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Notochord and spinal malformations

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Fourteen spinal malformations were found in 328 spontaneously aborted human embryos and fetuses. Of these, 13 were investigated histologically and histomorphometrically and compared with 47 age- and stagematched normal specimens to document any possible relationship between spinal and notochordal malformations. Although six notochordal malformations, such as bifurcation, were found in 14 spinal malformations, the respective sites did not correspond. Abnormality of notochordal tissue – such as degeneration and delayed development, which was observed in nine of 14 spinal malformations – was not restricted to the site of the spinal malformation. Moreover, tissue changes responsible for spinal malformations usually did not occur in direct contact with the notochord. On the other hand, malformed notochords were found in normal specimens. We therefore were unable to confirm in human specimens that notochordal abnormalities induce spinal malformations, a fact well established in lower vertebrates.

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The pathogenesis of congenital vertebral malformations in humans remains unclear because pertinent observations during the early embryonic stages are rare (Tanaka & Uhthoff 1981a, Tanaka & Uhthoff 1981b). Results of experimentally induced malformations in lower vertebrates have been used to elucidate the pathogenesis (Nogami & Ingalls 1967, Theiler et al. 1975, Watterson et al. 1954) and an inductive role has been attributed to the notochord (Bancroft & Bellairs 1976, Cooper 1956, Ruggeri 1972). Based on observations made in older human fetuses and neonates, Shapiro & Eyre (1981) hypothesized that congenital scoliosis is induced by a malformed notochord. Our observations of the human notochord and its effects on normal spinal development (Goto & Uhthoff 1986) did not lend support to their hypothesis. We investigated the state of the notochord in cases of malformation of the anterior spinal column.

Materials and methods

In 328 spontaneously aborted human embryos and fetuses (crown-rump length 7 to 490 mm, estimated age of gestation according to Patten (1968) 5 weeks to full-term) 14 instances of spinal malformations were found. Special attention was paid to 13 specimens (age of gestation 5½ to 13 weeks) because their age permitted us to classify them according to their notochordal development (Goto & Uhthoff 1986). They were compared with 47 age-matched normal specimens. The preparation of specimens has been published previously (Goto & Uhthoff 1986). The development age was determined by the mean segment height of the fourth to eighth thoracic vertebra (S. H.: distance between the middle of adjacent intervertebral discs).

The development of the notochord and its surrounding tissue at the site of spinal malformation was compared with that at non-malformed sites, as well as with age-matched normal specimens.

Besides determining the mean S. H., two measurements were made in serial sections of all specimens showing a continuous notochordal sheath (Stages 2 to 5) using a Zeiss microscopic ruler: 1. Diameter of the notochordal sheath (outer wall) at the

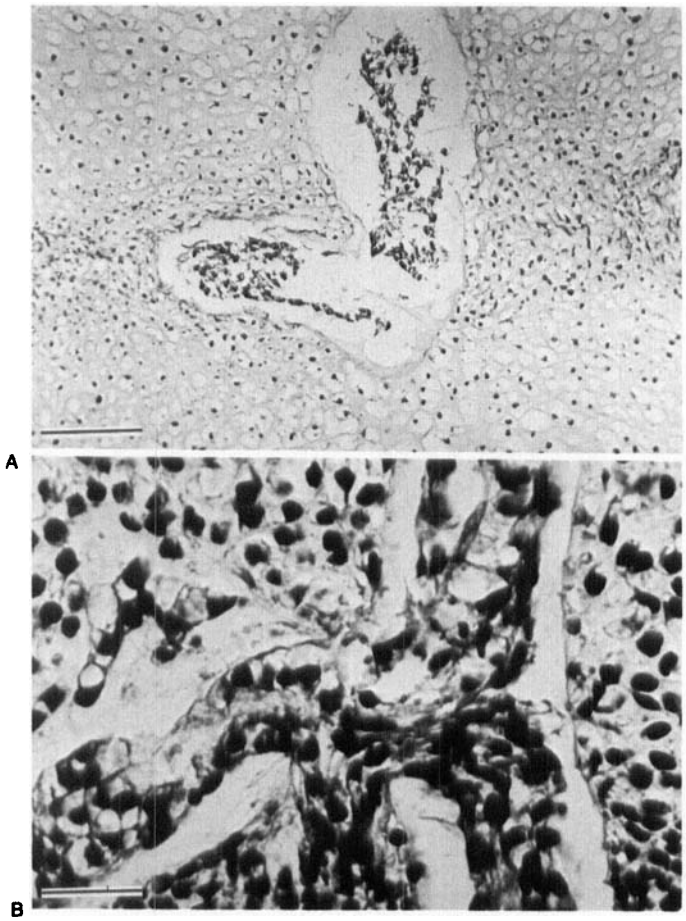

Figure 1. Photomicrographs of bifurcated notochords at non-malformed site in a malformed case, A, Case 11, mean S. H. 866 μ, estimated age 8½ weeks, Stage 5, frontal section (scale 100 μ), and in a normal case, B, mean S. H. 272 μ age 6 weeks, Stage 2, frontal section (Mann Dominici, scale 25 μ).

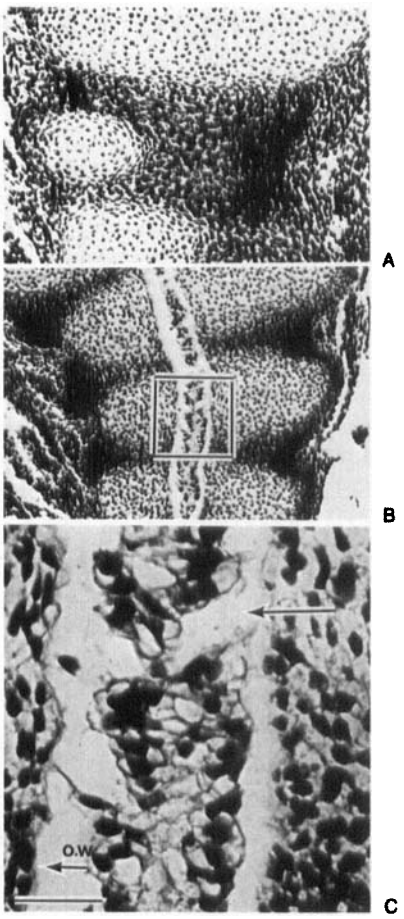

Figure 2. Photomicrographs of a malformation in Stage 2, Case 4, mean S. H. 350 μ, age 6½ weeks, frontal section. Mixed type at the sacrococcygeal level. A and B, identical level but at different depths. C, notochordal cells with cytoplasmic vacuoles, partly absent inner wall (large arrow) and clear outer wall (o.w.) of the sheath similar to normal notochordal tissue of this stage.
(A, B. Goldner's trichrome, scale 100 μ, C. Goldner's trichrome, scale 25 μ).

disc level. 2. Maximum width of the area occupied by small hypertrophic cells around the notochord at the disc level.

Results

The estimated ages of 14 malformed specimens ranged from 5½ to 16 weeks, and their mean S. H. were between 151 and 2140 micrometers. Out of 328 specimens, 219 were less than 16 weeks old. Therefore, in our material the incidence of malformation during the embryonic and early fetal periods amounted to 6.4 per cent. Their distribution according to notochordal stages was as follows: Stage 1, no malformation seen; Stage 2, four malformations; Stage 3, three malformations; Stage 4, three malformations; Stage 5, three malformations. The remaining malformed specimen was 16 weeks old. Mean S. H. of each malformed specimen at the non-malformed T4 to T8 sites fitted into the range of normal specimens. However, when comparing the measurements for S. H. over the entire non-malformed part of the

spine, a greater variation was found for malformed specimens than for normal specimens. The sites of malformations were distributed over the entire extent of the spine. In addition five specimens (Cases 3, 8, 9, 12, and 13) showed malformations at two independent sites for a total 19 malformed sites. Eleven of them involved the thoracic spine.

Of the 14 malformed specimens six showed a bifurcated notochord (Figure 1a). All of them (Cases 7, 8, 9, 11, 12, and 13) were situated at non-malformed sites separated from the site of malformation at least by three levels. In one normal specimen a bifurcation was seen (Figure 1b, see also Figure 13 in Tanaka & Uhthoff 1981b). Here the notochordal tissue development was absolutely normal. In five malformed specimens, serial sections revealed absence of notochordal tissue at the malformed sites. However, its absence extended into the non-malformed sites. Enlargement of the diameter of the notochord at the disk level was delayed in every case of Stages 3 and 4, not only at the malformed sites, but also in four out of six cases at the non-malformed sites. An unusually large number of small hypertrophic cells surrounded the notochord at the disc level in almost every malformation, particularly at the malformed sites.

Two cases of malformation are shown to document our findings. Case 4 (stage 2) (Figure 2a, b, c), mean S. H. 350 μ, age 6½ weeks, mixed failure at sacrococcygeal region (Figure 2a). This case shows a notochordal development at the malformed site (Figure 2b and c) identical to that of normal specimens, consisting of notochordal cells with cytoplasmic vacuoles and partly absent inner wall at both the malformed and the non-malformed sites. Cartilaginous and mesenchymal tissue are clearly demarcated as in normal specimens.

Case 13 (Stage 5), mean S. H. 1088 μ, 12 weeks, failure of segmentation at T3 to T5 and mixed failure at the L4 to sacrum level with fused hemivertebra (Figure 3). At the non-malformed sites, a bifurcated mucous streak in the intervertebral body is present, whereas in some of the malformed vertebral bodies the mucous streak shows signs of dispersion. As seen in Figure 3, the notochord at the malformed lumbosacral site is obvious only where a fibrous disc space is present between the hemivertebrae. Also a shift in its location is seen. However, the notochordal tissue at this site is in the same stage of development as that at non-malformed sites. No abnormality of the sclerotomic tissue is obvious other than narrowing of the disc space and occasional hazy demarcation between vertebral body and disc at the malformed site.

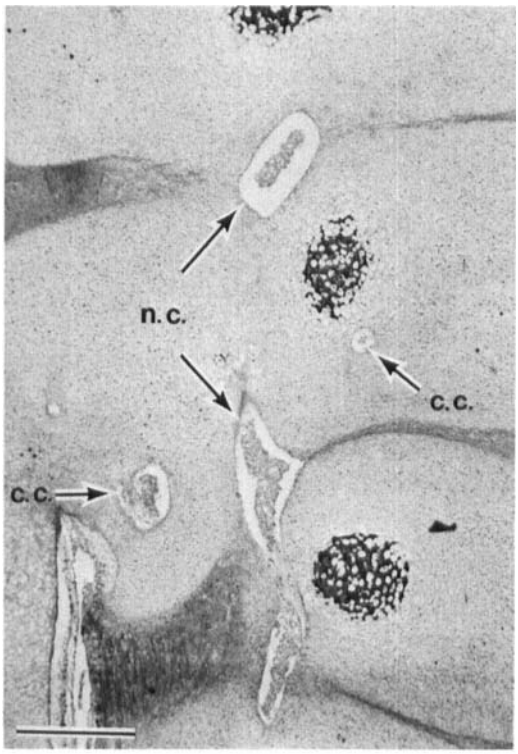

Figure 3. Photomicrograph of case 13, mean S. H. 1088 μ, age 12 weeks, Stage 5, frontal section. The presence of the notochord is limited to the level of the fibrous disks between hemivertebrae. Note the shift of the notochord and thus a displacement of the nucleus pulposus. (Scale 500 μ) n.c., notochord. c.c., cartilage canal. Goldner's trichrome.

Discussion

In his study of spinal development in humans, Sensenig (1949) described three developmental periods; namely, a membrane formation period, a cartilage formation period, and a bone formation period. Further research has confirmed an additional short phase of resegmentation situated between the membrane and

cartilage formation periods (Remak 1855) or critical segmentation (Winter 1973, Tanaka & Uhthoff 1981a) lasting from the 5th to the 6th week. In our staging of notochordal development, early chondrification and subsequent fibrous tissue formation correspond to stages 1 and 2. This early chondrification phase has been considered to be the most critical for the genesis of malformations (Winter 1973, Tanaka & Uhthoff 1981a), and the current study lends further support to this assumption.

The pathogenesis of malformations remains obscure, although induction by neighboring tissues, such as the notochord, has been strongly suspected. Tissue induction as a prerequisite to its differentiation has always been an important concept in embryology. Based on observations in lower vertebrates, induction of mesodermal cells to differentiate into various tissues composing the anterior part of the spine has been attributed to the notochord (Cooper 1965, Bancroft & Bellairs 1976, Ruggeri 1972). Consequently, notochordal abnormalities were thought to be responsible for the initiation of malformations in humans (Feller & Sternberg 1930, Shapiro & Eyre 1981).

However, our observations in normal embryos make the inductive capacity of the notochord (Goto & Uhthoff 1986) questionable. The current study lends further support to our hypothesis that in humans the notochord induces neither spinal development nor malformations. Various notochordal abnormalities, such as bifurcation, delayed development, marked regressive cellular changes, or probable absence of the notochord, were usually seen at sites free of malformation in malformed specimens and were even seen in normal specimens.

Moreover, 11 spinal malformations were observed away from the notochord and only three in close proximity to it. Therefore, the fact that malformations occur more frequently at sites away from the notochord than directly around it, speaks against the assumption that the notochord induces spinal malformations.

The only possible evidence of an inductive role of the notochord on the surrounding tissues in our malformed specimens was the wide unsegmented distribution of small hypertrophic cells around the entire notochord corresponding to area A (Goto & Uhthoff 1986). Its significance remains to be elucidated.

Thus, should the human notochord play an inductive role in spinal malformations, notochordal abnormalities must be found close to them. However, the contrary was observed in our material. When present, sites of notochordal abnormalities were usually separated from the sites of spinal malformation by normally developing tissue. However, we want to stress that this is a morphologic study. Consequently, our conclusions as to the function of the notochord are solely based on these observations.

Another interesting point that our study has shown is that the extent of malformation is much greater than a cursory observation may indicate. Measurements of the S. H. in malformed specimens showed a much wider spread in values over the entire column than was seen in normal specimens.

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