# Computational and Organotypic Modeling of Microcephaly

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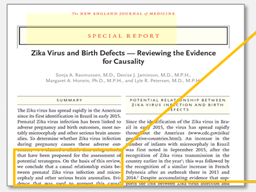
## Microcephaly and Maternal Zika Infection

- Cluster of birth defects in N Brazil linked to mosquito-borne Zika virus by the Brazilian Health Ministry (November) and validated by CDC (December) [1].

Science (2016)

N Engl J Med (2016)




"On the basis of this review, we conclude that a causal relationship exists between prenatal Zika virus infection and microcephaly and other serious brain anomalies."

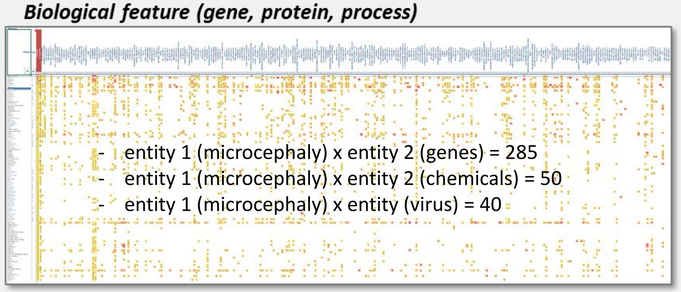
- Reduction in brain volume, ventricular dilations, brain calcifications, retinal defects, and placental insufficiency are all part of the congenital Zika story.
- A broader scientific need exists for Adverse Outcome Pathway (AOP) models of microcephaly because it has many possible causes [2]:
  - intrauterine infections (e.g., Rubella, CMV, ZikV)
  - inborn errors of metabolism (e.g., urea cycle, mitochondriopathies)
  - maternal smoking, drug and alcohol abuse
  - environmental chemicals (e.g., methylmercury)
  - genetic factors (autosomal recessive traits; microdeletions, duplications)
  - prenatal malnutrition, socioeconomic factors, ...
- OBJECTIVE: capture information on 'microcephaly' into an AOP framework.

## Microcephaly Information Retrieval

- MGI Mammalian Phenotype Browser: 'microcephaly' (MP:0000433) returns 85 gene associations including candidate genes for microcephaly in humans.
- ToxRefDB returns ~75 chemicals invoking dilated ventricles/hydrocephaly (39), and/or reduced brain size/cellular mass (40).
- *MicrocephalyConnections* tool sweeps literature (PubMed) to produce a multidimensional database of MeSH co-annotations (350,651 records).

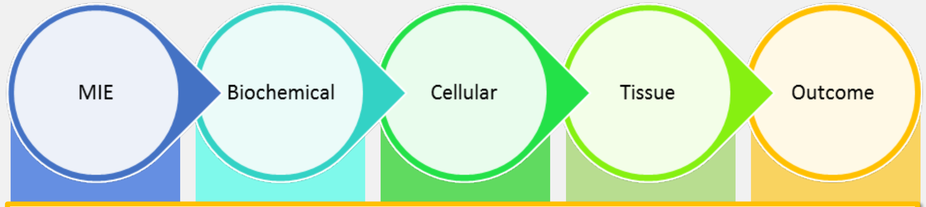
***Biological feature (gene, protein, process)***

***Stressor (chemical, virus)***




- ToxCast high-throughput screening (HTS) data for bioactivity profiling (https://actor.epa.gov/dashboard/), in progress.

## Adverse Outcome Pathway (AOP)




***KEY EVENT 4:*** *reduced neurogenic capacity in the ventricular zone of the brain, leading to cortical thinning and reduced brain size at the 2nd trimester.*

- Human brain size is determined by the number of neurogenic cells available to form neocortex and is a function of the precursor pool size of neuroprogenitor cells (NPCs) [3].
- NPCs self-replicate in the ventricular zone of the brain during 1st trimester; this growth period is followed by differentiation to form neocortex by the 2nd trimester [4].

***KEY EVENT 3:*** *altered neuroprogenitor growth kinetics, leading to hypoplasia of the neurogenic niche in the 1st trimester.*

- Zika virions infect hNPCs (but not hES cells or neurons) *in vitro,* and the resulting consequences on cell growth/apoptosis has a demonstrable effect on hNPC-derived neurosphere size [5,6,7].
- Chemical injury (alcohol, methylmercury) during rodent neurodevelopment alters NPC pool sizes via adverse effects on the growth kinetics (cell growth/migration/apoptosis) [8].

***KEY EVENT 2:*** *misorientation of hNPC mitotic division, leading to premature loss of neuroprogenitors from the proliferative cycle.*

- hNPCs divide symmetrically before switching to asymmetrical (neurogenic) divisions; premature switching (or apoptosis) results in loss of NPCs from the proliferative cycle [3,4].
- Alignment of the mitotic spindle determines the polarity of mitotic divisions to self-replicate hNPCs (equal division) or spinoff a daughter cell that enters the neurogenic lineage (unequal division) [9].

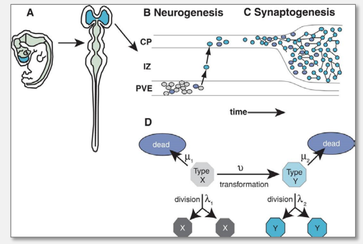
***KEY EVENT 1:*** *dysregulation of the centrosome cycle, leading to misalignment of the centrioles and the microtubule organizing center of the cell.*

- Many candidate genes for human 'primary microcephaly' function in the structural organization and regulation of the centrosome, containing two centrioles at right angles to each other [10].
- Transcriptomic analysis showed hNPCs express five genes for human primary microcephaly (MCPH1, ASPM, CENJ, STIL, CDSRAP2) as an indication of an centrosomal cycle for further investigation of this hypothesis.

***MOLECULAR INITIATING EVENT:*** *ToxCast bioactivity profiles are a resource for building predictive signature(s) for microcephaly.*

- 30 ToxRefDB chemicals that invoke decreases in brain developmental parameters had bioactivity profiles mapping to one or more targets in a ToxCast assay (top gene scores = p53, NRF2, PXR, AhR, HSF1, VDR, ...).
- Bioactivity profiles are being used to build predictive signatures for microcephaly that can be used in modeling the system, both *in silico* (computational) and *in vitro* (human brain mimics).

## Computational Model of Neurodevelopment




LEFT: model for the neurogenic switch to assess criticality of NPC loss on neurodevelopment [8]. BELOW: parameters to simulate NPC dynamics mathematically following chemical (or viral) exposure [9]. This model can be applied to simulate Key Events 3-4 in the AOP.

Parameterization and equations of the dynamic model for neurodevelopment

*Kolmogorov forward differential equation for transition probabilities*

Equation $\mathrm{d}P(x,y,t)/\mathrm{d}t = (x-1)\lambda_1(t)P(x-1,y,t) + (x+1)\mu_1(t)P(x+1,y,t) + (y-1)\lambda_2(t)P(x,y-1,t) + (y+1)\mu_2(t)P(x,y+1,t) + (x+1)\upsilon(t)P(x+1,y-1,t) - [x\lambda_1(t) + x\mu_1(t) + y\lambda_2(t) + y\mu_2(t) + x\upsilon(t)]P(x,y,t))$

Parameters $P(x,y,t) = P(\mathrm{X}(t)=x, \mathrm{Y}(t)=y|\mathrm{X}(t_0)=x_0, \mathrm{Y}(t_0)=0)$. X(t) and Y(t) denote the numbers of X and Y cells at time $t$, $x_0$ is the number of X cells at initial time $t_0$. $\lambda_1(t)$ and $\mu_1(t)$ are the reproduction and death rates for X cells, $\lambda_2(t)$ and $\mu_2(t)$ are the reproduction and death rates for Y cells, $\upsilon(t)$ is the rate of transformation of X cells to Y cells

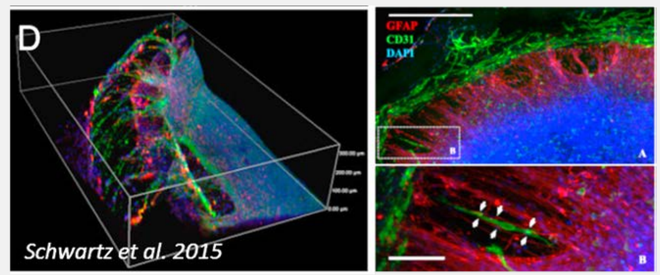
*Matrix to approximate solution of the above equation*

Matrix
$$\begin{matrix} \varepsilon_1(t) & 0 & 0 & 0 & 0 \\ \upsilon(t) & \varepsilon 2(t) & 0 & 0 & 0 \\ \lambda_1(t)+\mu_1(t)+\upsilon(t) & 0 & 2\varepsilon_1(t) & 0 & 0 \\ \upsilon(t) & \lambda_2(t)+\mu_2(t) & 0 & 2\varepsilon_2(t) & \upsilon(t) \\ -\upsilon(t) & 0 & \upsilon(t) & 0 & \varepsilon_1(t)+\varepsilon_2(t) \end{matrix}$$

Parameters $\varepsilon_1(t) = \lambda_1(t) - \mu_1(t) - \upsilon(t)$ and $\varepsilon_2(t) = \lambda_2(t) - \mu_2(t)$

## Human Brain Mimics

Dynamics of hNPC growth, migration, and apoptosis for the computational model can be assessed in miniorganoids developed from hNPCs + iPSC-derived endothelial and microglia [11]. Studies are planned to develop human brain mimics from microcephalic patient-derived iPSCs and to provide evidence for Key Events 1-3 in the AOP for chemicals and Zika.

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