DOCUMENT SUMMARY

This paper uses an innovative zebrafish model to investigate the function of the human-specific duplicated gene *SRGAP2C*, a key factor in human brain evolution. Its relevance to Enlitens is profound and twofold. First, it provides a powerful biological basis for neurodiversity, demonstrating how a single gene duplication can profoundly alter the balance of excitatory/inhibitory neurons, change the developmental trajectory of brain immune cells (microglia), and even impact the visual system, leading to a distinct neurotype with different sensory processing. Second, the study's entire premise—that the standard mouse model was insufficient for research due to "embryonic lethality"—is a powerful analogy for Enlitens' mission. Just as researchers needed a new model (zebrafish) to see what the standard model was hiding, Enlitens uses a new assessment model (the clinical interview) to understand the complexities of individuals that standardized tests were never designed to capture.

FILENAME

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METADATA

- **Primary Category**: NEURODIVERSITY
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CRITICAL QUOTES FOR ENLITENS

"However, the significance of SRGAP2 duplication beyond neocortex development has not been elucidated due to the embryonic lethality of complete Srgap2 knockout in mice."

"Using zebrafish, we showed that srgap2 knockout results in viable offspring that phenocopy "humanized" SRGAP2C larvae."

"Together, our functional characterization of zebrafish Srgap2 and human SRGAP2C in zebrafish uncovered novel gene functions and highlights the strength of cross-species analysis in understanding the development of human-specific features."

"The embryonic lethality of complete Srgap2 loss-of-function in mouse models 27 has limited global assessments of its functions in development."

"Here, we generated zebrafish srgap2 "knockout" models resulting in viable offspring, providing us an opportunity to characterize SRGAP2 developmental functions beyond the neocortex."

"Leveraging our viable larvae, we found zebrafish mutants exhibited increased susceptibility to seizures, a screen not possible in the embryonic-lethal mice, strengthening findings of SRGAP2 as an epilepsy gene 28."

"These results are largely consistent with a clinical report of early infantile epileptic encephalopathy in a human child carrying a reciprocal translocation disrupting SRGAP2 28, providing evidence that mutations of this gene may contribute to epilepsy."

"We note that the embryonic lethality of Srgap2 knockout mice has impeded similar evaluations in mammalian models to date."

"While we cannot rule out that mutant microglia were more activated, we propose microglia exhibited developmental delay similar to that observed in synaptic spine maturation in mice 21. Indeed, a recent preprint 85 showed similar microglia neoteny in SRGAP2C mouse and human cell models."

KEY STATISTICS & EVIDENCE

- **Excitatory/Inhibitory Imbalance**: Both *srgap2* knockout and *SRGAP2C*-injected larvae showed a ~20% increase in the ratio of excitatory to inhibitory neurons in single-cell RNA sequencing data. This was validated with imaging, which showed a ~29% increase in the ratio.
- **Seizure Susceptibility**: SRGAP2C larvae experienced spontaneous, unprovoked ictal-like electrical events, classifying them as epileptic, while control and knockout larvae did not. The LFP scores for SRGAP2C larvae were in the range observed in zebrafish models of established epilepsy genes.
- **Altered Microglia Morphology**: Microglia in both knockout and humanized models showed significantly reduced ramifications (increased sphericity) compared to controls at 3 and 7 days post-fertilization. Control microglia became more ramified as they matured, while mutant microglia retained a more spherical, immature shape, suggesting arrested development.
- **Increased Visual Sensitivity**: Both knockout and humanized larvae showed a significant increase in response to light stimulus compared to controls, suggesting higher sensitivity to light changes.
- **Enhanced Optomotor Response**: A larger percentage of knockout and humanized larvae showed a positive optomotor response (aligning with moving stripes) compared to the control group, suggesting more sensitive neuronal responses to visual cues.
- **Cross-Species Gene Overlap**: There was a significant overlap in differentially expressed genes between human/primate microglia and the zebrafish SRGAP2 mutants (Fisher's test odds ratio = 2.77, p-value = 0.0046). A similar significant overlap was found for retinal cells (69 overlapping genes, Fisher's test odds ratio = 6.23, p-value < 2.2×10^{-16}).

METHODOLOGY DESCRIPTIONS

Using a "Non-Standard" Model to Overcome Limitations

The paper's core methodological innovation is the use of zebrafish to overcome the limitations of the standard mouse model, which is directly analogous to Enlitens' use of clinical interviews to overcome the limitations of standardized tests. "However, the significance of SRGAP2 duplication beyond neocortex development has not been elucidated due to the embryonic lethality of complete Srgap2 knockout in mice." "The embryonic lethality of complete Srgap2 loss-of-function in mouse models 27 has limited global assessments of its functions in development. Here, we generated zebrafish srgap2 "knockout" models resulting in viable offspring, providing us an opportunity to characterize SRGAP2 developmental functions beyond the neocortex." "We compared phenotypes with SRGAP2C-expressing "humanized" larvae by performing morphological, gene expression, cellular, molecular, and behavioral assays." "We consistently observed concordant effects in srgap2 knockout and SRGAP2C-humanized larvae across all assays, demonstrating that human-specific SRGAP2C antagonizes zebrafish Srgap2 functions."

THEORETICAL FRAMEWORKS

Gene Duplication as a Driver of Human-Specific Traits

The study is framed within the theory that the duplication of genes, particularly those unique to the human lineage, is a major driver of human evolution and the development of human-specific traits, especially in the brain. "Recent expansion of duplicated genes unique in the Homo lineage likely contributed to brain evolution and other human-specific traits." "One hallmark example is the expansion of the human SRGAP2 family, resulting in a human-specific paralog SRGAP2C." "Together, these studies support the contribution of SRGAP2C to the emergence of unique neuronal features and cognitive capacities in humans."

Neoteny in Brain Development

The paper discusses the concept of neoteny (the retention of juvenile or immature features) as a key outcome of *SRGAP2C* function. This is a crucial concept for reframing developmental "delay" as a potentially adaptive trait that increases plasticity. "As a result, expressing human-specific SRGAP2C in mouse models consistently phenocopies Srgap2 knockdown/knockout, including increased rate of neuronal migration, neurite outgrowth, increased density of dendritic spines, and neoteny in the spine maturation process 21." "These results align with results observed in Srgap2 knockdown or SRGAP2C-expressing mouse embryos that exhibit neoteny of synaptogenesis 18." "While we cannot rule out that mutant microglia were more activated, we propose microglia exhibited developmental delay similar to that observed in synaptic spine maturation in mice 21. Indeed, a recent preprint 85 showed similar microglia neoteny in SRGAP2C mouse and human cell models."

POPULATION-SPECIFIC FINDINGS

Cross-Species Analysis of Microglia

The study directly compares microglia between humans (who have SRGAP2C) and other primates (who do not), finding that the changes observed in the "humanized" zebrafish model reflect actual evolutionary differences in these cell types. "To examine if SRGAP2/C might contribute to human-specific microglia membrane dynamics, we re-analyzed published single-cell transcriptomes of 610,596 prefrontal cortex cells from human, chimpanzee, macaque, and marmoset 64." "In line with its conserved "core" characterization 54, SRGAP2 exhibited highest expression in the microglia clusters in all primates (Figure 4C), including human- and hominidae- specific microglia subclusters (Figure 4D, Note S2, Table S18)." "Taking an analogous pseudo-bulk approach to our zebrafish analysis, we compared gene expression of human (+SRGAP2C) versus chimpanzee, macaque, or marmoset (-SRGAP2C "controls") microglia." "Human DEGs were consistent with reduced microglia ramifications, including downregulation of genes associated with cell projection and the plasma membrane (Table S19)." "These results highlight that the alterations of microglial cell shape observed in our zebrafish SRGAP2C "humanized" models recapitulate human-specific biological processes that occur in microglial cells."