kriegbork and Puzansky found no long-term improvement in the appearance of oxycephalic patients following surgery. In other studies, linear craniectomy of unilateral or bilateral coronal synostosis prevented subsequent cranial distortion in only 60% of the cases. The Hyp mice can be used as a model to study the effectiveness of a craniectomy in dealing with the cosmetic deformity associated with premature coronal synostosis in rickets. In addition, the rate and manner of bone healing at the craniectomy site can be studied to see if early refusion is a problem. Mice resemble humans in that adult cranial bones do not normally regenerate. For this reason, mice may be more suitable for experimental work in this area than cats, rabbits, or rats, in which the cranial bones do regenerate in young adults.

Events Preceding Craniosynostosis in VDRR

The role of VDRR in the development of craniosynostosis in humans is ambiguous. Of the patients with simple hypophosphatemic VDRR described by Reilly, approximately one-third were diagnosed as having craniosynostosis. This anomaly was seldom detected before 18 months of age. The late onset of craniosynostosis in these patients may be due to the fact that the reduced plasma phosphate level, which is the hallmark of the disease, often does not develop until 6 to 12 months after birth. Similarly, in the Hyp mice, craniosynostosis was detected relatively late, 4 weeks after birth. This development of premature suture fusion appeared to be correlated with a significant reduction in plasma phosphate levels during the 20- to 49-day age period.

The first indication of impending fusion of the coronal suture in the Hyp mice occurred at 3 weeks of age when transsutural fibers appeared: obliquely oriented dense collagen fiber bundles extending uninterrupted across the suture between the adjacent bones. Although the arrangement of collagen fibers varies even within the same suture, transsutural fibers are present only when growth is ceasing or has already ceased. The appearance of transsutural fibers may precede irregular bone formation and osteophyte production, such as Sitsen observed along the margins of cranial sutures in rachitic patients.
A synostosis is often already present by 3 weeks of age in the sagittal, mid-palatal, and interfrontal sutures of normal rats, but not in transversely oriented sutures. Transversely oriented sutures, such as the coronal and frontonasal, remain open to allow for differential growth in the development of a long, narrow skull. This same general growth pattern is normally present in the mouse. The premature closure of the coronal suture in the Hyp mouse apparently alters this pattern and results in cranial distortion in the form of a short, wide, high skull.

**Similarities of Humans and Mice with X-Linked Hypophosphatemia**

Although great care must be taken when attempting to extrapolate the results from animal studies to human patients, the X-linked hypophosphatemic mouse model appears to be useful because: 1) the primary defect in both affected humans and mice is increased renal excretion of phosphate due to decreased tubular reabsorption; and 2) many of the physical deformities secondary to hypophosphatemia, such as cranial vault deformities, retarded facial growth, and dental problems, are similar in affected humans and mice. Craniosynostosis can now be added to the list of similarities between human VDRR patients and Hyp mice. These similarities mean that the Hyp mice can be used in a number of ways to study the pathogenesis and treatment of craniosynostosis and associated craniofacial anomalies, particularly as they relate to VDRR.

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**References**

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