Acta Genet Med Gemellol 36:433-436(1987)

The Possible Role of Homeotic Genes in the Causation of Malformations in Monozygotic Twins

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Abstract. It is proposed that the malformations observed to occur with increased frequency in monozygotic twins are similar to the types of malformations caused by mutation in homeotic genes in animals.

Key words: Twins, Malformations, Homeotic genes, Animal models

Dysmorphologists and gemellologists for many years have found the study of congenital malformations in twins to be a very fruitful endeavor. It is the purpose of this paper to propose a new, testable hypothesis for some of the malformations observed to occur with increased frequency among monozygotic (MZ) twins.

The most recent comprehensive review of malformations in twins was that of Schinzel and Smith [5]. The paper reported their personal observations of malformations found to occur more frequently among MZ twins tham among singletons, and reviewed the considerable literature on the subject. Table 1 is a summary of their paper. It is notable that the defects occur early in embryonic development. It is also notable that in the majority of cases, the twins are discordant for the malformation; for example, Nance [3] estimated the concordance rate for neural tube defects within MZ twin pairs to be 6%.

Few hypotheses have been proposed to explain these observations. For the most part, authors have postulated that whatever causes twinning also causes the malformations, or that somehow the process of twinning itself leads to the malformation. Nance's proposal that a heritable cytoplasmic abnormality contributes to neural tube defects in MZ twins

Table 1. Malformations which occur with increased frequency in monozygotic twins*

Sirenomelia
Sacrococcygeal teratoma
Exstrophy of the cloaca
Neural tube defects
VATER association
Holoprosencephaly
Renal agenesis
Anal atresia
TE fistula

* Adapted from Schinzel and Smith [5].

was one of the few concrete hypotheses offered [3].

Recent discoveries about the genetic control of embryonic development provide new ideas and techniques to approach the questions about malformations in twins. Work with the nematode *C. elegans* [1] and with *Drosophila* [2] has shed much light on the mysteries of early development. Such studies have been able to identify the molecular structure of genes involved in regulating pathways in embryonic development. One characteristic of these genes, across species (including man), has been the presence of a conserved gene sequence termed the "homeo-box" [2]. Many homeotic genes have been sequenced, and their polypeptide products identified.

The functions of these controlling, homeotic, genes have been deduced from studies of the effects of mutations in these genes (Table 2). Some mutations seen in *C. elegans* are heterochronic, and alter the timing of initiation of cells proceeding down a particular developmental pathway, leading to delayed completion of the pathway, or failure of completion of the pathway, which can have far-reaching of local consequences [1]. Other heterochronic mutations can result in premature termination of a developmental pathway. Abnormal segmentation of the embryo, as seen in the abnormally shortened ftz mutation in *Drosophila,* can occur [4]. The most spectacular mutations results in the development of a body structure in the wrong location; antennapedia mutants in *Drosophila* are examples of this [2].

It appears that homeotic genes influence development by controling the transcription of specific genes. The proteins encoded by homeo-boxes are basic and appear to bind to DNA [2]. These proteins may turn genes on or off by binding to the DNA. It therefore appears that there must be some equilibrium relationship between the quantity of homeo-box-derived proteins in the cell and the nuclear DNA which determines if genes are turned on. It is not yet known if homeotic gene products are active only in the cells in which homeotic genes are turned on, or if homeo-box products diffuse from cell to cell, or if there are other genes which control homeotic genes. It is known that homeotic genes are active at the pregastrulation stage in *Drosophila* genes [4].

Many of the defects observed in MZ twins can be thought of as occurring as a results of processes similar to those seen in heterochronic and homeotic mutants in animal

Table 2. Characteristics of reported heterochronic and homeotic mutations in animals

Abnormal segmentation
Premature completion of a developmental pathway
Delayed completion of a developmental pathway
Development of a body structure in the wrong location

models (Table 3). Anencephaly and myelomeningocele can easily be explained as failure to complete a developmental pathway in time, either because of delay in starting, or because of retarded cell proliferation. Holoprosencephaly can be explained as premature termination of a developmental pathway. Sirenomelia is difficult to classify; conceivably it could result from failure of segmentation, or from premature termination of programmed cell death. Klippel-Feil malformation and the VATER association seem to be segmentation defects.

Previous speculation that malformations in MZ twins were causally related to the process of twinning take on a new attractiveness in light of new knowledge about homeotic genes. It seems not inconceivable that fission of the zygote can lead to unequal distribution of cytoplasmically transmissable compounds such as homeo-box-derived proteins. Maldistribution of these essential controlling compounds could then upset the regulatory balance between these proteins and the DNA in individual cells. In some instance, more homeo-box derivatives will be resynthesized in time to restore the balance before important developmental pathway need to be turned on, while in other instance the lag time will result in failure to begin or end a particular developmental task.

Techniques have been developed for identifying homeotic gene activity and the presence of homeo-box-derived products *in situ* in animal embryos. Regions of active translation of homeotic genes in the embryo can be identified with labelled cDNA probes which hybridize to the mRNA [2]. Since the homeo-box-derived proteins have been identified, it should be possible to identify locations in embryos where specific control proteins are present with monoclonal antibodies and to qualitatively estimate their concentration by Western blotting. With the availability of in vitro fertilization, it should

Table 3. Types of early developmental defects seen with increased incidence among MZ twins

Failure to complete a pathway
Anencephaly
Myelomeningocele
Early completion of a pathway
Sirenomelia
Holoprosencephaly
Segmentation abnormality
Klippel-Feil
VATER association

not be too complicated to manipulate animal zygotes, separating cell masses at various stages of early organization and determining how homogeneously a particular homeotic protein is distributed between the resultant half zygotes, or blastocysts. Development of the half embryos would be observed in order to determine if inequal distribution of the particular homeotic protein produces abnormal structural development.

This hypothesis is not offered as the "unified field theory" to explain all malformation in twins. It is offered as a new area for investigation. It is apparent that many other factors operate in defects in MZ twins, such as porencephaly and gastroschisis [5].

Acknowledgements: The author thanks Flora McClure for her capable assistance in the preparation of this manuscript.

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