Computational and Organotypic Modeling of Microcephaly

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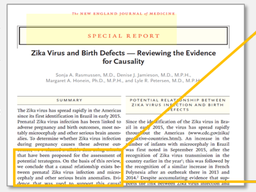
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Microcephaly and Maternal Zika Infection

- 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)



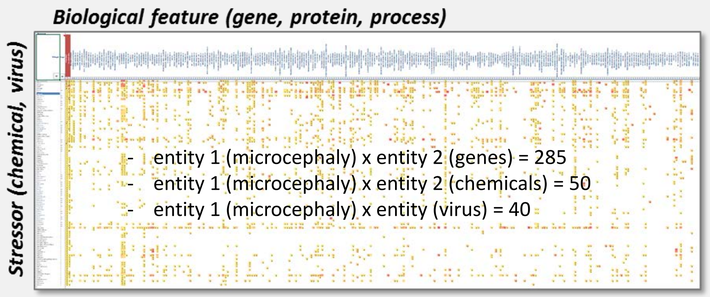
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"On the basis of this review, we conclude that a causal relationship exists between prenatal Zika virus infection and microcephaly and other serious brain anomalies."

- 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.

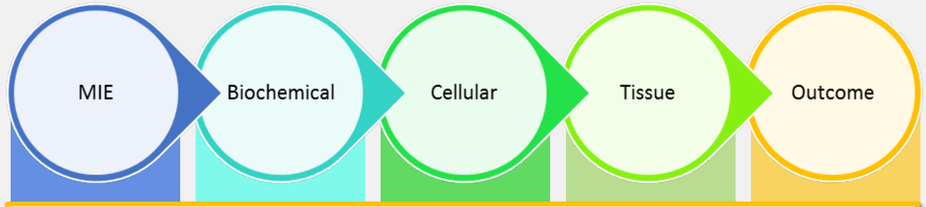
Microcephaly Information Retrieval

- 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).



- 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].

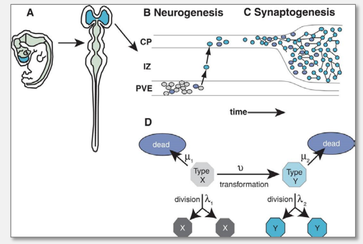
KEY EVENT 1: *dysregulation of the centrosome cycle, leading to misalignment of the centrioles and the microtubule organizing center of the cell.*

- 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

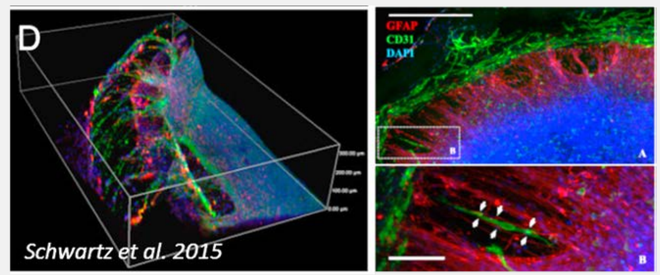
Matrix to approximate solution of the above equation

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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Support is acknowledged from EPA/NCCT/CSS and co-operative agreements with EPA-G2013-STAR-L1 grants 835737 (Univ. Wisconsin) and 835738 (Univ. Washington).