



"Achondroplasia Gladiator"—
Bibliothèque Nationale, Paris

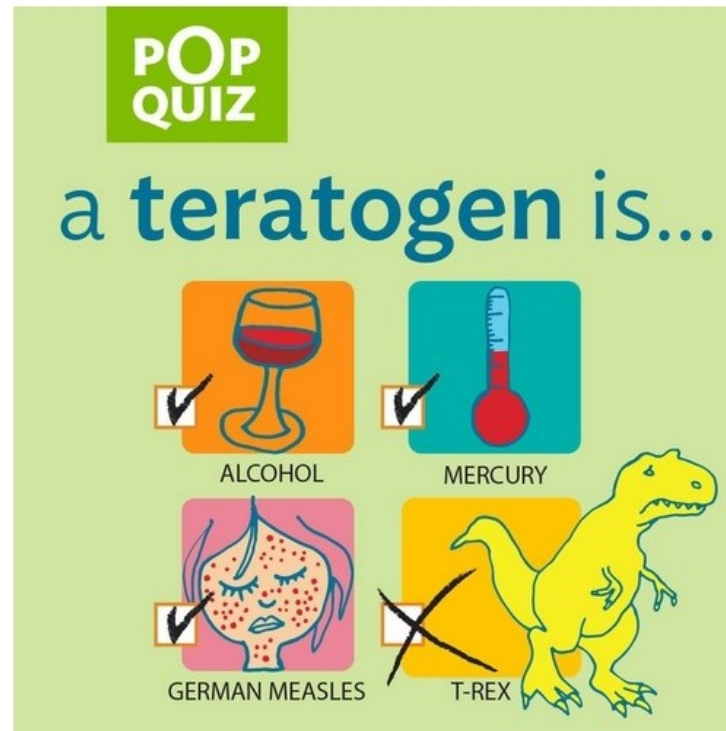
Introduction to teratology

Petr Vaňhara

Department of Histology and
Embryology LF MU

Embryology is the study of normal intrauterine embryonic and 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

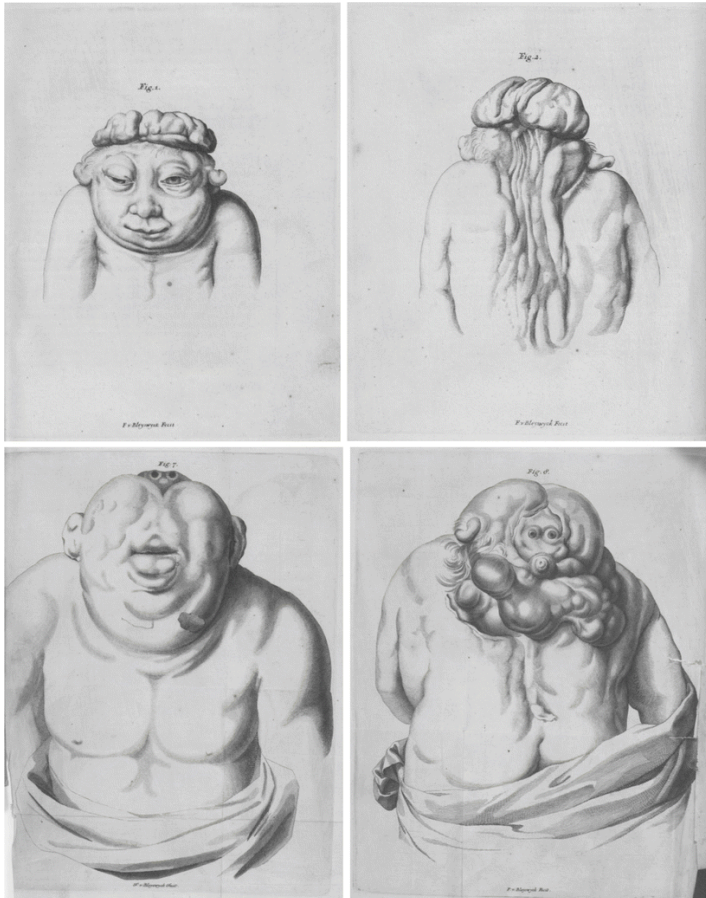
τέρας (Greek) *teras* = monster

Everything looking abnormal



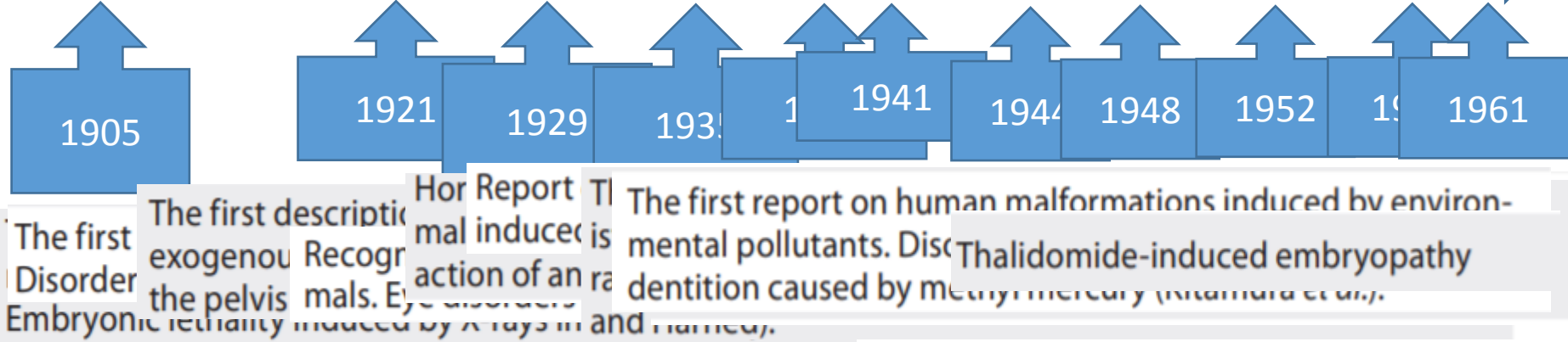
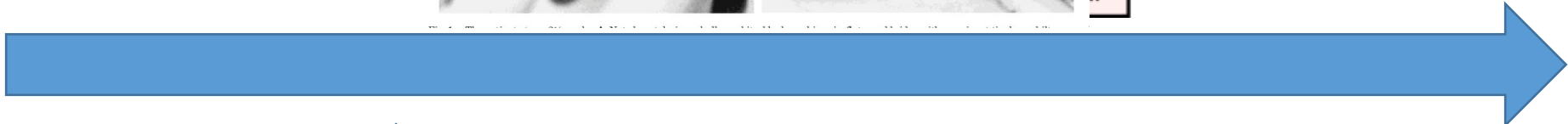
Historical context

Since the 17th 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



What is a teratogen?

- X-Rays
- Lipid diet
- Hypovitaminosis
- Sex hormones
- Virus infection
- Chemical substances
- Medications
- Environmental pollutant (methyl mercury)

Teratogens around us

Physical

- Ionizing irradiation (UV, RTG, α , β , γ),
- Temperature
- Mechanical factors in utero (pes equinus, amnion bands)



Chemical

- Nucleotide analogues (chemotherapy)
- Organometal compounds
- Hormones and endocrine disruptors
- Medications (aminopterin, thalidomide, antiepileptic drugs, anticoagulants, vitamin A)
- Environmental pollutant (methylmercury, lead, heavy metals)
- Alcohol, drugs, solvents
- Many others



Biological

- Infection agents (Toxoplasmosis, Rubella, Cytomegalovirus, Herpes, Syphilis)
- Disease of mother (diabetes, thyroid conditions, autoimmune disorders, PKU)



What does a teratogen do?

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



INTRODUCTION TO TERATOLOGY

Teratogen	Vrozená vada
Infekce	
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
Fysikální činitele	
rtg záření	microcephalia, spina bifida, rozštěp patra, defekty končetin
hypertermie	anencephalia, spina bifida, mentální retardace, defekty obličeje, srdeční malformace, omphalokéla, defekty končetin
Chemické látky	
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 štěrbiny, 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
Hormony	
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

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).

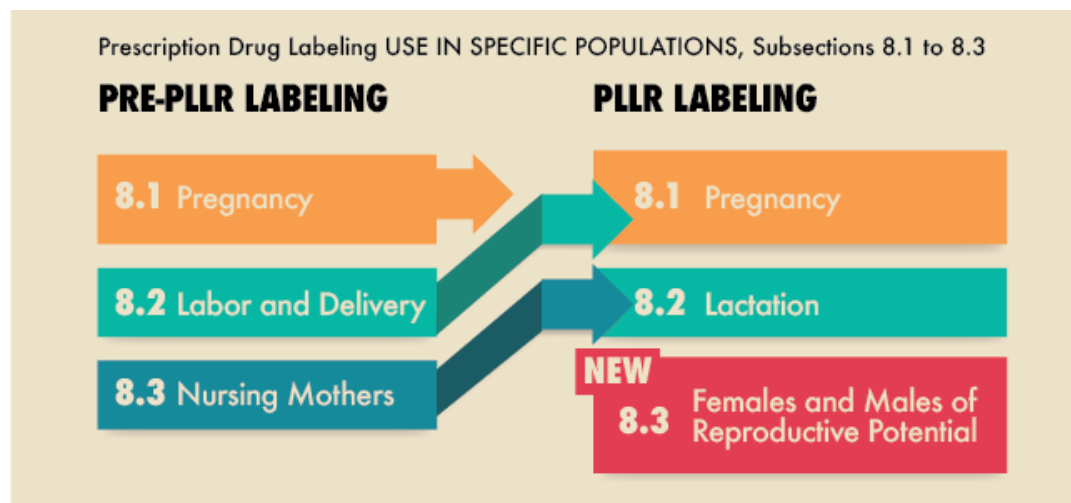
Category X: Studies in animals or human beings have demonstrated fetal abnormalities, or there is evidence of fetal risk based on human experience, or both, and the risk of the use of the drug in pregnant women clearly outweighs any possible benefit. The drug is contraindicated in women who are or may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

How to classify a teratogen?

Pregnancy and Lactation Labeling Rule (PLLR) since 2015 in USA

Instead of using an arbitrary lettering system, the PLLR provides detailed risk summaries and more comprehensive information derived from clinical experience (if available), animal data, and concerns related to the pharmacologic activity of the drug.

In addition, the label includes information on the risks associated with untreated illness. This information helps to put the potential effects of the drug into perspective with the goal of providing a more individualized risk-benefit analysis.



https://eu-rd-platform.jrc.ec.europa.eu/eurocat/prevention-and-risk-factors/medication-during-pregnancy_en

How to avoid teratogen?

- Talk with your healthcare provider about any medications you're taking.
- Avoid cigarettes, alcohol and recreational drugs.
- Don't take any supplements, medications or prescription drugs without checking with your healthcare provider.
- Avoid cleaning litter boxes.
- Avoid hot tubs, saunas and anything that raises your internal body temperature.
- Remove tuna, swordfish and other fish high in mercury from your diet.
- Talk with your supervisor or human resources about harmful chemicals in your workplace.



How to identify a teratogen?

<5%

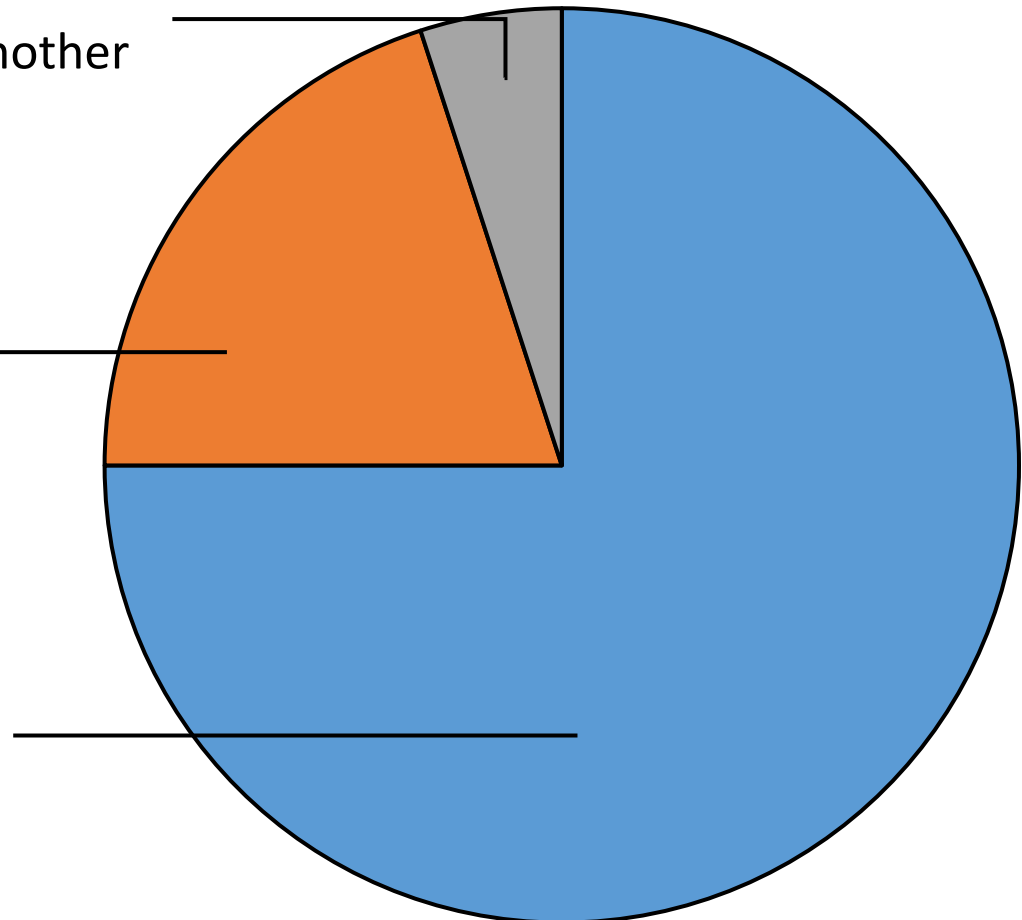
- Environmental
- Intrauterine infections
- Metabolic disorder of mother
- Drugs and medications
- Radiation

20-25%

Genetical

65-75%

Multifactorial or unknown

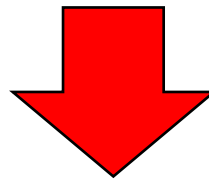


How to identify a teratogen?

- Animal studies
- Observations from human exposure

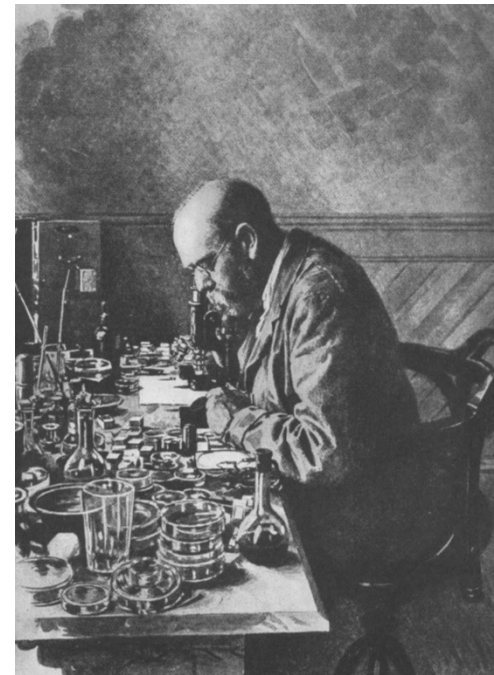
Koch's Postulates in microbiology:

- *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 or group of defects (syndrome).
- The increase of defects is associated with the use of a new drug or the widespread exposure 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

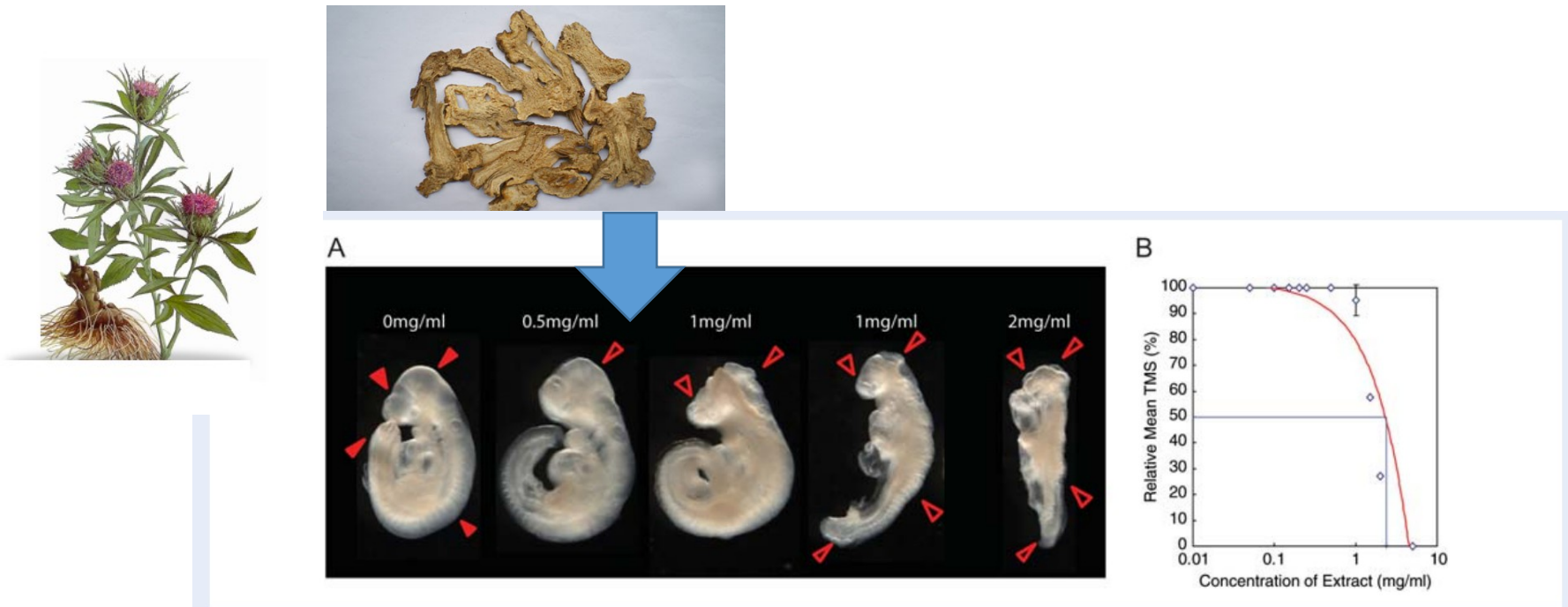


Figure 1 Embryotoxicity of LAR in mouse embryo culture. **(A)** Abnormal embryonic development and anomalies were observed in treatment groups from 0.5 to 2 mg/ml of LAR. Representative embryos are presented. Closed arrows represent the normal structures. Open arrows represent the malformed structures, 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/gas034

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^{1,†}, L. Li^{1,†}, A. Borchert², Clara B.S. Lau³, P.C. Leung³, and C.C. Wang^{1,4,*}

¹Department of Obstetrics and Gynaecology, The Chinese University of Hong Kong, Prince of Wales Hospital, Shatin, New Territories, Hong Kong; ²Institute of Biochemistry, University of Medicine Berlin, Charité, Berlin, Germany; ³Institute of Chinese Medicine, The Chinese University of Hong Kong, Shatin, New Territories, Hong Kong; ⁴School of Biomedical Sciences, The Chinese University of Hong Kong, Shatin, New Territories, Hong Kong

*Correspondence address. Tel: +852-2632-2810; Fax: +852-2636-0008; E-mail: ccwang@cuhk.edu.hk
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)

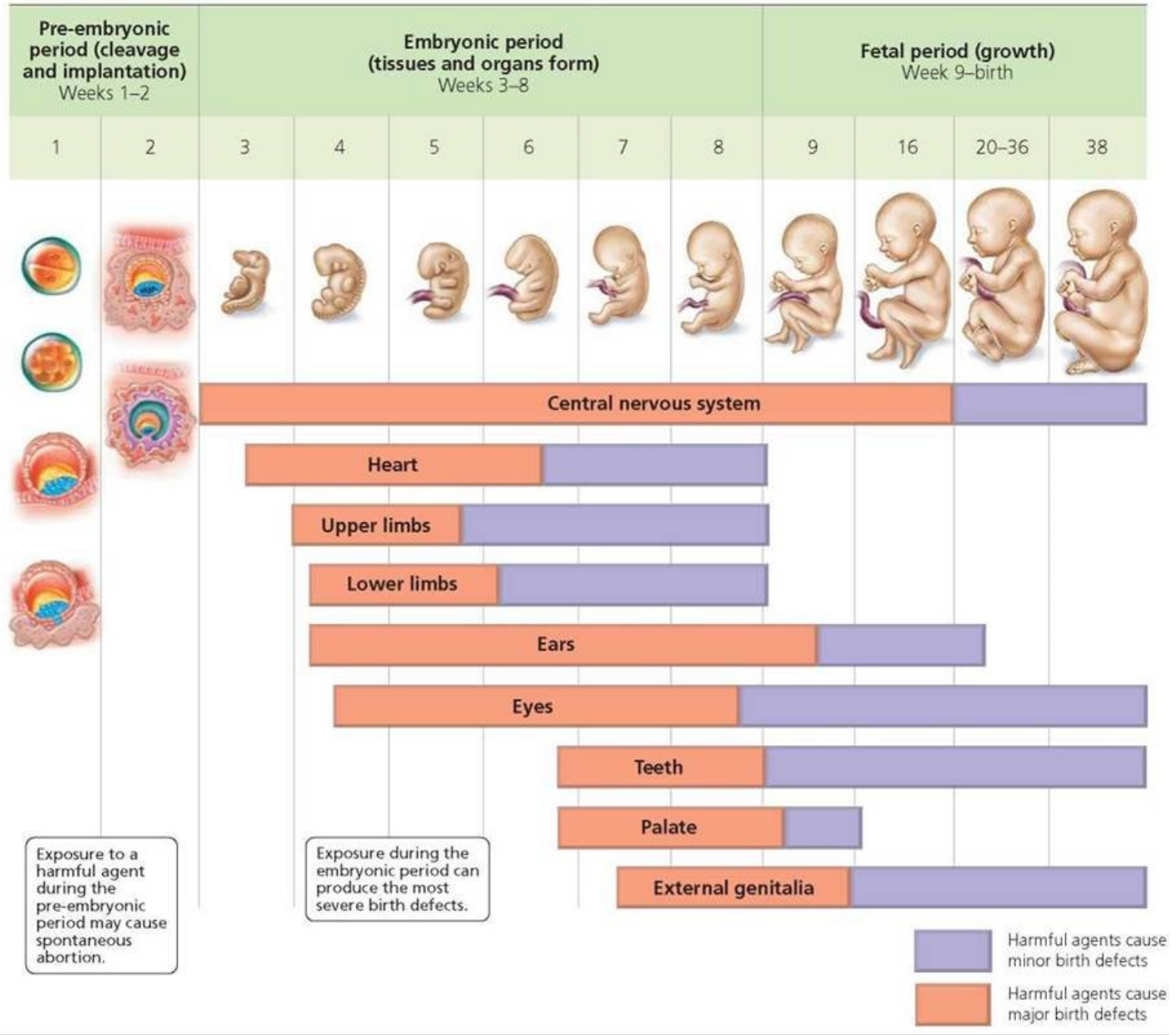
1. Susceptibility to teratogenesis depends on the **genotype** of the conceptus and the way in which this interacts with environmental factors.
2. Susceptibility to teratogenic agents varies with the **developmental stage** at the time of exposure.
3. Teratogenic agents act in **specific ways** (mechanisms) 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

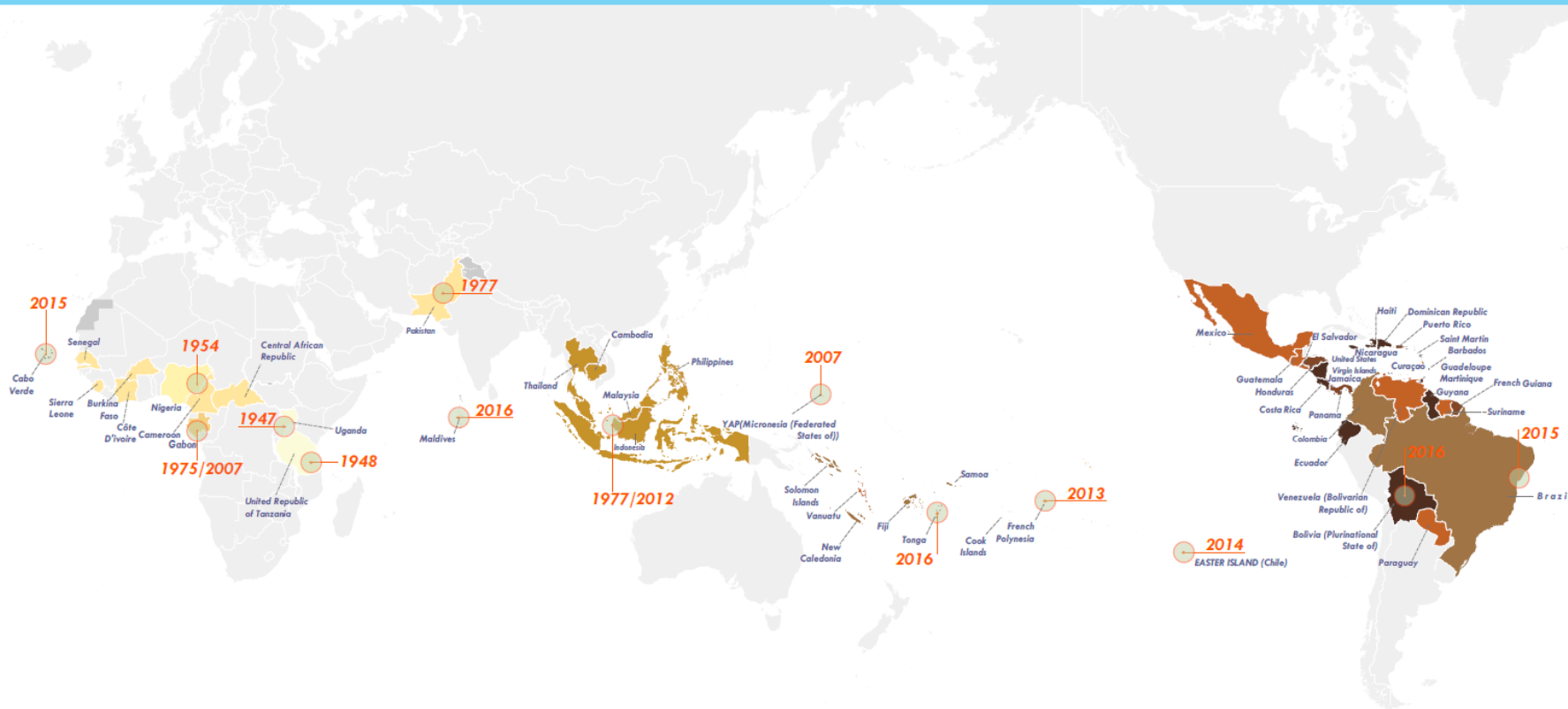
2010





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).

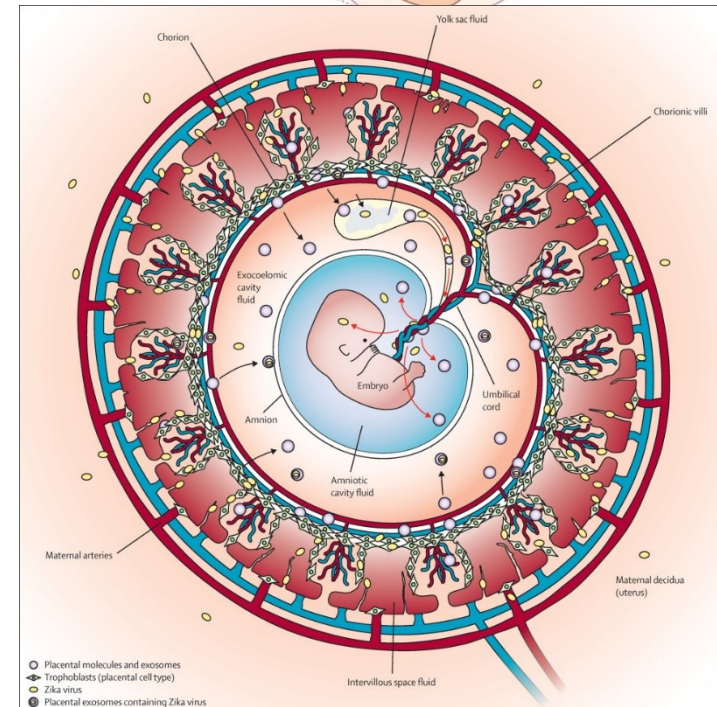
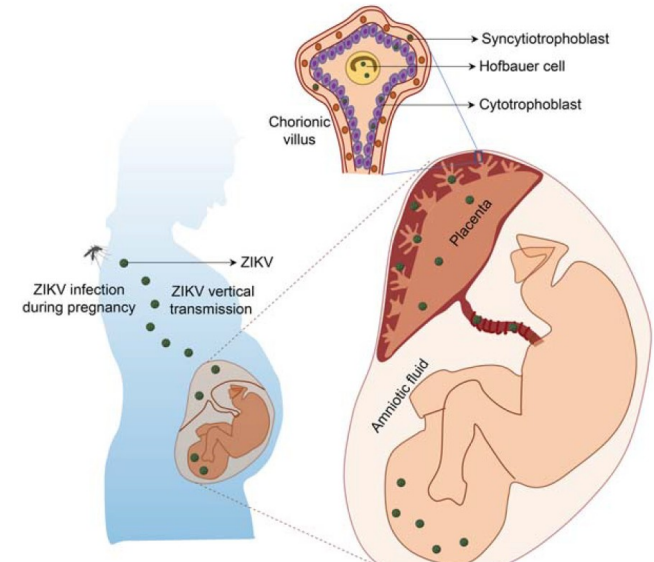
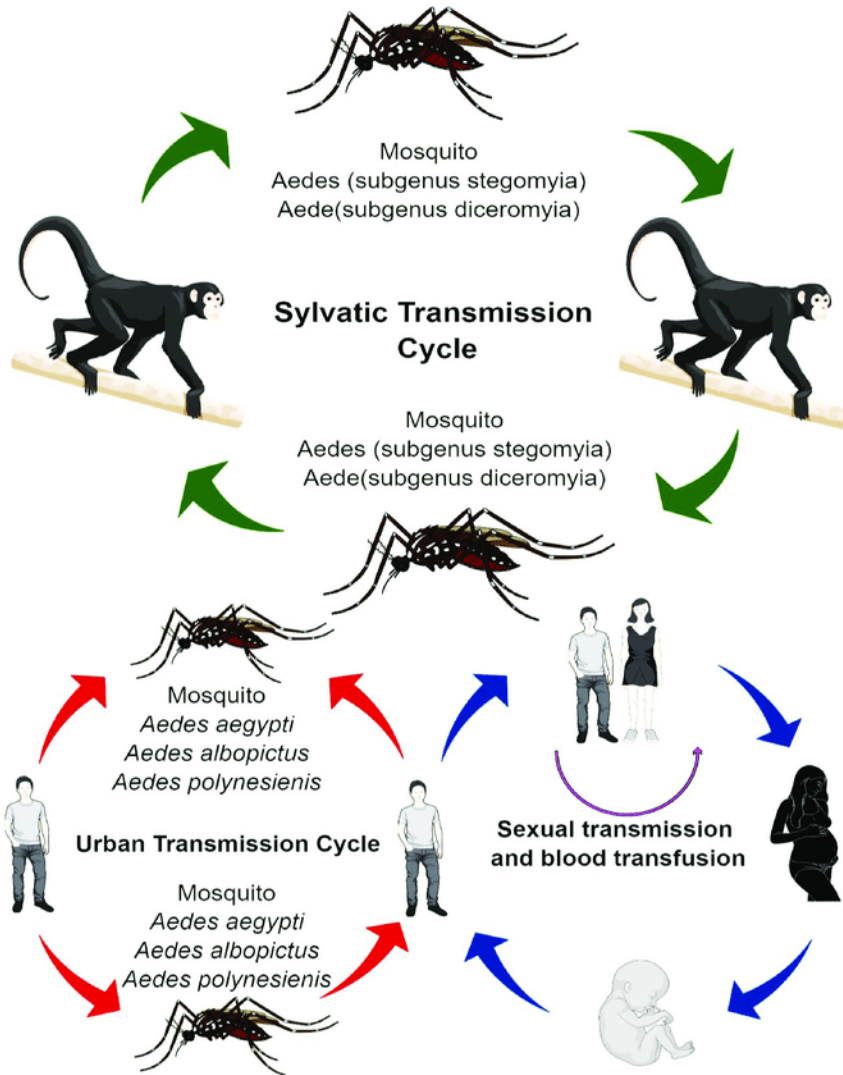
Coherence: Coherence between epidemiological and laboratory findings increases the likelihood of an effect.

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

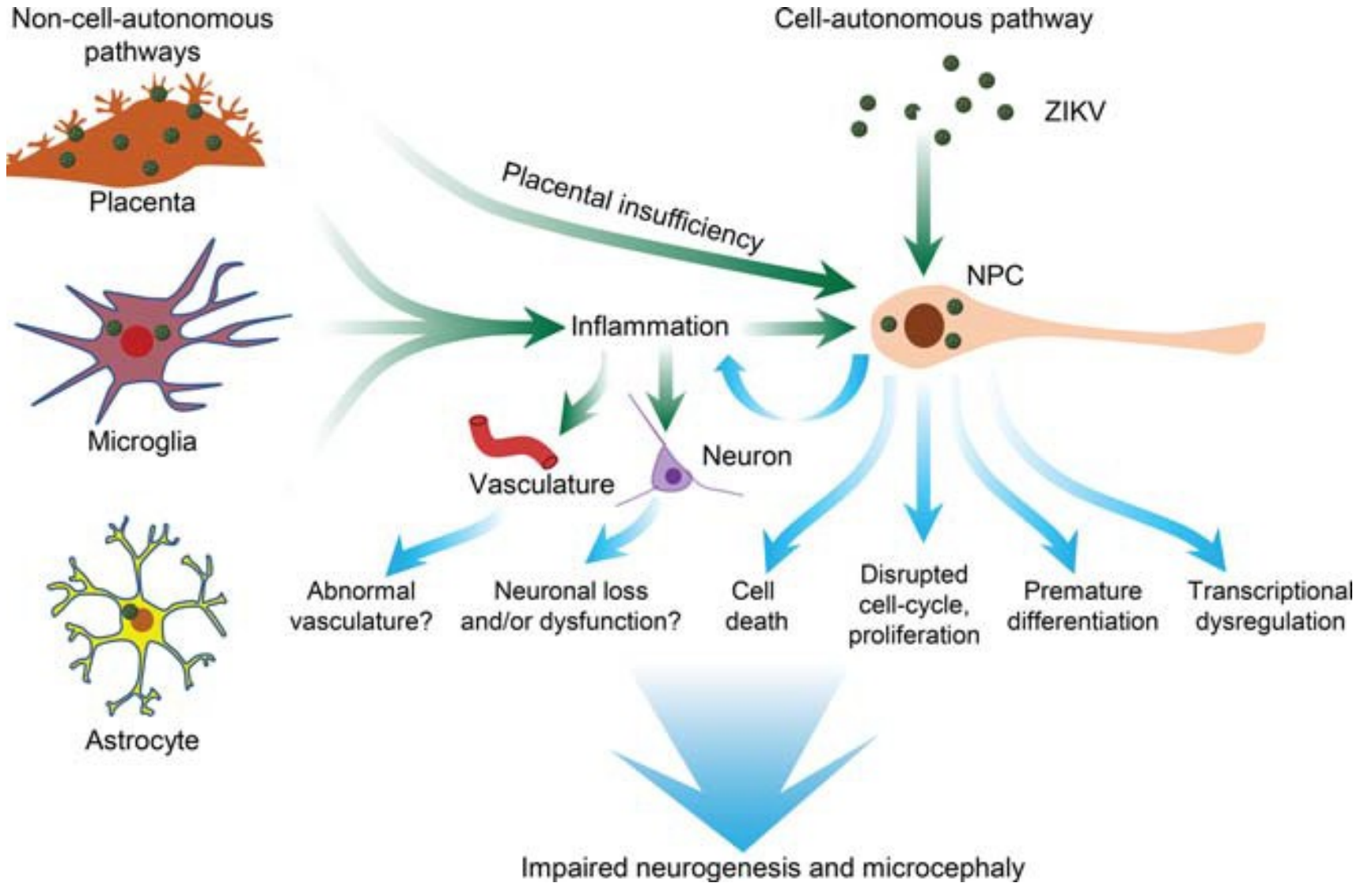
Analogy: The use of analogies or similarities between the observed association and any other associations.

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



Thank you for attention

pvanhara@med.muni.cz

RESEARCH

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

Rebecca M. Tashiro, Yuanhong Han, María J. Monteros, Joseph H. Bouton, and Wayne A. Parrott*