Causal Inferences from ToxCast Data: molecular pathways and cellular processes for cleft palate

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Overview

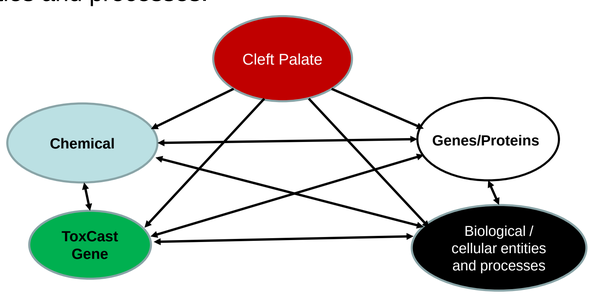
Cleft palate (CP), a common human birth defect, is often observed in prenatal developmental toxicity studies. Sixty-three chemicals in ToxCastDB have been linked to CP in ToxRefDB or the biomedical literature. These compounds are structurally diverse and thus may perturb prenatal development in mechanistically diverse ways.

An integration and visual analysis of the high-throughput screening data with chemical structure features along with application of automated literature mining provide a new approach to elucidate molecular pathways and cellular processes in adverse outcome pathways leading to cleft palate.

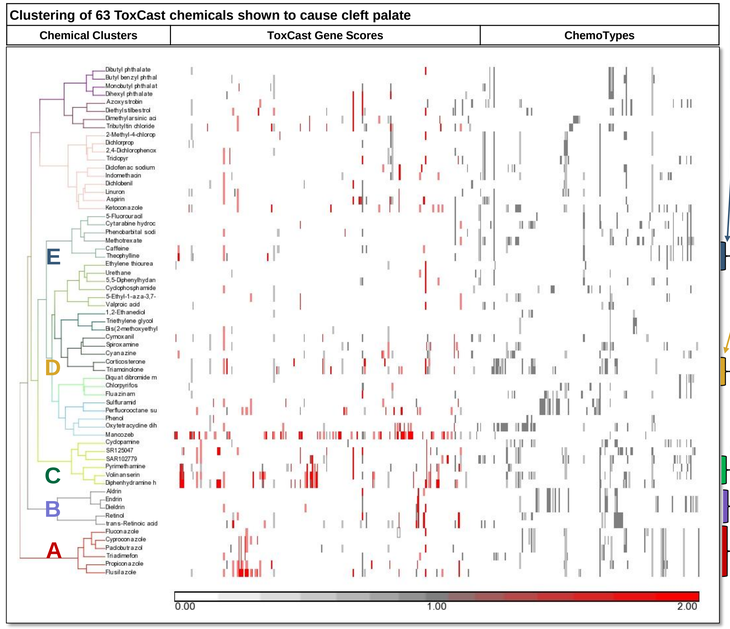
Methods

Construction and clustering of dataset with ToxCast results and chemical structural descriptors	
287 ToxCast Gene Scores	**229 chemical structural descriptors (chemotypes)**
0 No activity	0 Chemical does not have chemotype
1 Activity in cytotoxicity region (nonspecific)	1 Chemical has chemotype
2 Activity in pre-cytotoxicity region (specific)	

The dataset was clustered using Partek (Pearson's dissimilarity and Ward's method) to identify coherent clusters. Comprehensive mapping was performed on each cluster to mine the literature for relationships between chemical, ToxCast assay target (gene), cleft palate, genes associated with cleft palate and biological / cellular entities and processes.



Results



Cluster E: Genes contributing to cluster

Symbol	Name
ADORA2A	adenosine A2a receptor
COL3A1	Collagen, type III, alpha 1

Cluster D: Genes contributing to cluster

Symbol	Name
COL3A1	Collage, type III, alpha 1
IL6	Interleukin 6
CSF1	Colony stimulating factor
NR3C1	Glucocorticoid receptor
SAA1	Serum amyloid A1

Cluster C: Genes contributing to cluster

Symbol	Name
ADRA1B	adrenergic receptor, alpha 1b
ADRA2B	adrenergic receptor, alpha 2b
HRH2	Histamine receptor H2
HTR2C	Serotonin receptor 2C
HTR7	Serotonin 7
SCN1A	Sodium channel, voltage-gated, type I a subunit
SIGMAR1	Sigma non-opioid intracellular receptor 1

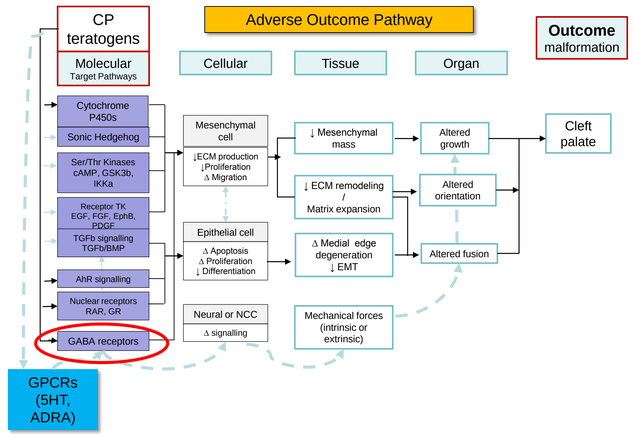
Cluster B: Genes contributing to cluster

Symbol	Name
RARG	**Retinoic acid receptor gamma**
RXRB	Retinoic X receptor beta
NR1I2	Nuclear receptor subfamily 1, group I, member 2
RARB	Retinoic acid receptor beta
RXRA	Retinoic X receptor alpha

Cluster A: Genes contributing to cluster

Symbol	Name
CYP2A2	cytochrome P450, family 2, subfamily a, polypep 2
CYP2B1	cytochrome P450, family 2, subfamily b, polypep 1
CYP2C19	cytochrome P450, family 2, subfamily C, polypep 19
CYP2C13	cytochrome P450, family 2, subfamily c, polypept 13

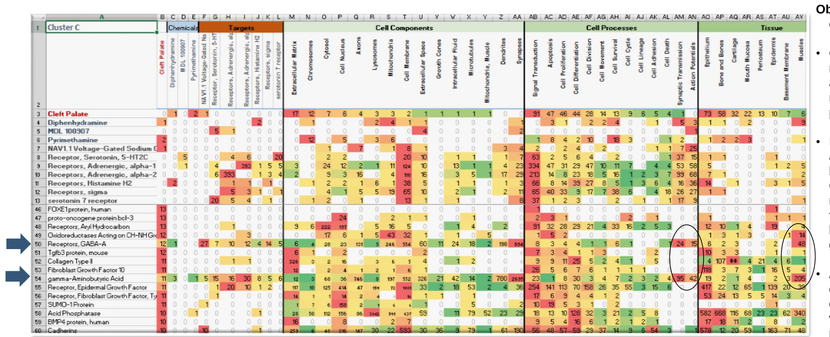
Enhancing an AOP framework for cleft palate



Literature Mining Example: 'Cluster C' *e*-library highlights

Observations and connections:

- The 3 chemicals in Cluster C are not clearly associated with CP in the literature, nor are their targets.
- ToxCast targets for Cluster C are, however, connected through literature to GABA, a receptor known to be linked mechanistically with CP.



Observations and connections:

- GABA and its receptor is highly associated with neuromuscular physiology.
- Evidence points to a connection between CP and GABA-dependent neuromuscular function and cycles back to the ToxCast gene target.
- *Hypothesis:* chemicals in this cluster invoke CP via a GABA-ergic pathway, and the downstream biological effects are mediated by neuromuscular or neural crest contractility.

Conclusions

- We clustered 63 cleft palate toxicants by integrative mining of ToxCastDB bioactivity and chemotype.
- For 5 of the most coherent clusters, literature mining found plausible links between CP and ToxCast target.
- One example (Cluster C) identified a putative AOP for GABA signaling and CP.
- A computational approach integrating *in vitro* profiling data, chemical structure descriptors, and knowledge from the literature is generalizable to any system.

DISCLAIMER: does not reflect EPA policy

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