Monozygotic Twinning

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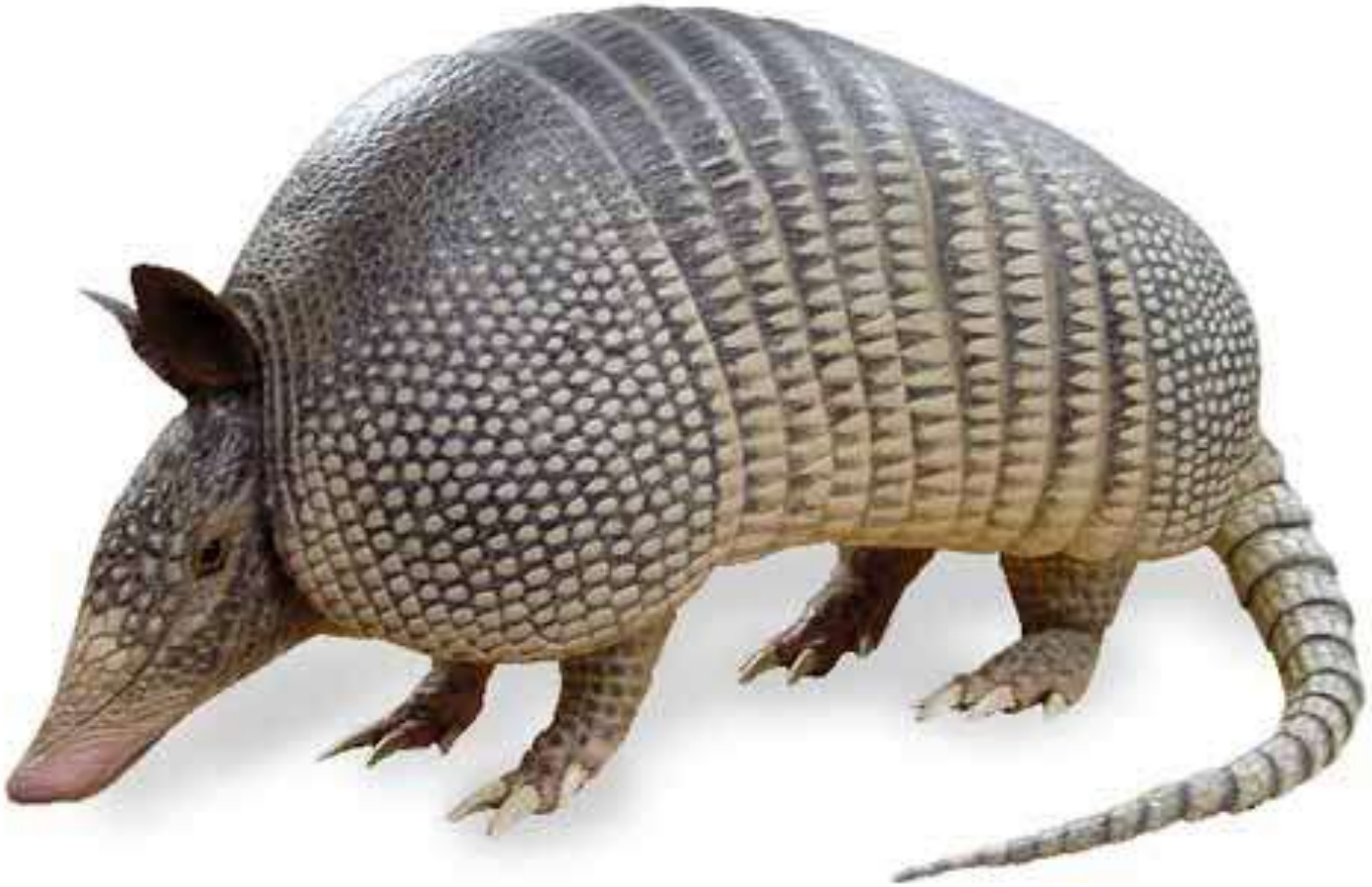
Zygosity

Zygosity refers to whether twins are:

- Monozygotic twins (MZ): arise by the fertilization of one ovum by one sperm to give one zygote , that later divides to form 2 or more embryos
- Dizygotic twins (DZ): arise by the fertilization of 2 different ova by 2 different sperms to give two embryos

No naturally occurring animal models for MZ twinning except in Armadillos



Incidence of twinning

- General incidence of both DZ and MZ spontaneous twinning is about 1 in 80 live births.
- Highest incidence is among Black Africans (40/1000 live births), lowest in Asia (6/1000), Europe and North America (10-20/1000 live births).
- Incidence of MZ twins is thought to be constant throughout the world at about 4/1000.
- Incidence of DZ twins varies between populations. DZ twinning is associated with increased levels of FSH in the mother, with higher parity, with older age of mother and with a tall heavy built of mother. Spontaneous DZ twinning is decreasing in frequency in the West due to decreasing maternal age and lower fertility.

Weinberg's method to estimate number of MZ twins versus DZ twins (rough population estimate):

Unlike sex twins are DZ

Assuming that DZ twins are half like sex and half unlike sex

If A = like sex twins

B = unlike sex twins

MZ twins = A-B

DZ = 2B

- This assumption is not very accurate because the actual frequency of same sex twins is higher in DZ twins than expected. Also rarely MZ twins are unlike sex e.g. 46,XY and 45,X.

- It is difficult to assess the number of twin pregnancies at conception. It is estimated that about 70% of twin pregnancies diagnosed by ultrasound before the 10th week of gestation miscarry or convert to singleton (vanishing twin)

Recognition of zygosity

1. Physical characteristics after birth.
2. Biochemical characteristics : for example blood types, enzyme polymorphisms, HLA types.
3. DNA fingerprinting : It is better to take a skin biopsy than to test blood because of the possible chimerism in blood cells.

Chimerism and mosaicism

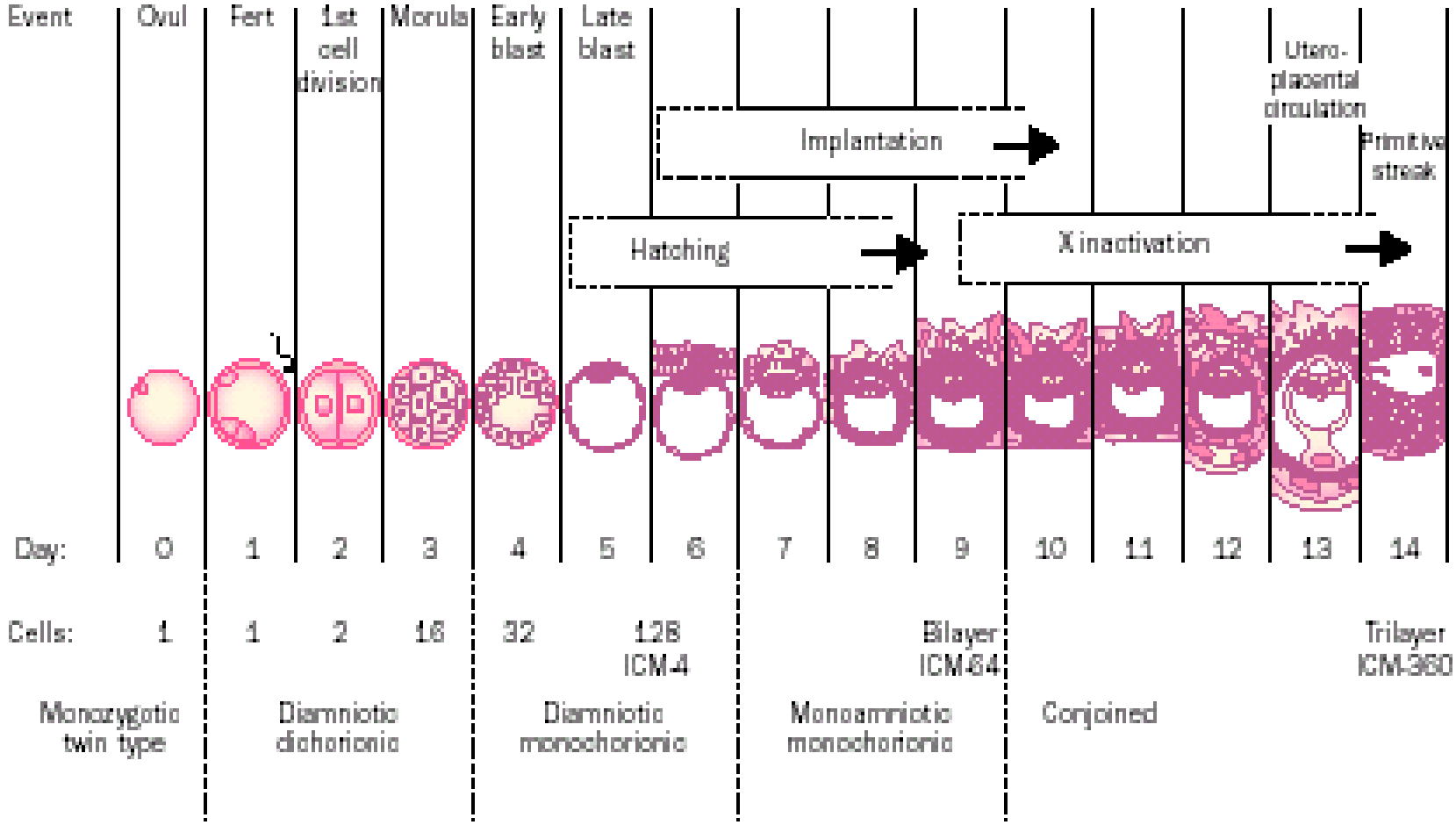
- Chimerism means presence of cells in one individual derived from two genetically distinct sources. For example chimerism of blood cells occurs in DZ twins due to intrauterine vascular connections.
- Mosaicism means the presence in an individual of 2 cell lines derived from the same zygote . For example a mosaic Turner female with 45,X/46,XX cell lines or a mosaic Down syndrome with 46,XY/47,XY,+21 cell lines.

Type of chorion

- Placental membranes begin to form by day 4 in human development
- DZ twins are dichorionic diamniotic

Type of MZ twins	Time of division into twins from fertilization	% of MZ twin pregnancies surviving to birth
Dichorionic diamniotic	0-3 days	25-30%
Monochorionic diamniotic	4-7 days	70-75%
Monochorionic monoamniotic	Up to 14 days	1-2%

Timing of MZ twinning



Classification of Congenital abnormalities

- **Malformation:** occur due to abnormal development. Structures may fail to form, may form incompletely or may develop with the wrong configuration. eg. Neural tube defects, congenital heart defects. Most occur before 8 weeks of gestation.
- **Sequence:** pattern of multiple defects that result from a single primary malformation. eg. Spina bifida leading to hydrocephalus , talipes and hip dislocation.
- **Deformation:** alteration in shape or position of body parts due to mechanical forces and usually occur after organogenesis during the later part of pregnancy. Forces may be external as in abnormal shape of uterus and oligohydramnios or internal as in fetal oedema. eg talipes, congenital hip dislocation.
- **Disruption:** result from destructive processes that alter a structure after it has formed normally, e.g. amniotic bands.
- **Aplasia/hypoplasia & dysplasia:** absence, insufficient or disorganised cellular proliferation of a tissue or organ eg. hypoplasia of corpus callosum

Congenital abnormalities in twins

- At about 30 weeks, both MZ and DZ twins start to slow down in their weight gain as compared to singletons.
- The rate of congenital abnormalities among MZ twins is around 10% among live births. (rate among singletons is 2-3% among live births).
- Anomalies specific to MZ twins include : Acardiac twin which occurs in 1:35,000 births and conjoined twins occurring in 1:100,000 births.
- Fetus papyraceous occurs when one of the twins dies later than the first trimester and so is not completely absorbed.

Congenital abnormalities in twins

- **Deformations** such as clubfoot, hip dislocation… are associated with intrauterine crowding.
- **Malformations** such as congenital heart defects may be associated with placental vascular connections.
- **Disruptions** such as limb reduction defects may be related to shared placental circulation.

Discordance and Concordance in twins

- Discordance means that a character or trait or disease is different in the pair of twins.
- Discordance between DZ twins could be related to both genetic and environmental factors.
- Since MZ twins share much of their genetic material, discordance among them is usually attributed to environmental factors.
- Discordance rate between MZ and DZ twins has been used to evaluate heritability of complex traits and diseases such as congenital abnormalities, diabetes, ,schizophrenia…

Recent work, suggests that MZ twins are not identical, and genetic differences exist between them

MZ twins are often discordant

We cannot assume that the different tissues and organs of each twin will be genetically identical.

70% of MZ twins and 8% of DZ twins have had *in utero* vascular connections, which means that sampling of blood from either twin may not reveal discordance.

Many ***genetic*** forms of discordance have been described within MZ twin pairs and may even ***play a role*** in causing MZ twinning. These may be related to

1. Epigenetic mechanisms
2. Somatic mutations in genes or chromosomes
3. Mosaicism
4. Cytoplasmic inheritance

Intra-uterine ***environmental*** differences in the allocation of the number of cells and in the placental vascular supply to each twin, as well as stochastic (*random*) developmental events, may lead to major discordance at birth between the twins of a MZ pair.

Epigenetic mechanisms

Definition:

Heritable phenomena that regulate gene expression without involving changes to the DNA code.

One of the epigenetic mechanisms is *DNA methylation*

- Methylation of DNA is involved in establishing and maintaining a particular state of gene expression during differentiation including early development.
- Methylation of genomic DNA may affect a variety of processes related to gene expression including *X chromosome inactivation,* and *genomic imprinting.*

X-chromosome inactivation

Several authors have suggested that skewed X-chromosomal inactivation may play a role in female MZ twinning. Skewed X-inactivation means that during embryogenesis, two different loci were to arise: one expressing the maternal X, the other expressing the paternal X. A number of female MZ twins have been discordant for various X-linked recessive diseases, suggesting, and often demonstrating, non-random X-inactivation.

X-inactivation does not explain all female monozygote twinning, but could be responsible for the excess of MZ female twins.

Genomic imprinting

Genomic imprinting involves allele-specific methylation, depending on whether a particular gene is inherited from the mother or from the father, and accounts for 'parent of origin' differences in gene expression.

Gametes are known to undergo genome wide demethylation before new imprints are established.

Etiology of MZ twinning: proposed mechanisms

Abnormalities or early rupture of the Zona pelluicda may lead to herniation of blastocyst and separation of inner cell mass into two clumps

Twinning itself may be a type of congenital anomaly so that the fertilized ovum resulting in twins develops in a different way than the fertilized ovum that results in singleton

Skewed X-inactivation

Etiology of MZ twinning: proposed mechanisms

Cells within the blastocyst develop in such a way as to become discordant, recognizing each other as foreign and so separate into two separate cell masses and thus develop into 2 embryos. (from 8 cell stage to about 360 cell stage in the inner cell mass, before appearance of primitive streak):

- Mutation of a gene
- Development of a chromosome abnormality
- Change in gene control processes e.g. methylation

MZ twins and IVF

- **With IVF ,1-2% of pregnancies are MZ twins.**
- **It must be noted that the figure of 4/1000 for spontaneous MZ twins is based on live birth data ,but the MZ twinning rate reported after assisted conception is based on detection of cardiac activity between 6 and 9 weeks gestation.**
- **The risk of monoamniotic twinning is increased following IVF/embryo transfer.**

MZ twins and IVF

In human assisted reproduction, the *phenomena that may influence MZ twinning include*

1. **ovulation induction might lead to hardening of zona pellucida**
2. **zona pellucida manipulation eg puncturing**
3. **changes in the configuration of the inner cell mass (ICM) resulting from certain culture conditions**

MZ twins resulting from assisted reproductive technique may not have the same mechanisms as spontaneously occurring MZ twins

Familial MZ twinning

- Few families have been reported in which MZ twinning occurs more frequently than expected.
- Interestingly there does not seem to be an increase in congenital anomalies among MZ twins in these families.
- Transmission was reported to occur both from the maternal and paternal side of the family.
- A possible mode of inheritance was suggested which is AD with variable penetrance.
- Therefore there must be an inherited defect causing or predisposing to MZ twinning.

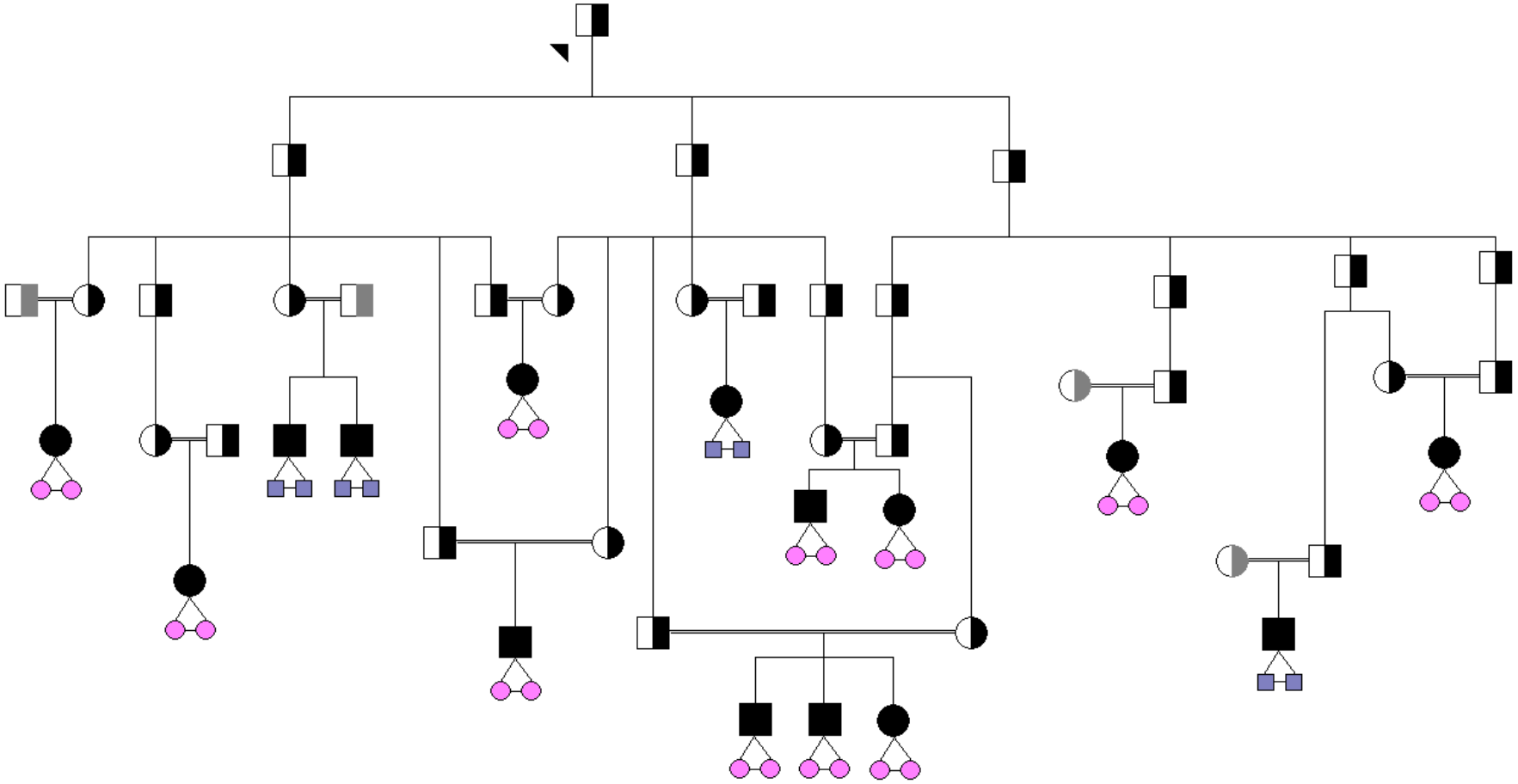
Familial MZ twinning

What is this defect???

- In one of the zona pellucida (ZP) proteins ----- fragile ZP –early hatching----early separation of blastocyst clumps?
- Skewed X-inactivation leading to two different clumps in inner cell mass?
- Fertilisation of old ova ----hard ZP-----fracture----cuts blastocyst in two?
- Cytoplasm of old ova----errors in methylation programming?
- Defective cell-cell adhesion , for example abnormalities in cadherin protein?

A rise in MZ twins was induced in mice by hypoxia and changes in temperature.

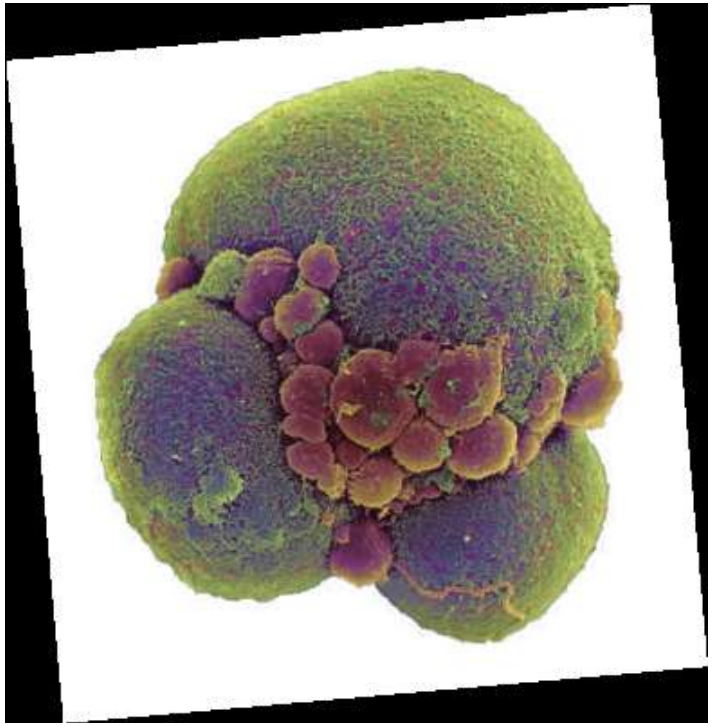
Pedigree with familial monozygotic twinning



(Nature, volume 458, 16 April 2009)

One theory of twinning holds that subtle differences force cells in early embryos to repel one another and establish two separate cell masses. Bruno Reversade speculates that a mutated candidate gene might deprive cells in the embryo of their ability to adhere tightly to each other, resulting in a split.

DNA samples are being gathered from large families with multiple MZ twins and tested using new genetic and biology techniques to help in highlighting the etiology of MZ twinning.



The causes of MZ twinning in human beings remain a mystery.

Solving this mystery could clarify many of the processes of early development in humans