Pathogenesis of preaxial polydactyly of the hand in human embryos

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SUMMARY

Hand plates with preaxial polydactyly from 13 human embryos of the Carnegie stages 17–23 were examined macro- and microscopically. Morphological features in early pathogenesis of preaxial polydactyly are (1) an abnormal extension and a delayed involution of the apical ectodermal ridge on the preaxial border of the hand plate in stages 17 and 18, (2) a precocious development of an interdigital notch between the duplicated thumbs in stages 17 and 18, and (3) bifurcation of the distal part of the first digital ray in stage 19. A disorder of the interaction between limb ectoderm and mesoderm is considered to be the pathogenetic event.

INTRODUCTION

Polydactyly is one of the common malformations in man and experimental animals. It is a heterogeneous anomaly occurring either on the preaxial or postaxial part of the hands or feet. Incidence and type of polydactyly have been found to vary between different racial groups (Jones, 1971). In the Japanese, preaxial polydactyly predominates over postaxial (Kobayashi, 1942).

The pathogenesis of preaxial polydactyly in experimental animals has been studied both in genetic polydactyly (Grüneberg, 1963; Zwilling, 1969) and teratogen-induced polydactyly (Kanamori, 1958; Kameyama, Hayashi & Hoshino, 1973), whereas little has been reported on the pathogenesis of preaxial polydactyly in man. Lineback (1921) described a case of preaxial polydactyly in a 22 mm human embryo. However, the 22 mm stage is not early enough for observations of the initial morphological changes in the formation of polydactyly.

A large number of human embryos, both normal and abnormal, have been collected in the Department of Anatomy, Faculty of Medicine, Kyoto University since 1961 (Nishimura et al. 1966; Nishimura, Takano, Tanimura & Yasuda, 1968). Among embryos at the stage of digital formation and beyond, cases of preaxial polydactyly of the hand have been found at a rate of about 4 per 1000 (Yasuda & Uwabe, 1967). These polydactyloous embryos have provided a

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unique opportunity to study early processes involved in the formation of preaxial polydactyly in man. This paper is concerned primarily with macro- and microscopic observations on hand plates with preaxial polydactyly from 13 human embryos of the Carnegie stages 17–23.

**MATERIALS AND METHODS**

The 13 embryos were from the collection of the Human Embryo Centre at the Department of Anatomy, Faculty of Medicine, Kyoto University (Director: Prof. H. Nishimura). Details of the collection have been described elsewhere (Nishimura et al. 1966, 1968). The specimens were fixed in Bouin’s fluid for a day and transferred to 10% formalin for storage. The specimens were staged according to Streeter’s criteria (1948, 1951) and examined for possible external malformations under a binocular dissecting microscope. Hand plates exhibiting preaxial polydactyly were removed from the trunk and photographed. They were then dehydrated, embedded in paraffin by standard methods, serially sectioned at 10 μm in a palmar plane, and stained either with hematoxylin and eosin, or by Mallory’s azan staining.

A human hand plate first exhibits digital rays at stage 17. At this stage an early indication of preaxial polydactyly can be detected with certainty. Hence only embryos of stages 17 and over, which were of good histological quality, were included in the present study, although there were several specimens of stage 16.

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**Table 1. Embryos with preaxial polydactyly (stages 17–23)**

<table>
<thead>
<tr>
<th>Specimen no.</th>
<th>Stage</th>
<th>CRL* (mm)</th>
<th>Affected side†</th>
<th>Associated malformations</th>
</tr>
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<tbody>
<tr>
<td>9928</td>
<td>17</td>
<td>—</td>
<td>r</td>
<td>—</td>
</tr>
<tr>
<td>14742</td>
<td>17</td>
<td>8·2†</td>
<td>rl</td>
<td>Holoprosencephaly</td>
</tr>
<tr>
<td>19347</td>
<td>17</td>
<td>10·8</td>
<td>l</td>
<td>Holoprosencephaly</td>
</tr>
<tr>
<td>519</td>
<td>18</td>
<td>12·6†</td>
<td>l</td>
<td>—</td>
</tr>
<tr>
<td>3416</td>
<td>18</td>
<td>—</td>
<td>rl</td>
<td>—</td>
</tr>
<tr>
<td>3488</td>
<td>18</td>
<td>—</td>
<td>r</td>
<td>—</td>
</tr>
<tr>
<td>9054</td>
<td>18</td>
<td>—</td>
<td>r</td>
<td>r Cleft foot</td>
</tr>
<tr>
<td>9403</td>
<td>18</td>
<td>—</td>
<td>r</td>
<td>—</td>
</tr>
<tr>
<td>9953</td>
<td>18</td>
<td>10·8†</td>
<td>rl</td>
<td>Holoprosencephaly</td>
</tr>
<tr>
<td>7220</td>
<td>19</td>
<td>—</td>
<td>rl</td>
<td>Horseshoe kidney</td>
</tr>
<tr>
<td>475</td>
<td>20</td>
<td>—</td>
<td>r</td>
<td>—</td>
</tr>
<tr>
<td>15357</td>
<td>20</td>
<td>19·7</td>
<td>l</td>
<td>—</td>
</tr>
<tr>
<td>8003</td>
<td>23</td>
<td>23·9†</td>
<td>l</td>
<td>—</td>
</tr>
</tbody>
</table>

* Crown rump length measured after fixation of undamaged embryos.
† CRL of these embryos is smaller than the standard given by Streeter (1948, 1951). However, re-examination of morphological features of these embryos verified the correctness of the staging.
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† r, right; l, left; rl, bilateral.
Preaxial polydactyly in human embryos

Fig. 1. A dorsal view of a normal hand plate at stage 17.
Fig. 2. A dorsal view of hand plates of no. 9928. Preaxial polydactyly on the right.
Fig. 3. A section of the right hand plate of no. 9928.
Figs. 4–6. Serial sections through the proximal and distal protrusions with epidermal thickenings (arrows). The two thickenings are not continuous.
Fig. 7. Structure of the epidermal thickening.

with probable preaxial polydactyly. Hand plates from staged embryos without external malformation were used as normal controls.

The term ‘stage’ is preferably used in this report instead of Streeter’s ‘horizon’ according to the suggestion by O’Rahilly (1972).

OBSERVATIONS

Table 1 presents a summary of the 13 embryos with preaxial polydactyly. Macro- and microscopic findings at each stage are given below. In the following
description, the proximal thumb (protrusion) means the most radial finger, and the distal thumb (protrusion) means the finger between the most radial finger and the index finger. The second, third, fourth and fifth fingers denote the index, middle, ring and little fingers, respectively.

**Stage 17**

Normally the preaxial margin of a hand plate at this stage has a smoothly rounded contour (Fig. 1). In the specimens diagnosed as preaxial polydactyly, the preaxial margin was undulated by two protrusions separated by a shallow notch (Fig. 2). There were slight individual differences in the comparative size of the proximal and distal protrusions, and in the distance between the two protrusions. On the right hand of no. 9928, which was at the end of this stage, the two protrusions were almost equal in size, 0.25 mm apart, and separated by a definite notch of 0.05 mm deep (Fig. 2). The left hand of no. 14742 showed a shape similar to that of no. 9928 except for a small extra protrusion on the proximal part of the proximal thumb. The protrusions on the right hand of no. 14742 were 0.4 mm apart, and the notch between them was not apparent. The left hand of no. 19347 had two protrusions separated 0.35 mm by a shallow notch. The proximal protrusion was larger than the distal one.

Microscopic examination revealed a conspicuous epidermal thickening at the tip of these protrusions (Fig. 3). As in a normal hand plate at this stage, a band of thickened epidermis, the apical ectodermal ridge (a.e.r.) still remained along the apical rim of these polydactylous hand plates. Peculiarily, however, the a.e.r. grew thicker at its preaxial-proximal end, forming the distal protrusion (Fig. 4). On the more proximal rim, there was another epidermal thickening (Fig. 6), which was not continuous with the above mentioned a.e.r.

The epidermal thickening at the tip of the protrusion showed typical a.e.r. structure. It consisted of a few layers of cuboidal to columnar cells with oval nuclei and darkly staining basophilic cytoplasm, and was covered by a layer of flat peridermal cells (Fig. 7). The entire epithelium was about 35 μm at its thickest part. Pyknosis was occasionally found in the a.e.r. Epidermis covering the notch between the protrusions consisted of two layers of cells, i.e. a flat peridermal layer and a basal layer of cuboidal cells with round nuclei and lightly staining cytoplasm. These features of the epidermis covering the precociously formed notch were comparable to those of the epidermis covering interdigital notches developing normally during stage 18 and after.

Mesenchyme in the hand plates showed condensations for finger rays. In no. 14742, which was younger than the other two, only three rays for the fifth, fourth and third fingers were recognizable. No. 9928 and no. 19347 showed five digital rays, but the mesenchymal condensation for the thumb was rather vague. In the thumb area no definite difference was noticed between the mesenchyme just beneath the a.e.r. and that covered by the thinned epidermis.

The marginal vein was observed along the rim of the hand plate as in a normal
Preaxial polydactyly in human embryos

Fig. 8. A dorsal view of a normal hand plate at stage 18.

Fig. 9. A dorsal view of hand plates of no. 9953. Bilateral preaxial polydactyly.

Fig. 10. A section of the right hand plate of no. 9953.

Figs. 11–12. Sections through the proximal and distal protrusions with epidermal thickenings (arrows).

Fig. 13. Structure of the epidermal thickening. Pyknotic cells are seen in the thickening.

hand plate. No appreciable change in the pattern of the marginal vein was detected in the preaxial region beneath the abnormal epidermal thickenings.

**Stage 18**

In normal development the formation of interdigital notches begins at this stage (Fig. 8). On polydactylous hand plates the tip of the first digit was usually bifurcated by a more conspicuous notch than the notches between the normally developing fingers (Fig. 9). Differences were noted both between individuals and
between the two hands of one individual in the comparative size of the proximal and distal thumbs, in the distance between the two thumbs, and in the depth of the extra interdigital notch. In no. 3416 the left extra interdigital notch was more developed than the right.

Examination of histological sections demonstrated the abnormal epidermal thickenings at the tip of the bifurcated thumb in no. 9054 and no. 9953 (Fig. 10). Normally the a.e.r. disappears in hands by this stage. There was no conspicuous epidermal thickening at the tip of the second to fifth fingers in these two specimens. Examination of serial sections showed that the epidermal thickenings were isolated structures limited to the tip of the bifurcated thumb (Figs. 11 and 12). In the thickened epidermis pyknotic nuclei were abundant, and cells in mitosis were seldom seen. No obvious remnant of the a.e.r. was detected in the other four specimens, though epidermis covering the tip of the proximal and distal thumbs was characterized by basal cells with oval nuclei and darkly staining cytoplasm. Features of the epidermis covering the extra notch were similar to those observed in the previous stage.

In the mesenchyme there were five definite digital rays in all of the hand plates examined. The long axis of the first digital ray pointed to the tip of the distal thumb without exception. No bifurcation was seen in the distal portion of the first digital ray.

**Stage 19**

The external configuration of a normal hand plate at this stage is characterized by deepening of the interdigital notches. The angles formed between opposite borders of neighbouring fingers, however, are still more than 90° (Fig. 14). On both hand plates of no. 7220, there were proximal and distal thumbs separated by a conspicuous interdigital notch (Fig. 15). On the right the proximal thumb was larger than the distal one, while the situation was reversed on the left. The extra notch on the right hand was deeper than that on the left.

Histological examination showed no conspicuous epidermal thickening in either hand (Figs. 16, 17 and 18). Nevertheless, part of the epidermis covering the tip of the proximal thumb of both hands had a basal layer of cells with oval nuclei and darkly staining cytoplasm (Figs. 18 and 19), which were characteristics of cells in the epidermal thickening.

In the mesodermal components, all five metacarpals and the second to fifth proximal phalanges were found to have started chondrification. Bifurcation of the first digital ray was clearly demonstrated on the right hand and each of the distal and proximal thumbs had a definite blastemal condensation (Figs. 16, 17 and 18). On the left hand, a dense mesenchymal condensation was observed in the distal thumb, but there was no obvious blastemal condensation in the proximal thumb.
Fig. 14. A dorsal view of a normal hand plate at stage 19.

Fig. 15. A dorsal view of hand plates of no. 7220. Bilateral preaxial polydactyly.

Fig. 16. A section of the right hand plate of no. 7220.

Fig. 17. A section through the tip of the distal thumb.

Fig. 18. A section through the tip of the proximal thumb. A part of the epidermis is darkly stained (arrow).

Fig. 19. Structure of the darkly stained epidermis.

**Stage 20**

A normal hand plate at this stage has deep interdigital notches which reach the level of the middle phalanx. The angles formed between opposite borders of neighbouring fingers are acute (Fig. 20). On the right hand of no. 475 and on the left hand of no. 15357 the proximal thumb was almost equal in size to the distal thumb, and was attached perpendicularly to the root of the distal thumb (Fig. 21).
Fig. 20. A dorsal view of a normal hand plate at stage 20.
Fig. 21. A dorsal view of hand plates of no. 15357. Preaxial polydactyly on the left.
Fig. 22. A section of the left hand plate of no. 15357.
Fig. 23. A section through the distal and proximal thumbs.
Fig. 24. A section through the proximal thumb.

Microscopically there was no evidence of epidermal thickening or of a darkly stained basal layer at the tip of the proximal and distal thumbs (Figs. 22, 23 and 24). Chondrification of the skeletal elements was advanced as compared with the previous stage. In no. 475 the first metacarpus was widened at the distal end where the proximal phalanges of the duplicated thumbs were articulated. In no. 15357 the first metacarpus was not enlarged, and the proximal phalanx of the proximal thumb was in contact with the base of the proximal phalanx of the distal thumb (Figs. 22 and 23). The proximal phalanges of both thumbs were chondrifying.
Preaxial polydactyly in human embryos

Stage 23

Normally separation of the fingers by interdigital notches has been completed by this stage and phalanges in each finger are identifiable externally (Fig. 25). On the left hand of no. 8003 the proximal thumb, which was about half the size of the distal thumb both in length and diameter, was attached to the distal thumb at the interphalangeal joint (Fig. 26).

Histological examination revealed no epidermal abnormality. The single cartilaginous phalanx in the proximal thumb was articulated to the radial side of the distal end of the first proximal phalanx (Figs. 27 and 28).
DISCUSSION

My observations show the morphological features of the early pathogenesis of preaxial polydactyly of the hand in man to be (1) an abnormal extension and a delayed involution of the a.e.r. on the preaxial border of the hand plate in stages 17 and 18, (2) a precocious development of an interdigital notch between the duplicated thumbs in stages 17 and 18, and (3) bifurcation of the distal part of the first digital ray in stage 19.

In human upper limbs the a.e.r. first appears in stage 12 and disappears in stage 17 (O'Rahilly, Gardner & Gray, 1956; Swinyard & Pinner, 1969). The two protrusions observed in the preaxial region of the polydactylous hands at stage 17 suggest an excessive growth of the a.e.r. and an abnormal extension of its preaxial-proximal end. The a.e.r. observed in two of the polydactylous specimens of stage 18 demonstrates involution has been delayed.

The importance of the a.e.r. in limb morphogenesis has been well documented (Saunders, 1969). Naujoks (1963) and Tondury (1969) have suggested that aberrations of the a.e.r. possibly lead to limb deformities in man. However, there has been no observation in man directly supporting these suggestions. The present report is the first description of the association between an a.e.r. abnormality and a specific limb deformity in man.

The development of polydactyly following aberrations of the a.e.r. has been reported in experimental animals. Some polydactylous mutants in the chick have, as part of the sequence of events that lead to accessory distal elements in the wing, a stage in which the a.e.r. is more extensive than normal in a preaxial direction (Zwilling, 1969). In mice 5-fluorouracil induces preaxial polydactyly, of which the first detectable changes are preaxial spacing of the a.e.r. and its delayed involution in foot plates (Kameyama et al. 1973). These authors have attributed the fundamental process of formation of preaxial polydactyly to a disorder of the usual interactions between limb ectoderm and mesoderm. It is reasonable to assume that the same disorder takes part in the pathogenesis of preaxial polydactyly of the hand in man, because the aberrations of the a.e.r. are common between man and experimental animals, although the observations in mice were made on the hind limb. This assumption enables us to extrapolate results obtained in experimental animals to man.

The precocious formation of an interdigital notch between the proximal and distal thumbs observed in this study is not considered as the primary event leading to preaxial polydactyly, because there are types of preaxial polydactyly with duplication of the distal elements of the first digit without formation of an interdigital notch. The precocious notch formation may however provide some information about the mechanism involved in finger morphogenesis. Formation of interdigital spaces has been ascribed to a regional degeneration of mesenchyme (Milaire, 1965). Cell migration from interdigital to digital zones may play a role in the development of a digit (Kelley, 1970). In normal development interdigital
Preaxial polydactyly in human embryos

Epithelium invaginates into interdigital zones where the population of cells is sparse after the establishment of digital rays. Kelley (1973) found numerous cytoplasmic microfilaments oriented along the apical surface of the interdigital epithelium of human embryos in stages 17 and 18, and he suggested that interdigital epithelium might actively invaginate into zones of necrosis. In the process of formation of preaxial polydactyly in man the interdigital invagination of epithelium occurs prior to establishment of the digital ray in the presumptive thumb area. This finding supports the active invagination hypothesis of interdigital epithelium, and suggests that necrosis between the adjacent finger rays is not a prerequisite for the active invagination of epithelium. The relationship between the delayed involution of the a.e.r. and the precocious formation of the interdigital notch is unknown.

The bifurcation of the distal part of the first digital ray observed in stage 19 may be secondary to the differentiation and condensation of mesenchymal cells under the influence of the abnormal a.e.r.

There were some differences in the shape of the polydactylous hand plates between individuals and between the two hands of one individual. These differences in the early stage of limb morphogenesis are considered to lead to the variability of preaxial polydactyly in completed hands (Jones, 1971). The mass of the a.e.r. at the tip of the preaxial protrusions observed in stage 17 and the distance between the two protrusions may be the determinants of the type of polydactyly in the later stage.

The present observations indicate a possibility of detection of cases of preaxial polydactyly before stage 17 by looking for the preaxial protrusions along the border of hand plates. Observations of probable polydactylous hand plates at stage 16 are in progress to provide further information on the pathogenesis of preaxial polydactyly in man.

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REFERENCES


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