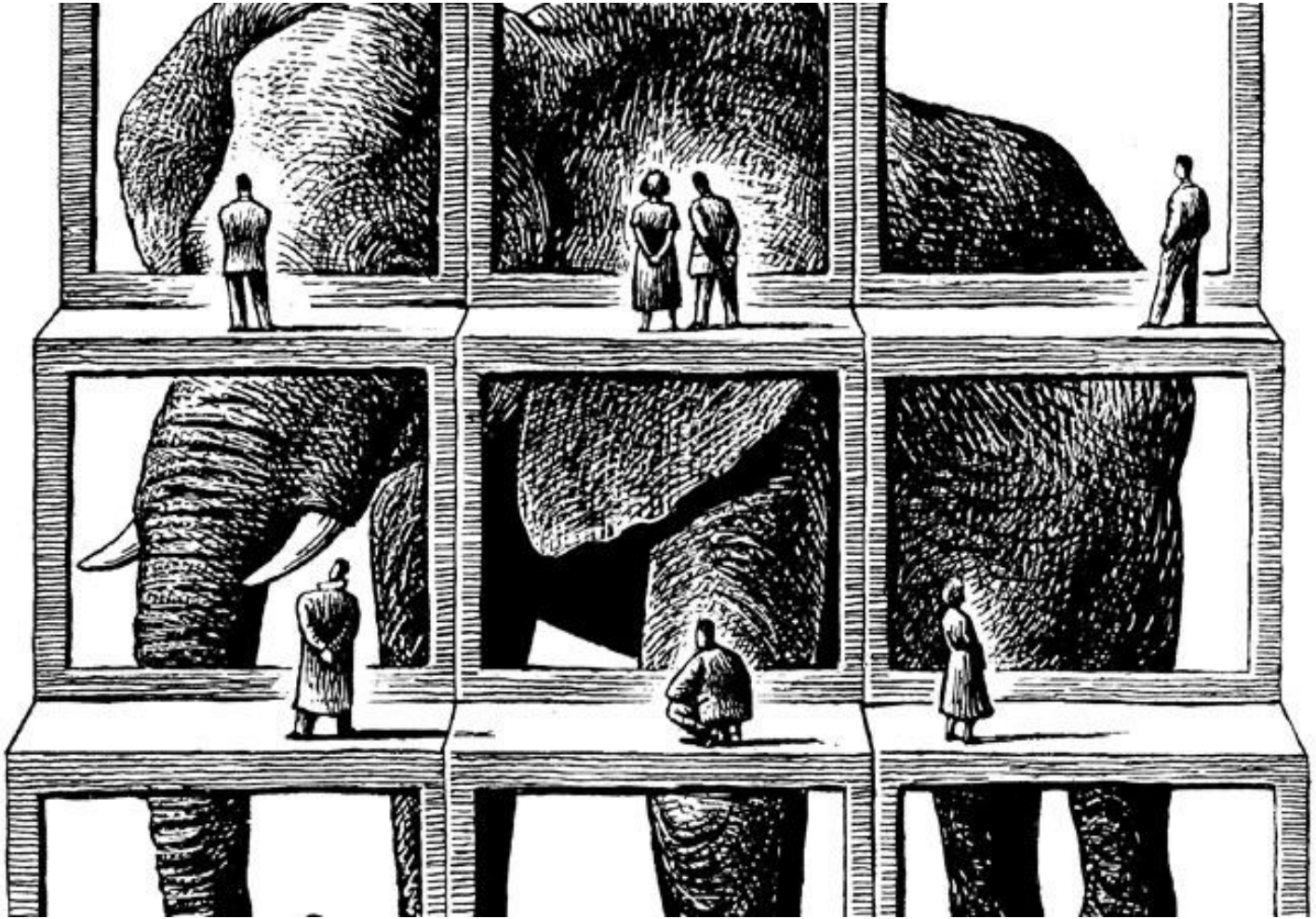


CONTEXT MATTERS!

Identification of cis-suppression of human disease mutations by comparative genomics

Group A

Team Members

Xiaoxiao Hou

Kevin Deng

Christine Mai

Pallavi Menon

Roadmap

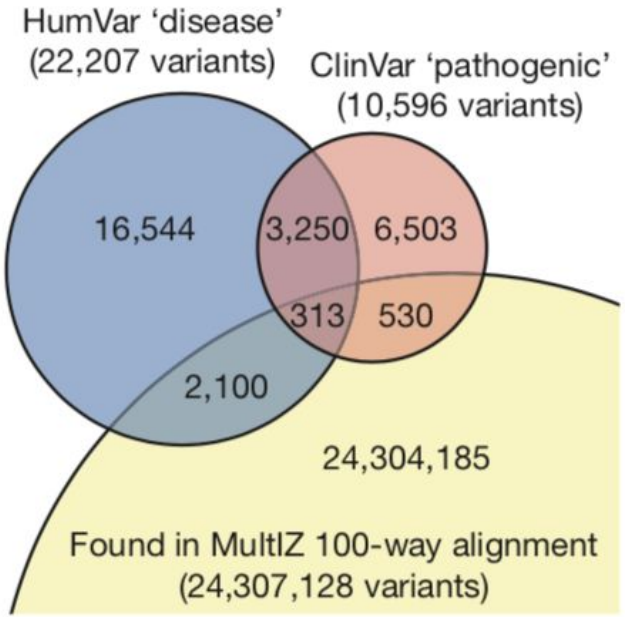
- Introduction to CPDs
- Structure of genetic interactions
- How CPDs arise
- One large-effect compensatory substitution
- Functional testing
  - BBS4 and RPGRIP1L
  - BTG2 zebrafish
- Conclusion

Acronyms

- CPDs - Compensatory Pathogenic Deviations
- SNVs - Single Nucleotide Variants
- WES - Whole Exome Sequencing
- MO-mediated - Morpholino-mediated

Genomic Context Matters

- Conserved regions => intolerant of variation but no genomic context of the mutated allele
- CPD: allele damaging in one sequence but neutral in orthologous sequence of diff. species
- Comparative genomics: fraction of pathogenic variants that are present as CPDs in other species ranges from 2–18%
- Two data sets of missense SNVs: HumVar and ClinVar 69,905 human missense mutations across 13,040 genes
- Pathogenic missense variants: 5.6% ± 0.5% of ClinVar variants and 6.7% ± 0.4% of HumVar variants found in the alignment of mammals



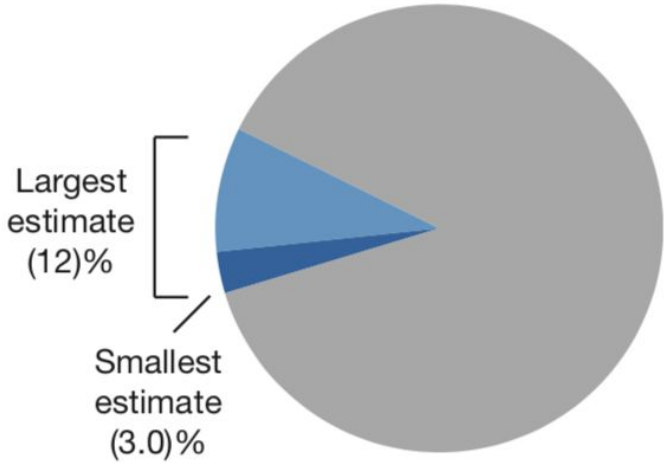
Testing for false pathogenic annotations

Cross referencing with population freq. data

Requiring variants in multiple species

Alt. alignment methodology

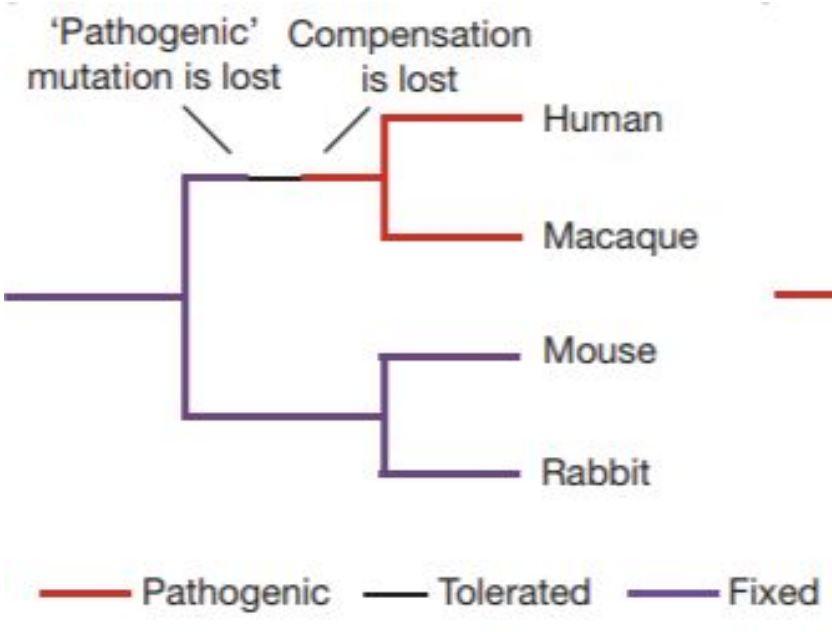
Alignment quality



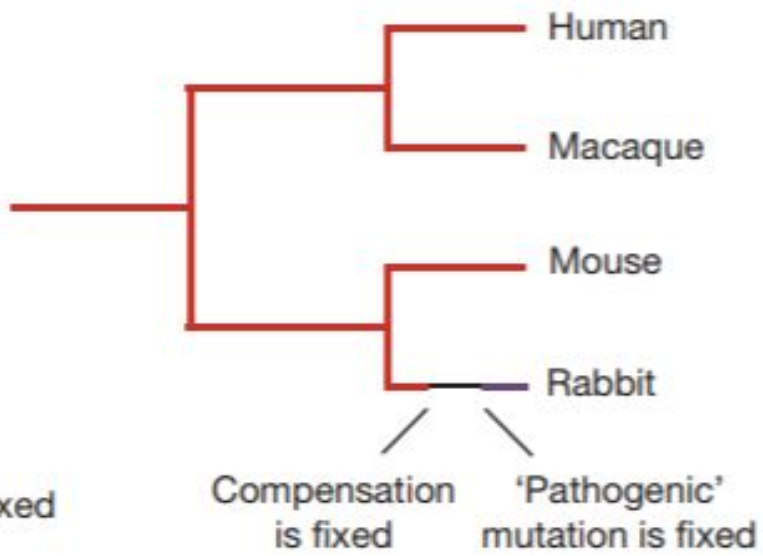
Structure of the genetic interactions

- Distribution of CPDs across the orthologous sequences reveals differences
- Examine sequence distance to estimate evolutionary time
- CPD presence necessitates all required compensatory substitutions - evolutionary time is the sum

How do CPDs arise?



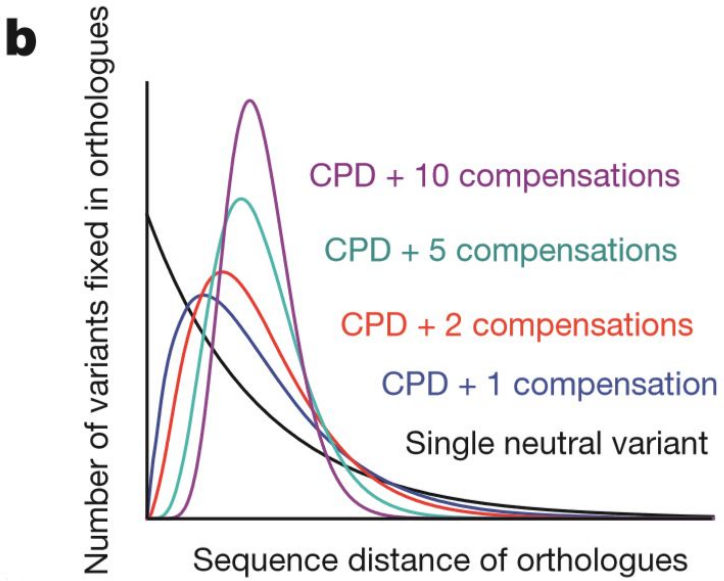
Option 1



Option 2

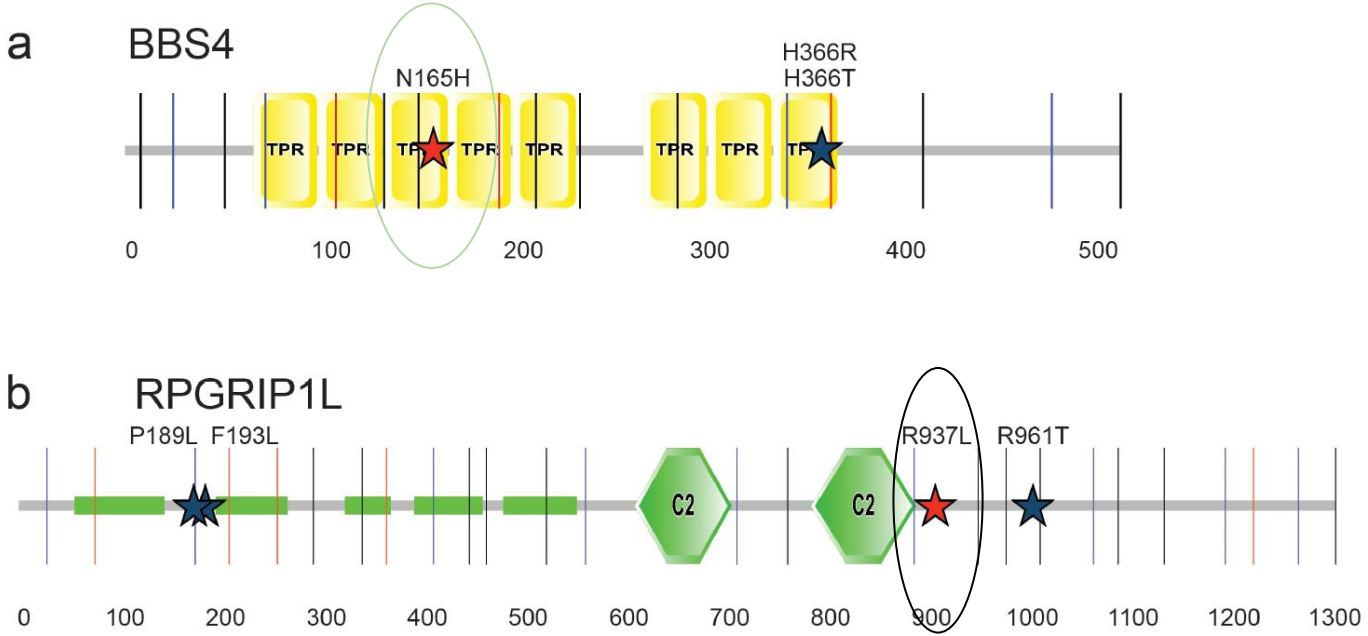
Hypothesis - most CPDs could be rescued by one large-effect compensatory substitution.

- Sequence distance used to approx. evolutionary time difference
- Shape of distribution reveals relative number of compensatory substitutions
- Observed CPD distribution on average has shorter evolutionary distances
- Convolution of 2 distributions fits best - pairwise compensatory interactions



BBS4 and RPGRIP1L involved in ciliopathies

Contribute to Bardet–Biedl syndrome and Meckel–Gruber syndrome



Multiple species with the human mutant allele fixed

a

BBS4	N160	A161	L162	N163	L164	N165	H366
H. sapiens	N	A	L	N	L	N	H
T. belangeri	H	•	•	Q	•	H	N
O. cuniculus	S	•	•	Q	•	H	R
O. princeps	S	•	•	Q	•	H	R
E. telfairi	S	•	•	Q	•	H	R
X. tropicalis	L	•	•	Q	•	H	S
T. nigroviridis	R	•	•	Q	•	H	T

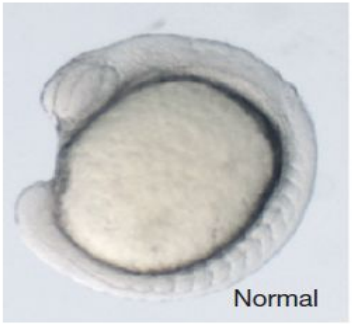
b

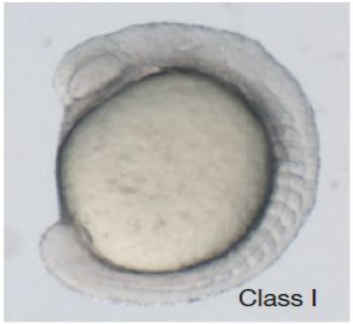
RPGRIP1L	P189	F193	I936	R937	S938	R961
H. sapiens	P	F	I	R	S	R
E. caballus	L	L	•	L	K	T
L. africana	L	L	•	L	•	T
P. capensis	L	L	•	L	N	T
T. manatus	L	L	•	L	•	T

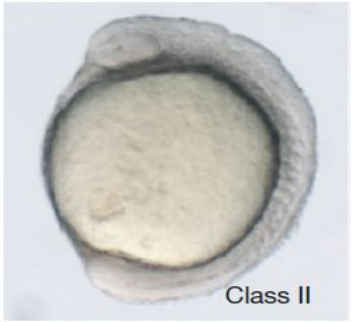
The pathogenic BBS4 165H allele is fixed in six species.

The pathogenic RPGRIP1L 937L allele is fixed in four species.

Established convergent extension defects



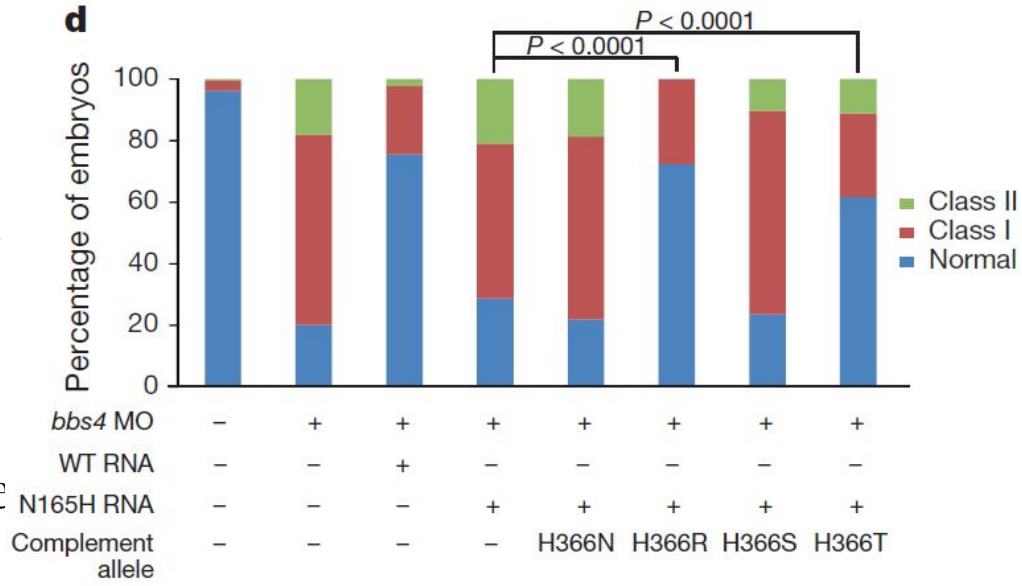




Examples of zebrafish convergent extension phenotypic groups

366R or 366T can rescue the loss of function observed in165H mutation

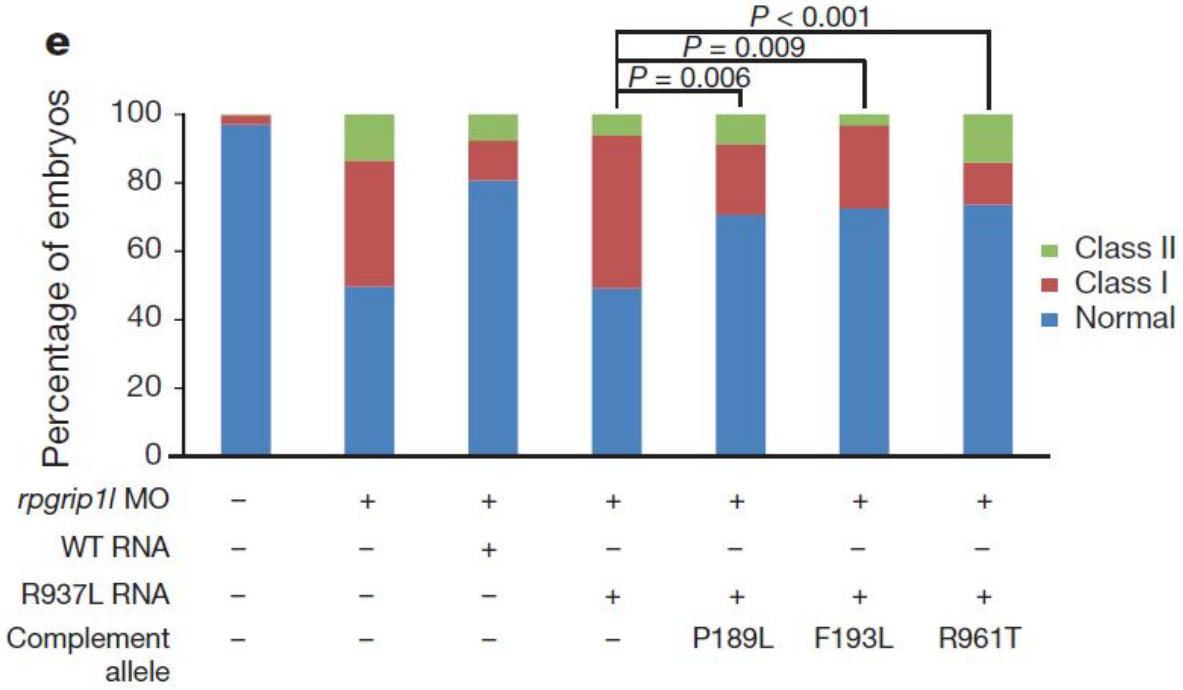
- Co-injection of MO with human wild-type mRNA rescued this phenotype,
- injection with human mutant mRNA showed no improvement
- The 165H/366N and the 165H/366S behaved as null,
- whereas 165H/366R was indistinguishable from wild type
- 165H/366T converted the functional null to a hypomorph



Mutation of 189L, 193L or 961T rescues the loss of function observed in 937L RNA

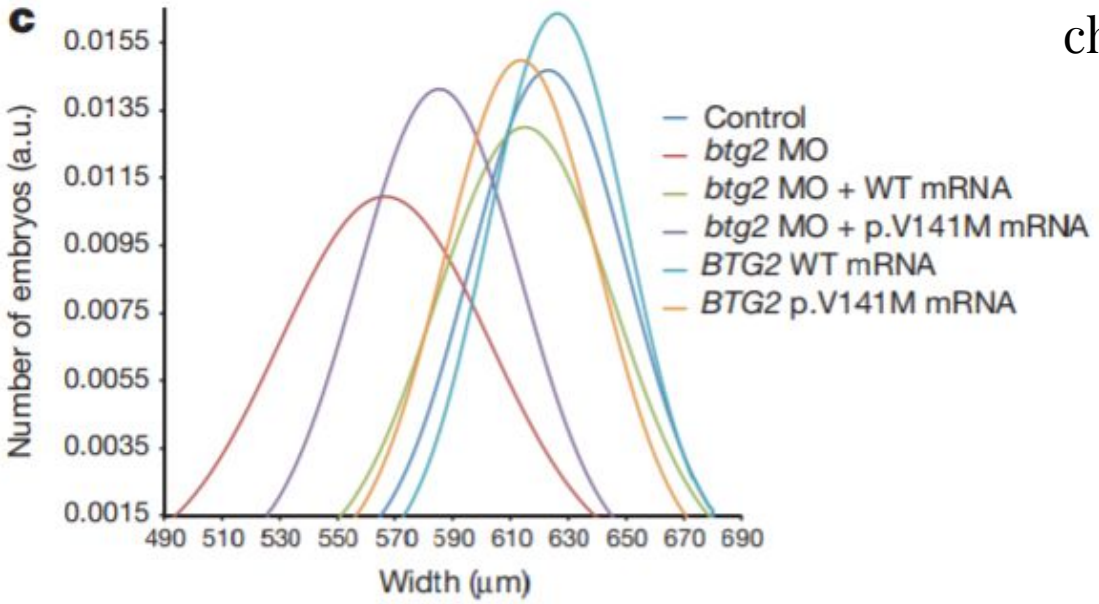
similar pattern for RPGRIP1L

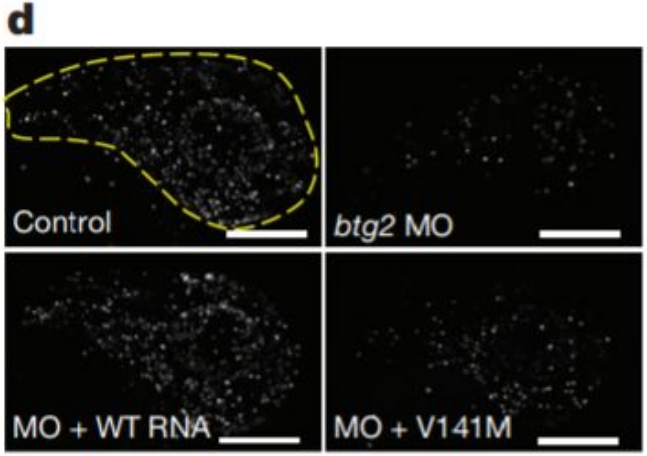
three complementing events (189L, 193L and 961T)



BTG2 Zebrafish

BTG2 is a regulator of cell cycle checkpoint in neuronal cells





V141 as a CPD site in primates

f

BTG2	R80	L128	Q140	V141	L142
H. sapiens	R	L	Q	V	L
P. troglodytes	•	•	•	•	•
G. gorilla	•	•	•	•	•
M. musculus	K	V	•	M	M
R. norvegicus	K	V	•	M	M
H. glaber	•	V	•	M	M
S. domesticus	K	V	•	M	M
B. primigenius	K	V	•	M	M
E. ferus caballus	K	V	•	M	M
F. catus	K	V	•	M	M
C. lupus familiaris	K	V	•	M	M
D. novemcinctus	K	V	•	M	M
G. gallus	K	P	•	M	M

- p.V141M allele was predicted computationally to be benign
- Likely reason: most BTG2 orthologues encode Met at orthologous position (exception of primates)
- Suggestion: V141 might be a CPD site in primates that branched from methionine

Conclusion

Genome editing to model human pathogenic mutations in a variety of model organisms has highlighted the critical need to not only pair computational predictions with functional studies, but also to evaluate the effect of human mutations in the context of the human sequence

"In the end, it looks like you can shield mutations with a single change elsewhere in the same gene, creating a single champion." -Katsanis

Acknowledgements

‘O Exalted One’

Dr. Nicholas Katsanis

World Hellenic Biomedical Association WHBA, Inc.

- **Authors of the Original Paper**
- **Esteemed Faculty**
- **Peers**