



*"Achondroplasia Gladiator"*—  
Bibliothèque Nationale, Paris

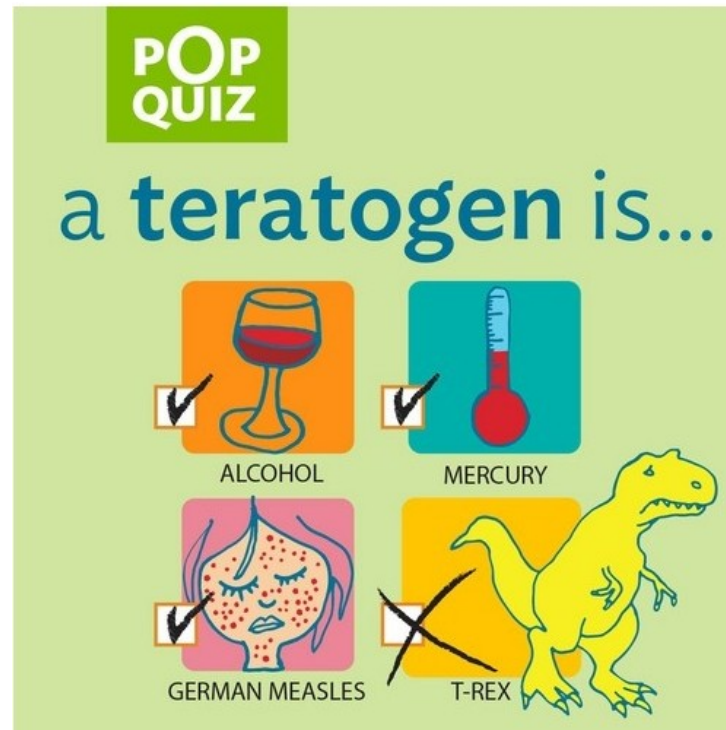
# Introduction to teratology

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Embryology LF MU

**Embryology** is the study of normal intrauterine embryonic or fetal development.

**Teratology** is the study of birth defects, and a **teratogen** is something that either induces or amplifies abnormal embryonic or fetal development and causes birth defects.



## Historical context

τέρας (Greek) *teras* = monster

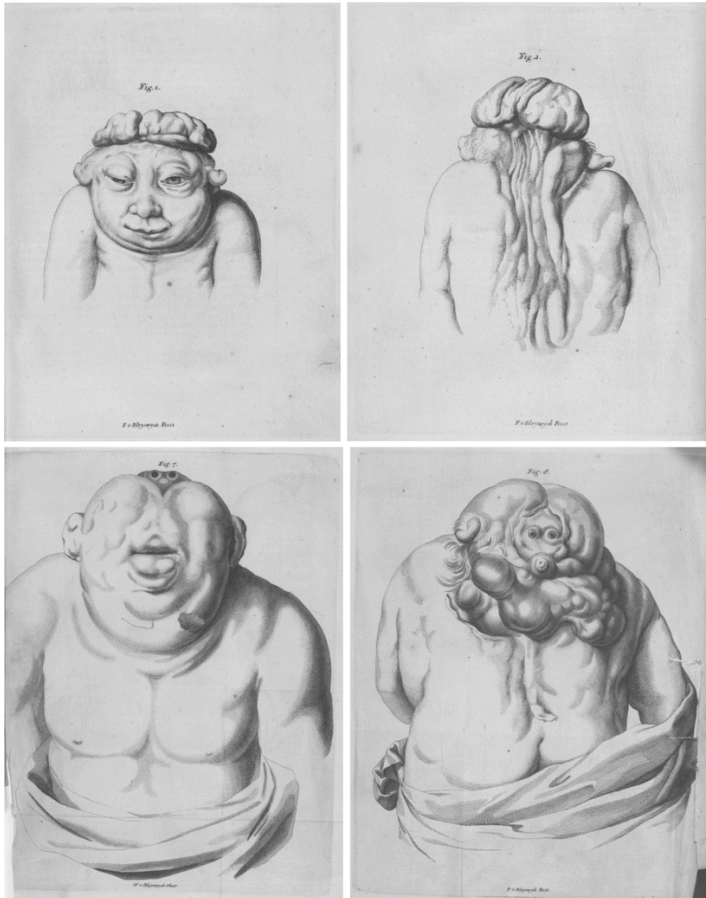
Everything looking abnormal





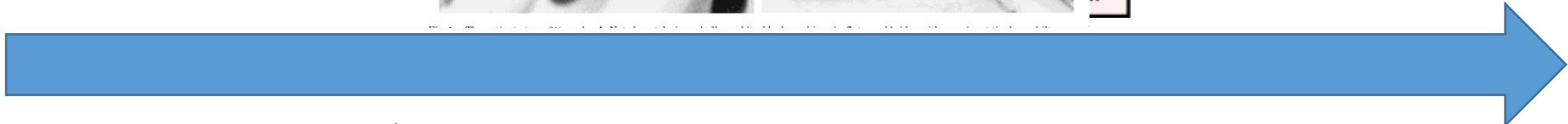
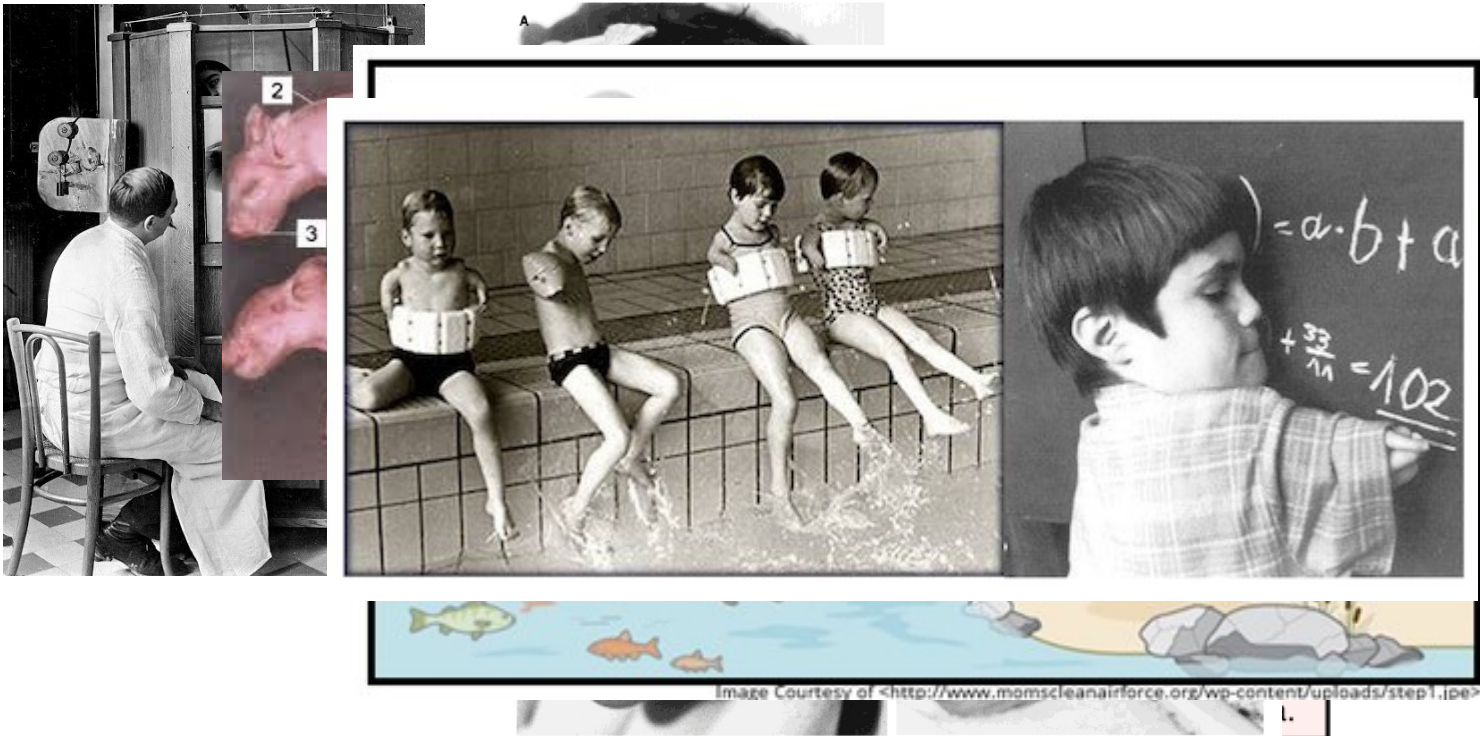
## Historical context

Since the 17<sup>th</sup> century related to abnormal births (development)



- **W. Harvey** (1578–1657) - used the term "developmental arrest",
- **C.F. Wolff** (1733–1794) - in his study on the intestine, the term "germ layer" was coined that has been in use to this day,
- **A. von Haller** (1708–1777) - was first to describe the development of the chicken heart,
- **I.G. de Saint-Hillaire** (1805–1861) - was first to introduce the term "teratology",
- **C. Dareste** (1822–1899) - discussed the modes of artificial induction of monstrosities (particularly by mechanical impulses during incubation of hen eggs),
- **R. Virchow** (1821–1902) - gathered a unique collection of rare developmental disorders of the human body in the "Museum of Pathology" in the Berliner hospital Charité,
- **E. Schwalbe** (1906–1999) - defined the expression "teratogenic termination point",
- **CH.R. Stockard** (1879–1936) - introduced the term "critical period".

# INTRODUCTION TO TERATOLOGY



1905	1921	1929	1931	1941	1944	1948	1952	1956	1961
The first description of the first exogenous embryonic retinopathy induced by X-rays in and named, "Madelung's disease".	The first descriptive report on human malformations induced by environmental pollutants. Discovery of the first report on human malformations induced by environmental pollutants. Discovery of the first report on human malformations induced by environmental pollutants.	Recognition of the first report on human malformations induced by environmental pollutants. Discovery of the first report on human malformations induced by environmental pollutants.	The first report on human malformations induced by environmental pollutants. Discovery of the first report on human malformations induced by environmental pollutants.	The first report on human malformations induced by environmental pollutants. Discovery of the first report on human malformations induced by environmental pollutants.	Thalidomide-induced embryopathy (Wiedemann et al.).	Minamata disease (Miyama et al.).			

## What is a teratogen?

- X-Rays
- Lipid diet
- Hypovitaminosis
- Sex hormones
- Virus infection
- Chemical substances (NaBr)
- Drugs (aminopterin, thalidomide)
- Environmental pollutant (methyl mercury)

## Physical

- Ionizing irradiation
- Temperature

## Chemical

- Nucleotide analogues
- Organometal compounds
- Hormones and endocrine disruptors
- Many others

## Biological

- Infection agents
- Disease of mother

## What does a teratogen do?

- Disrupts fine molecular or metabolic pathways
- Intra uterine growth retardation
- Failure of histogenesis, organogenesis
- Embryonic/fetal death



## How to identify a teratogen?

**<5%**

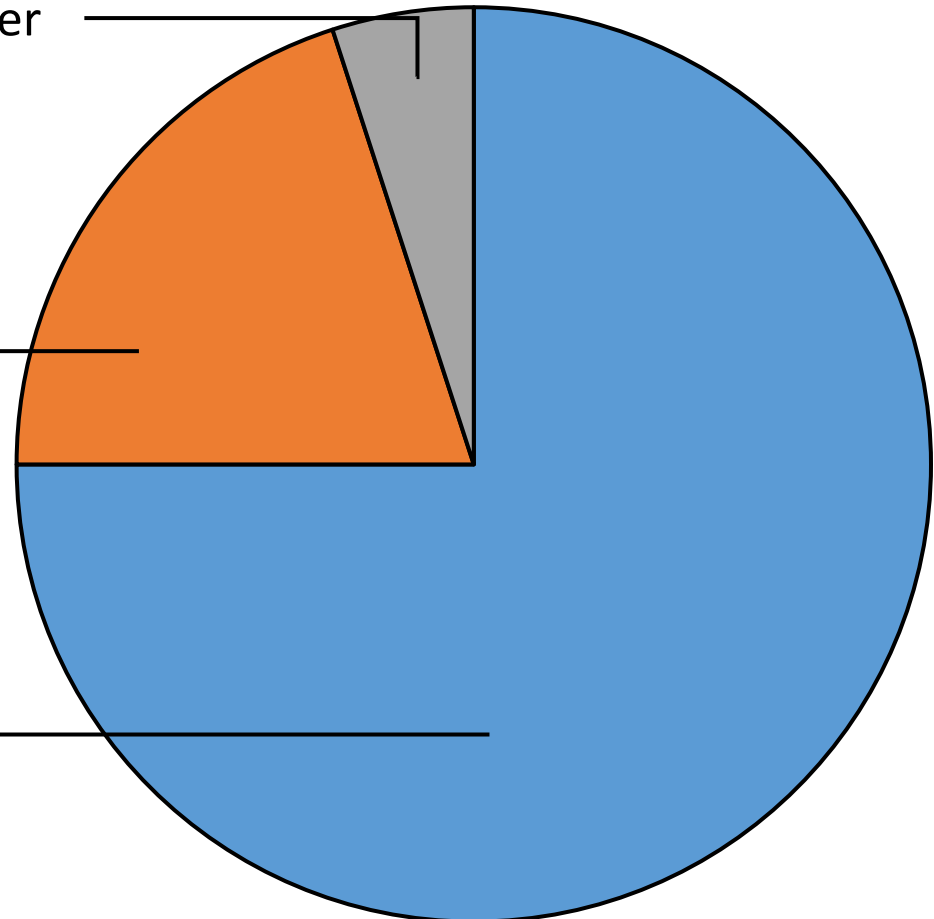
- Environmental
- Intrauterine infections
- Metabolic disorder of mother
- Drugs and medications
- Radiation

**20-25%**

Genetical

**65-75%**

Multifactorial or unknown



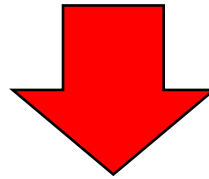


## How to identify a teratogen?

- Animal studies
- Observations from human exposure

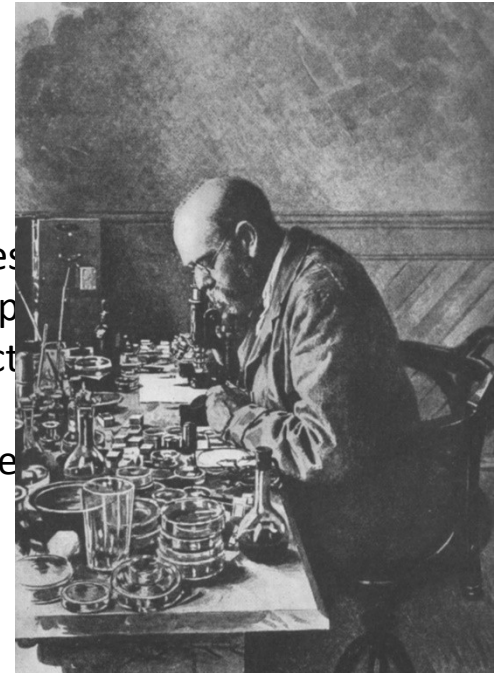
### *Koch's Postulates in microbiology:*

- *The microorganism must be found in abundance in all organisms suffering from the disease, but should not be found in healthy organisms.*
- *The microorganism must be isolated from a diseased organism and grown in pure culture.*
- *The cultured microorganism should cause disease when introduced into a healthy organism.*
- *The microorganism must be reisolated from the inoculated, diseased experimental host and identified as being identical to the original specific causative agent.*

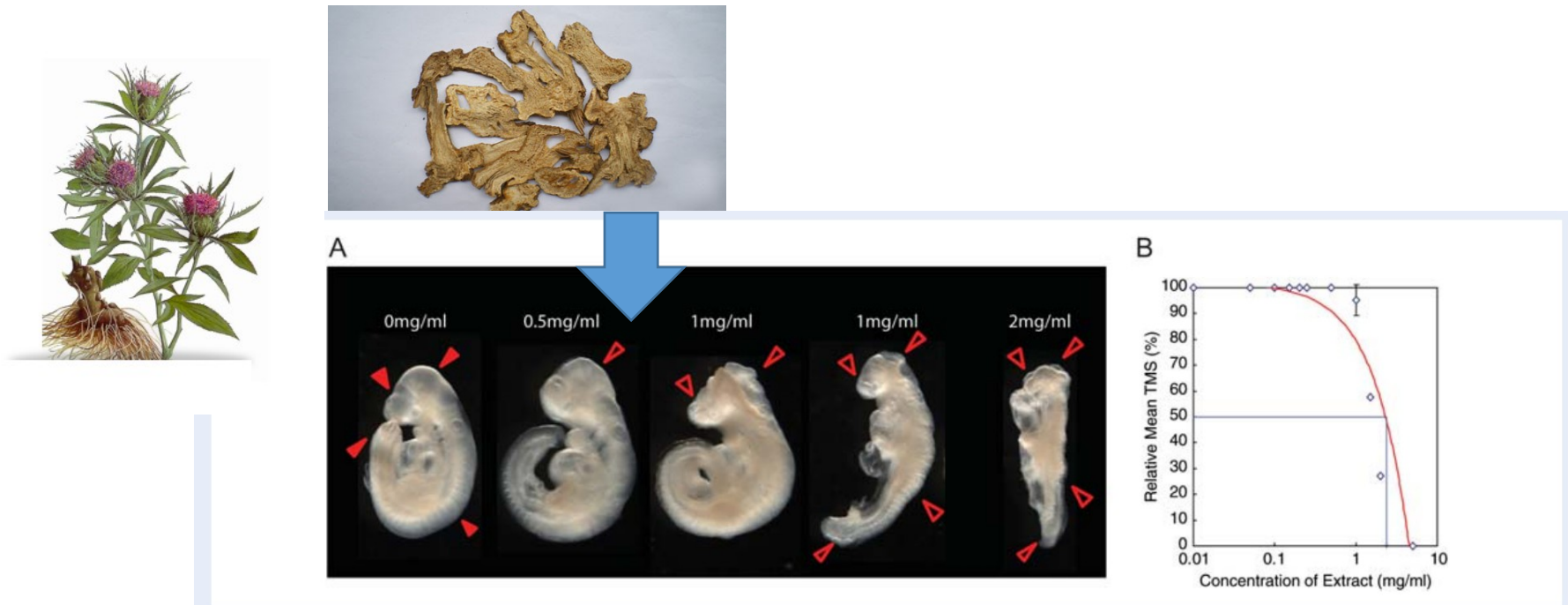


### **"Koch's Postulates,, adapted for teratology:**

- The agent must be present during the critical stage of development.
- The agent produces a particular pattern of birth defects in animal studies
- The agent crosses the placenta and there is a dose-response relationship
- There is an abrupt increase in the frequency of a particular defect (syndrome).
- The increase of defects is associated with the use of a new drug or the to a chemical or environmental change.
- There is an absence of other factors to explain the observations.
- The mechanism of teratogenesis makes biological sense.



# INTRODUCTION TO TERATOLOGY



**Figure 1** Embryotoxicity of LAR in mouse embryo culture. **(A)** Abnormal embryonic development and anomalies were observed in treatment groups from 0.5 to 2 mg/ml of LAR. Representative embryos are presented. Closed arrows represent the normal structures. Open arrows represent the malformed structures, including microencephaly, opened neural tube defects and sacral regression. Magnification  $\times 25$ . **(B)** Dose-response curve of LAR on embryo development. The minimum concentration of LAR which resulted in a 50% reduction in relative TMS in the embryos was indicated.  $n = 10$  embryos per each LAR concentration.

Molecular Human Reproduction, Vol.18, No.12 pp. 585–592, 2012  
Advanced Access publication on August 19, 2012 doi:10.1093/molehr/gar034

MHR

ORIGINAL RESEARCH

## Molecular studies of the congenital malformation induced by *Largehead Atractylodes Rhizome*, the most commonly used Chinese medicine for threatened miscarriage

L.Y. Tang<sup>1,†</sup>, L. Li<sup>1,†</sup>, A. Borchert<sup>2</sup>, Clara B.S. Lau<sup>3</sup>, P.C. Leung<sup>3</sup>, and C.C. Wang<sup>1,4,\*</sup>

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Submitted on March 27, 2012; resubmitted on August 3, 2012; accepted on August 9, 2012

# How to identify a teratogen?

- Animal studies
- Observations from human exposure
- **Understand the biological context**

## Wilson's Six Principles of Teratology (1977)

1. Susceptibility to teratogenesis depends on the **genotype** of the conceptus and the way in which this interacts with environmental factors.
2. Susceptibility to teratogenic agents varies with the **developmental stage** at the time of exposure.
3. Teratogenic agents act in **specific ways** (mechanisms) on developing cells and tissues to initiate abnormal embryogenesis (pathogenesis).
4. The **final manifestations** of abnormal development are death, malformation, growth retardation, and functional disorder.
5. The access of adverse environmental influences to developing tissues depends on the **nature of the influences** (agent).
6. Manifestations of deviant development increase in degree as **dosage** increases from the no-effect to the totally lethal level.

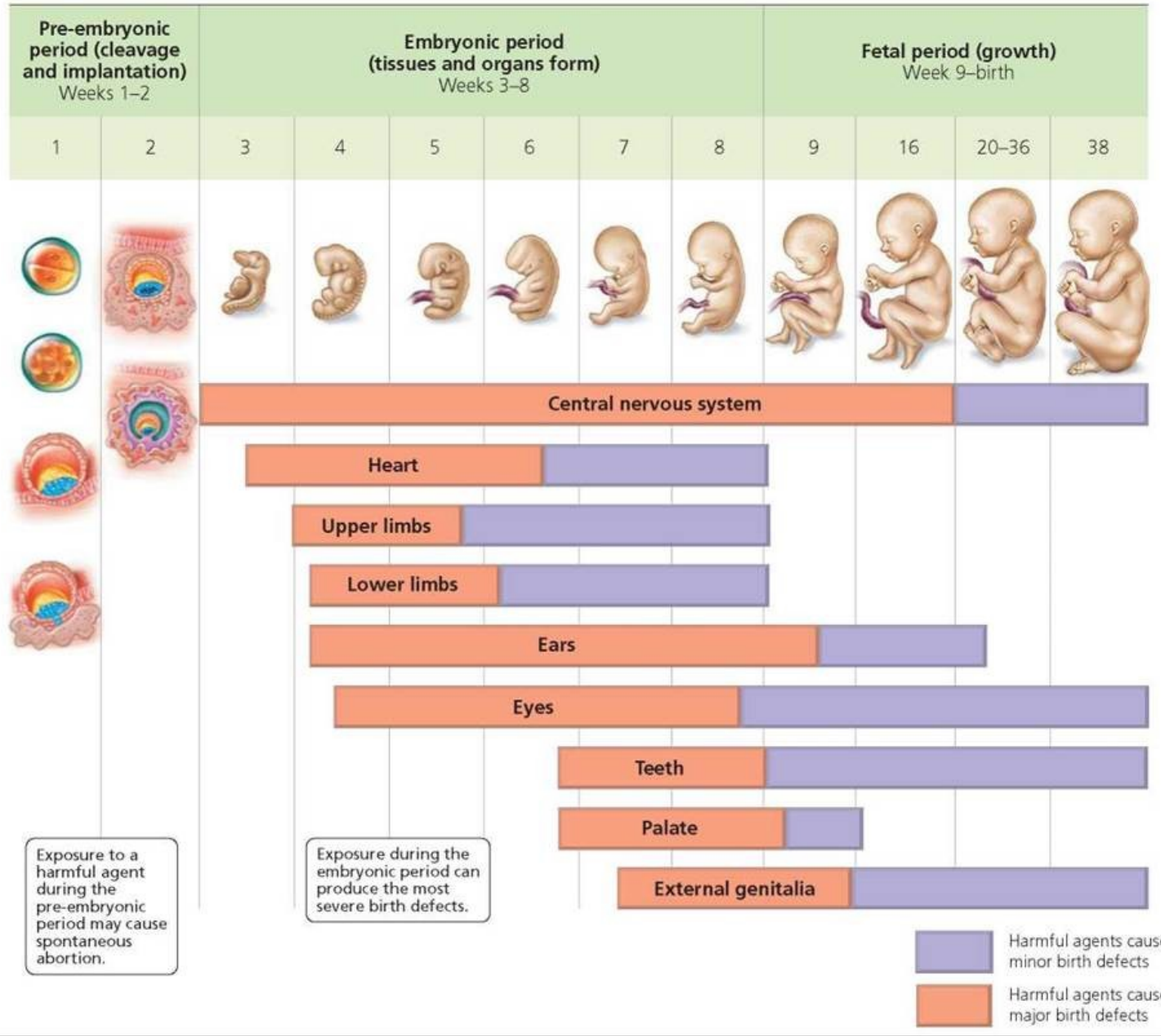
Handbook of  
Teratology

EDITED BY  
JAMES G. WILSON • E. CLARKE FRASER

2 Mechanisms  
and Pathogenesis



# INTRODUCTION TO TERATOLOGY



## Mechanisms of action?

- Mutation
- Chromosomal nondisjunction and breaks Mitotic interference
- Altered nucleic acid integrity or function
- Lack of precursors and substrates needed for biosynthesis
- Altered energy sources
- Enzyme inhibitions
- Osmolar imbalance
- Altered membrane characteristics

**1977**

- Epigenetic control of gene expression
- The effects of small regulatory RNAs
- The imbalance of gene products resulting from submicroscopic alterations of genomic structure such as copy number changes
- Alterations of the cytoskeleton
- Perturbations of the extracellular matrix
- Effects of mechanical forces on embryogenesis
- Disturbances of intracellular or intercellular signalling
- Dysfunction of molecular chaperones
- Effects on the distribution of molecules into subcellular compartments
- Alterations of the integrity of intracellular organelles

**2010**

## Teratogens around us

**physical** ionizing irradiation (UV, RTG,  $\alpha$ ,  $\beta$ ,  $\gamma$ ), temperature, mechanical factors (amniotic bands, pes equinus, ...)

**chemical** pharmacological drugs (antibiotics, antiepileptics, anticoagulants, cytostatics) solvents, alcohol, heavy metals, ...

**biological** pathogens (virus), disease of mother (diabetes, myasthenia gravis, PKU)



# INTRODUCTION TO TERATOLOGY

Teratogen	Vrozená vada
<b>Infekce</b>	
rubeola virus	katarakta, glaukom, srdeční vady, hluchota, abnormality zubů
cytomegalovirus	microcephalia, slepota, mentální retardace, odumření fetu
virus Herpes simplex	microphthalmia, microcephalia, retinální dysplasia
virus varicelly	hypoplasie končetin, mentální retardace, svalové atrofie
HIV	microcephalia, růstová retardace
<i>Toxoplasma gondii</i>	hydrocephalia, mozkové kalcifikace, microphthalmia
<i>Treponema pallidum</i>	mentální retardace, hluchota
<b>Fysikální činitele</b>	
rtg záření	microcephalia, spina bifida, rozštěp patra, defekty končetin
hypertermie	anencephalia, spina bifida, mentální retardace, defekty obličeje, srdeční malformace, omphalokéla, defekty končetin
<b>Chemické látky</b>	
thalidomid	defekty končetin, srdeční vady, hluchota, slepota, malformace dalších vnitřních orgánů
aminopterin	anencephalia, hydrocephalia, rozštěp rtu a patra
fentyoin	fetální hydantoinový syndrom, defekty obličeje, mentální retardace
kyselina valproová	defekty nervové trubice, kraniofaciální, srdeční a končetinové vady
trimethadion	rozštěp patra, srdeční, urogenitální a kosterní vady
lithium	srdeční malformace
amfetaminy	rozštěp rtu a patra, srdeční malformace
warfarin	chondrodysplasia, microcephalia
ACE inhibitory*	růstová retardace, odumření fetu
kokain	růstová retardace, microcephalia, abnormality chování, gastroschisis
ethanol	fetální alkoholový syndrom, krátké oční štěrby, hypoplasie maxily, srdeční malformace, mentální retardace
isotretinoin (analog vitamínu A)	embryopatie vyvolaná vitamínem A: malé abnormálně tvarované uši, mandibulární hypoplasie, rozštěp patra, srdeční vady, končetinové vady
průmyslová rozpouštědla	nízká porodní hmotnost, kraniofaciální defekty, defekty nervové trubice
organické sloučeniny rtuti	neurologické poruchy připomínající mozkovou obrnu
olovo	růstová retardace, neurologické poruchy

Teratogen	Vrozená vada
<b>Hormony</b>	
androgeny (ethisteron, norethisteron)	maskulinisace ženského zevního genitálu: splynulá labia, hypertrofický klitoris
diethylstilbestrol	hypoplasie varlat, malformace dělohy, vejcovodů a horní části vagíny, v dospělosti karcinom pochvy
diabetes mellitus	řada malformací, hlavně srdečních, defekty nervové trubice, syndrom kaudální regrese spojený s hypoplasii dolních končetin
obesita	srdeční vady, omfalokéla

\* ACE – angiotensin-konvertující enzym

- **Growth retardation**
- **Failure of histogenesis, organogenesis**
- **Embryonic/fetal death**



**Classification criteria?**

## How to classify a teratogen?



**Category A:** Controlled studies in women fail to demonstrate a risk to the fetus in the first trimester (and there is no evidence of a risk in later trimesters), and the possibility of fetal harm appears remote.

**Category B:** Either animal-reproduction studies have not demonstrated a fetal risk but there are no controlled studies in pregnant women or animal-reproduction studies have shown an adverse effect (other than a decrease in fertility) that was not confirmed in controlled studies in women in the first trimester (and there is no evidence of a risk in later trimesters).

**Category C:** Either studies in animals have revealed adverse effects on the fetus (teratogenic or embryocidal, or other) and there are no controlled studies in women or studies in women and animals are not available. Drugs should be given only if the potential benefit justifies the potential risk to the fetus.

**Category D:** There is positive evidence of human fetal risk, but the benefits from use in pregnant women may be acceptable despite the risk (e.g., if the drug is needed in a life-threatening situation or for a serious disease for which safer drugs cannot be used or are ineffective).

**Category X:** Studies in animals or human beings have demonstrated fetal abnormalities, or there is evidence of fetal risk based on human experience, or both, and the risk of the use of the drug in pregnant women clearly outweighs any possible benefit. The drug is contraindicated in women who are or may become pregnant.



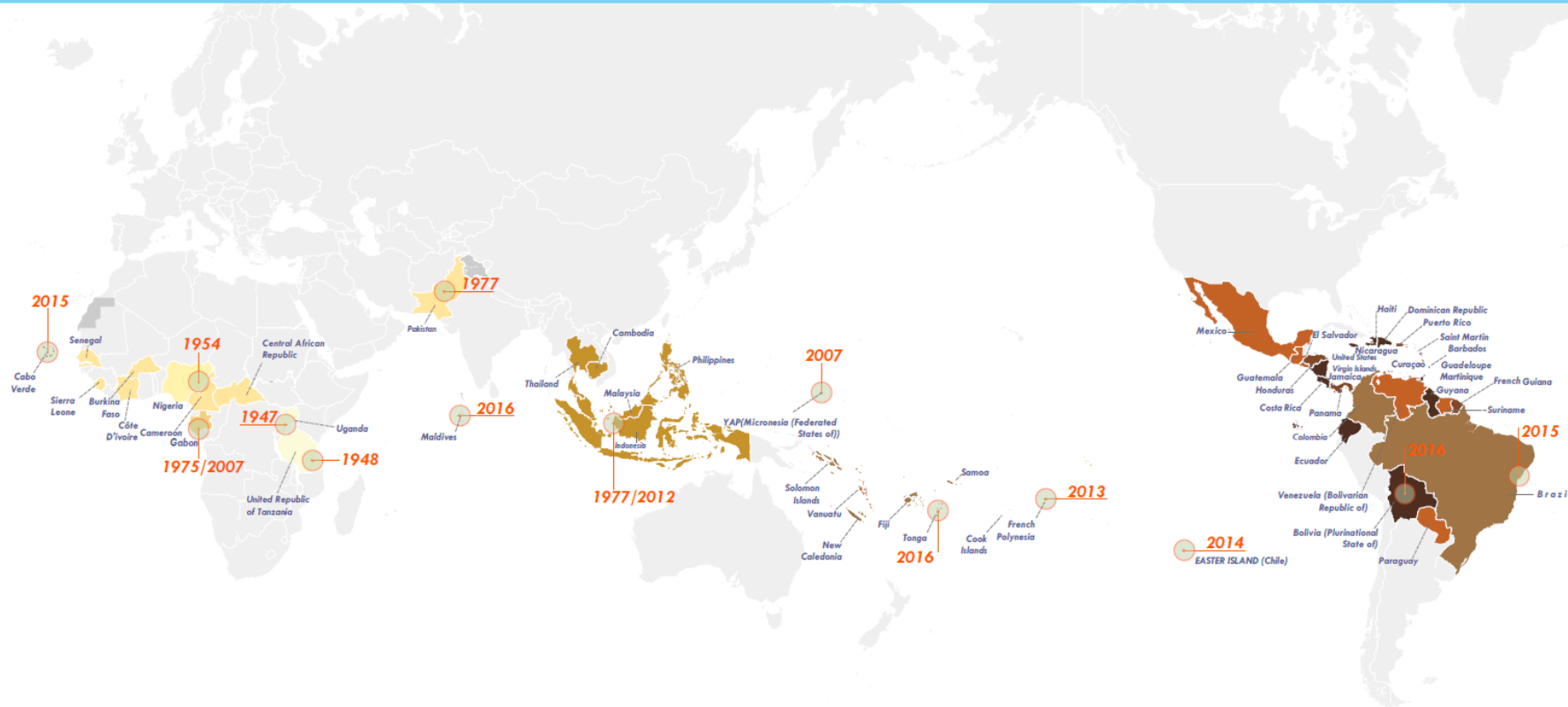






# CASE STUDY

## Countries and territories showing historical time-line of Zika virus spread (1947 - 2016)



Uganda  
United Republic of  
Tanzania

1947 - 1952

Nigeria

1954

Central African Republic  
Senegal  
Pakistan  
Burkina Faso  
Cote D'Ivoire  
Cameroon  
Sierra Leone  
Gabon  
Indonesia  
Malaysia  
Nigeria  
Costa Rica  
Cambodia

1960 - 1983

YAP (Micronesia  
(Federal states of))  
Gabon

2007 - 2009

French Polynesia  
EASTER ISLAND (Chile)  
Cook Islands  
New Caledonia  
Malaysia  
Philippines  
Cambodia  
Indonesia  
Thailand

2012 - 2014

Brazil  
Vanuatu  
Fiji  
Colombia  
Cabo Verde  
Samoa  
Solomon Islands

Jan - Oct 2015

El Salvador  
Guatemala  
Mexico  
Paraguay  
Suriname  
Venezuela (Bolivarian  
Republic of)

Nov - 2015

French Guiana  
Honduras  
Martinique  
Panama  
Puerto Rico

Dec - 2015

Bolivia (Plurinational  
State of)  
United States Virgin  
Islands  
Dominican Republic  
Costa Rica  
Guadeloupe  
Saint Martin  
Nicaragua  
Barbados  
Maldives  
Ecuador  
Guyana  
Jamaica  
Curaçao  
Samoa  
Haiti

Jan - 2016

Tonga

Feb - 2016

# Evidence for ZIKV induced microcephaly?



# How to identify a new teratogen?

- Observations from human exposure
- Understand the biological context
- Validate on animals

## Bradford Hill criteria



**Strength** (effect size): A small association does not mean that there is not a causal effect, though the larger the association, the more likely that it is causal.

**Consistency** (reproducibility): Consistent findings observed by different persons in different places with different samples strengthens the likelihood of an effect.

**Specificity**: Causation is likely if there is a very specific population at a specific site and disease with no other likely explanation. The more specific an association between a factor and an effect is, the bigger the probability of a causal relationship.

**Temporality**: The effect has to occur after the cause (and if there is an expected delay between the cause and expected effect, then the effect must occur after that delay).

**Biological gradient**: Greater exposure should generally lead to greater incidence of the effect. However, in some cases, the mere presence of the factor can trigger the effect. In other cases, an inverse proportion is observed: greater exposure leads to lower incidence.

**Plausibility**: A plausible mechanism between cause and effect is helpful (but Hill noted that knowledge of the mechanism is limited by current knowledge).

**Coherence**: Coherence between epidemiological and laboratory findings increases the likelihood of an effect.

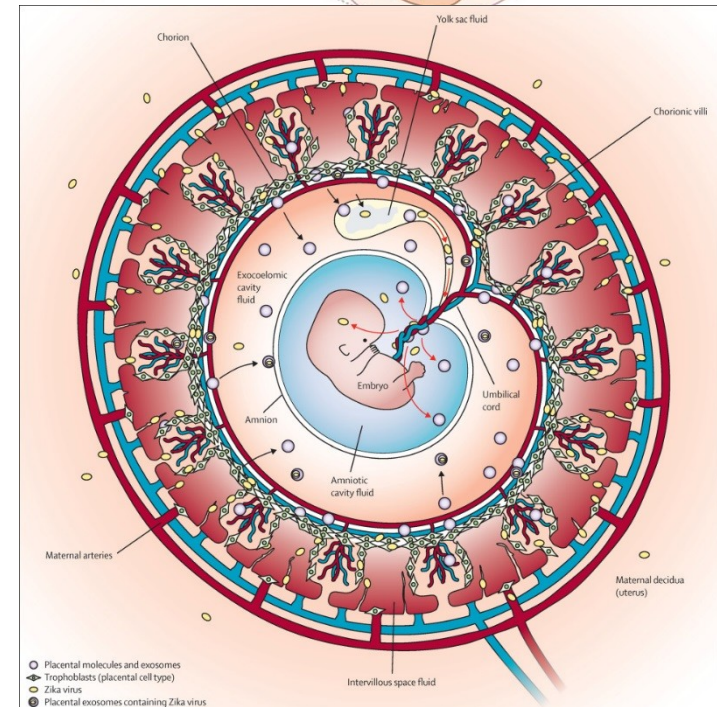
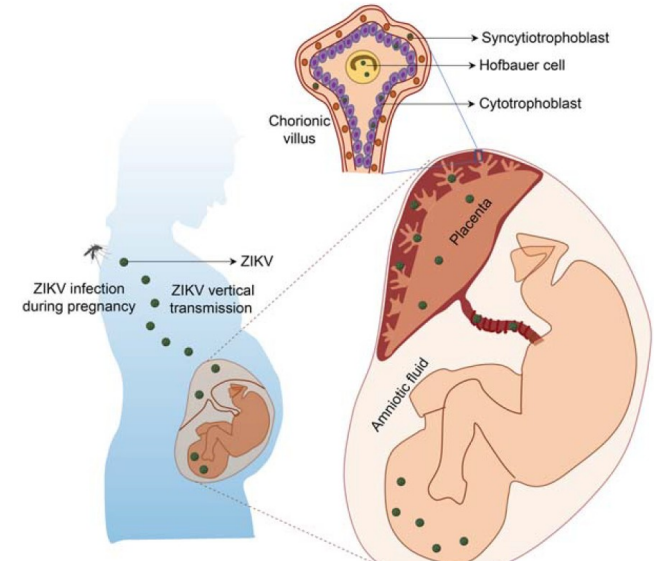
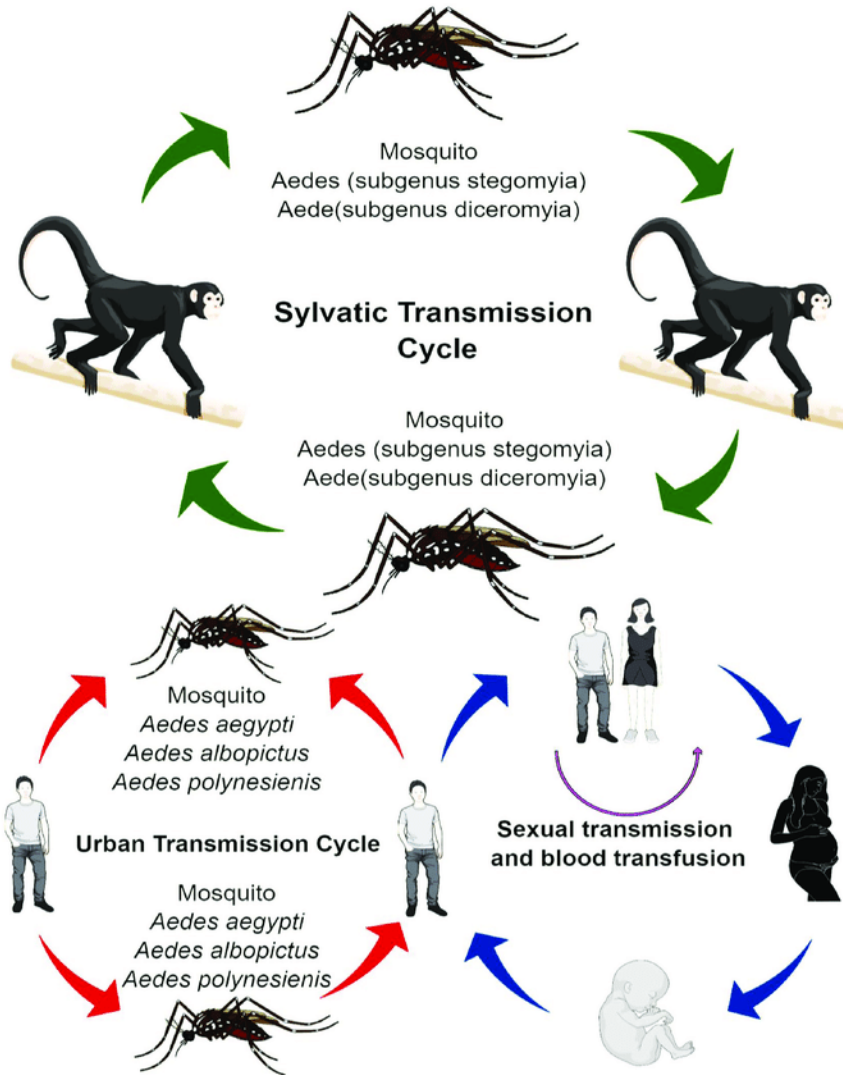
**Experiment**: "Occasionally it is possible to appeal to experimental evidence".

**Analogy**: The use of analogies or similarities between the observed association and any other associations.

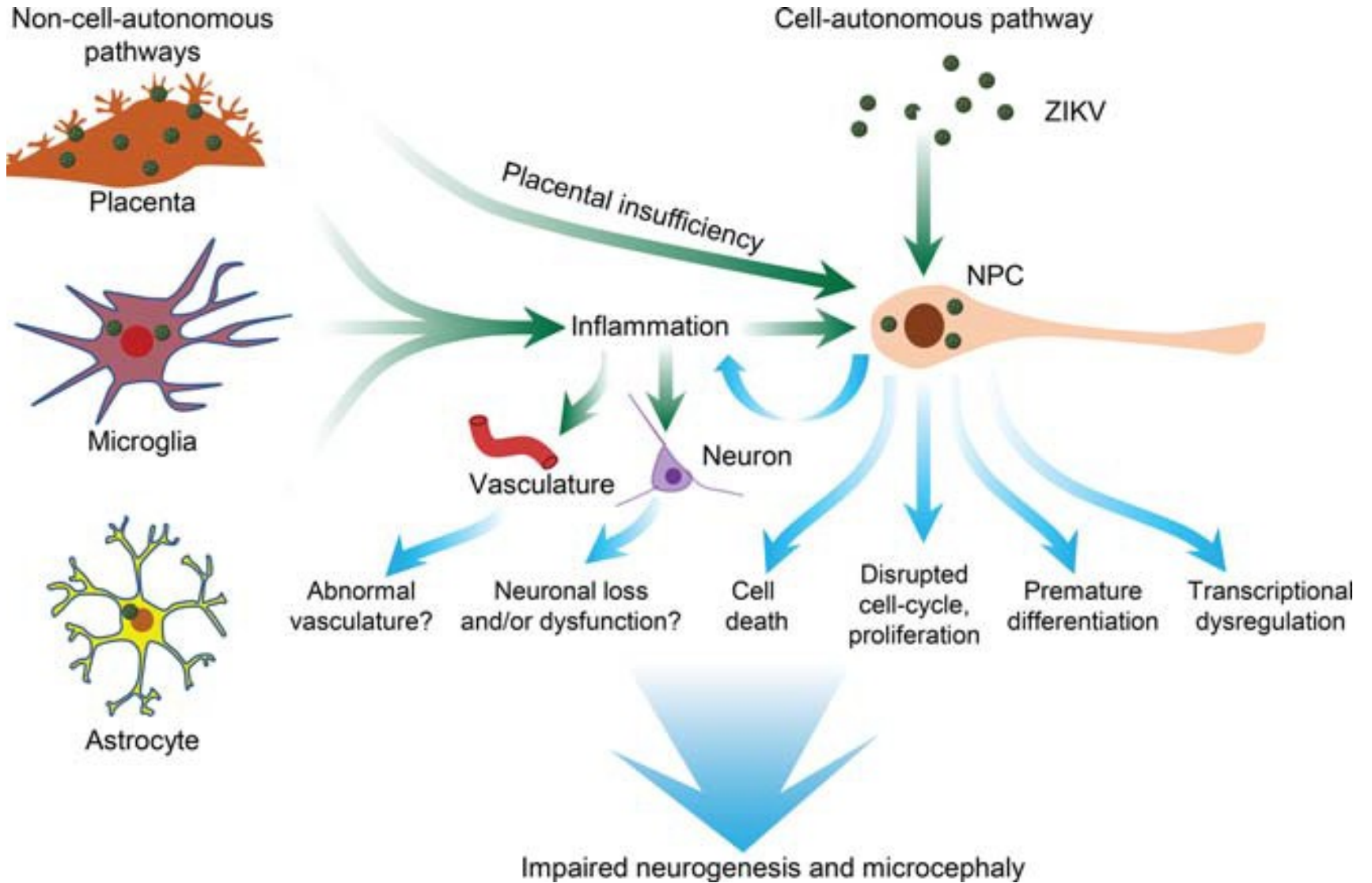
**Reversibility**: If the cause is deleted then the effect should disappear as well.



## ZKV transmission



# ZKV mechanism of action



## Take home message and further reading

- Teratology, teratogens → From Koch's postulates to Wilson's principles
- Mechanisms of action → any embryology and/or cell biology textbook
- Classification & clinical examples → any embryology textbook, FDA (EU) categories
- Identification, validation → ZIIKA forest virus story & Bradford Hill criteria

### **Suggested reading:**

- Friedman JM. The Principles of Teratology: Are They Still True? Birth Defects Research (Part A): Clinical and Molecular Teratology 88:766–768 (2010)
- Varga I. Embryology Teaching: An Often-neglected Part of the Medical Curriculum. Rev Arg de Anat Clin. 9(2):47-51 (2017)
- Ujhazy et al. Teratology – past, present and future. Interdiscip Toxicol. 5(4): 163–168 (2012)

Not every genetic anomaly results in a malfunction



X

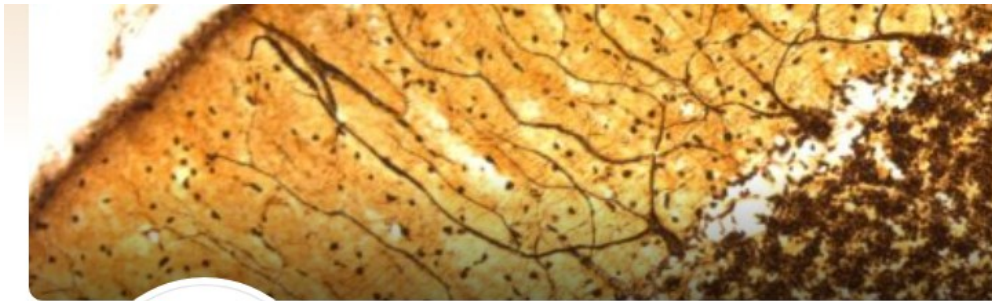


## RESEARCH

**Leaf Trait Coloration in White Clover  
and Molecular Mapping of the Red Midrib  
and Leaflet Number Traits**

# Thank you for attention

Questions? Comments?



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