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Zebrafish CRO
A Reliable Discovery Platform

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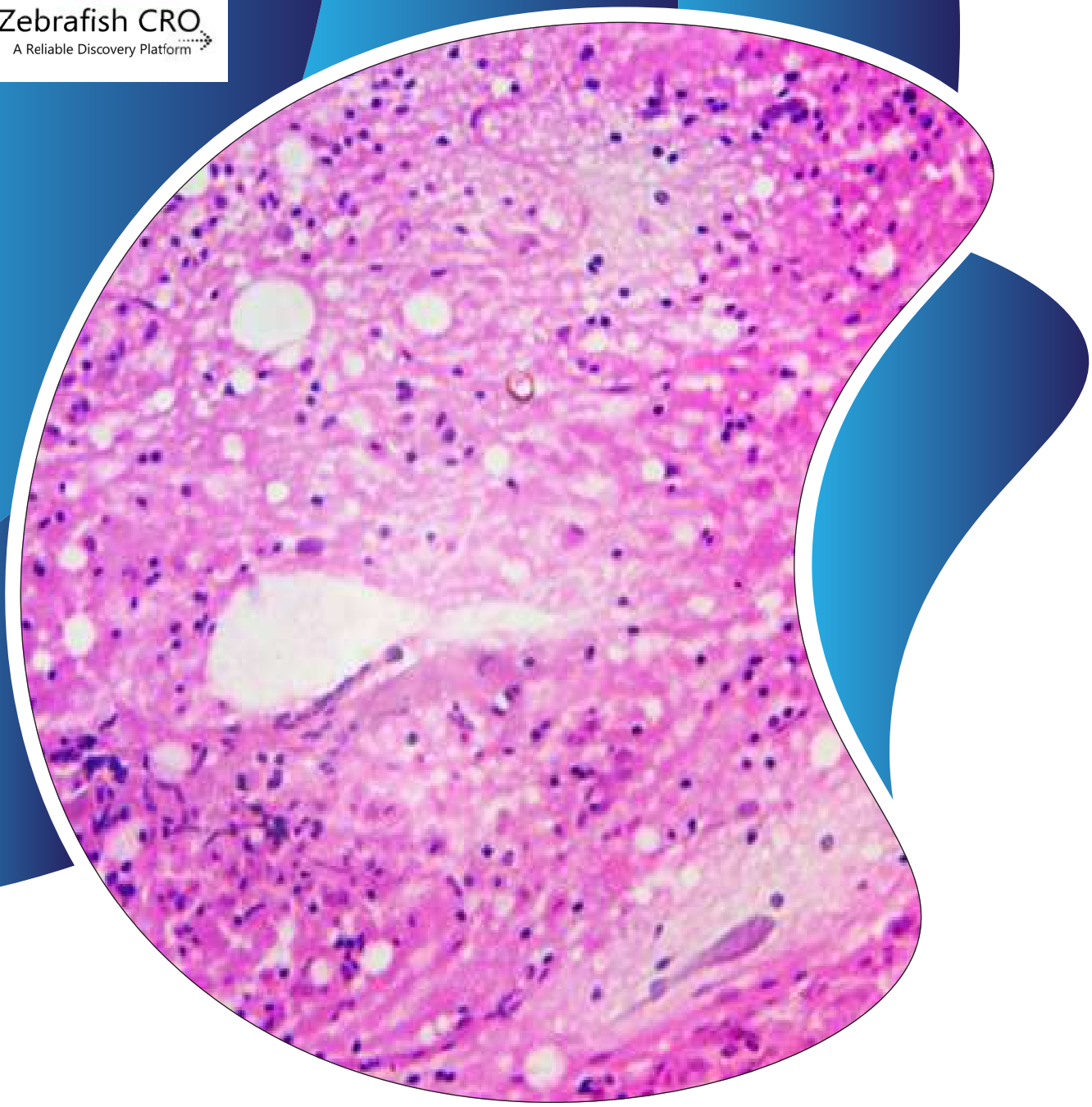

GBA1 VARIANTS

WITH CELL DEBRIS INSULTS DISPLAY

SEVERE MOTOR AND COGNITIVE DEFICITS

PENTAGRIT ZBERAFISH CRO
CHENNAI-INDIA

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Introduction:

Glucosylceramidase beta 1 (GBA1) variants are well characterized for their role in GBA1-PD. PD patients with GBA1 variant show a rapid decline in cognitive function and progress of disease with a higher severity in motor, freezing gait, cognitive and sleep disorders. More recently several studies have shown the role of cell debris to further accelerate the progress of disease. To recreate the pathogenicity of cell debris in the course of disease, here we evaluated the severity of PD progress on GBA variant L483P mutant with and without cell debris insults.

METHODS:

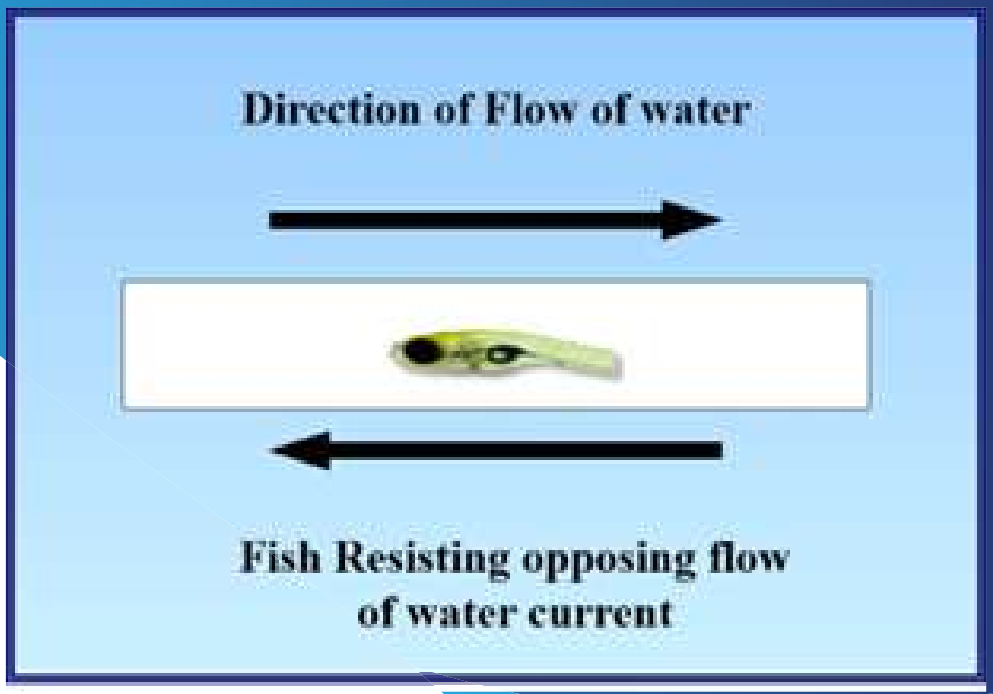
CELL DEBRIS INSULT

GBA1 variant L483P adults were raised until breeding stage and heterozygous offsprings were seeded with α-synuclein fibrils along with cell debris matter on day 5 post fertilization(dpf) through microinjection into the midbrain. Phenotype and pathology assessments were made on day 9 and 10 dpf.

Motor Co-Ordination:

To study motor coordination, larvae were introduced to the swim chamber (4 cm length and 4 mm diameter) and were allowed to acclimatize for 5 min before screening.

Following acclimatization, the larvae were subjected to swim against the inflowing water current in the chamber. The time taken by the larvae to fatigue and fall in the outflow of the chamber was measured.



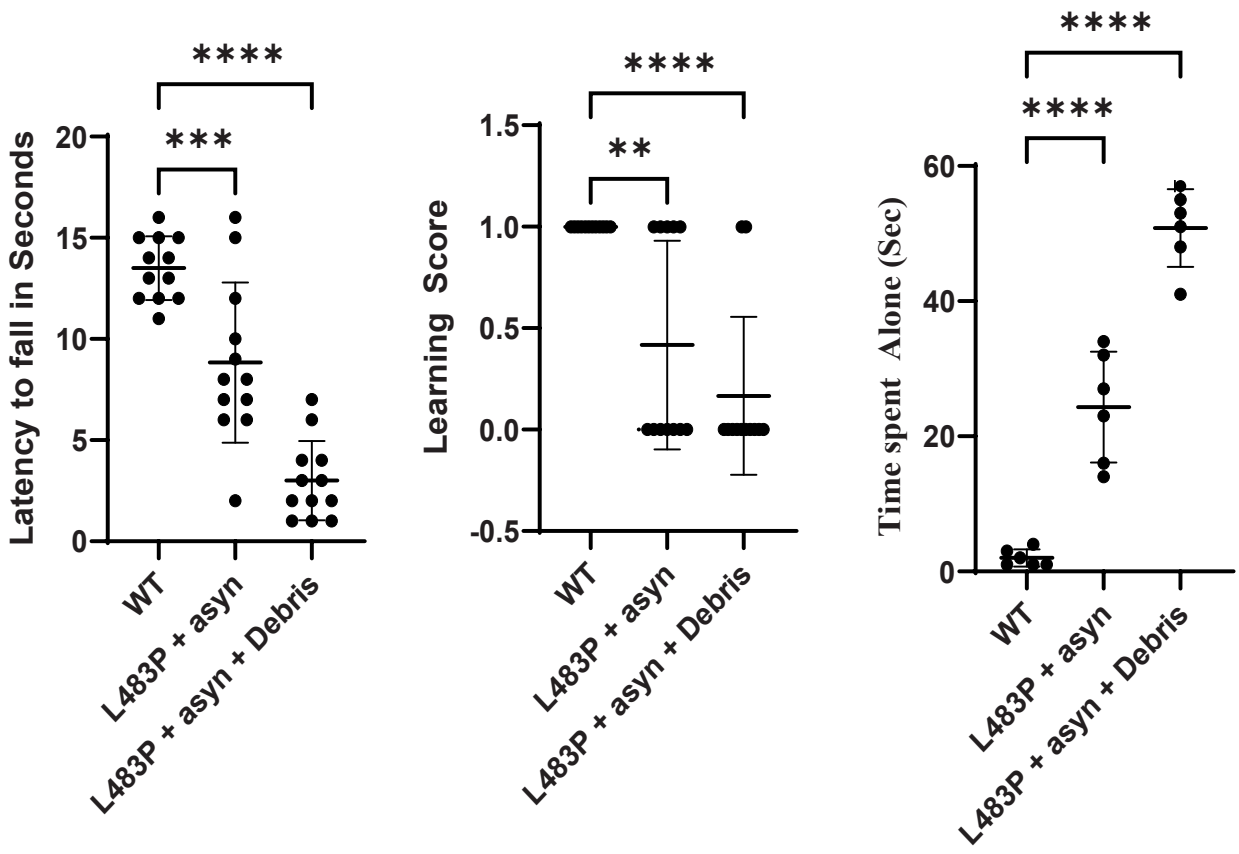
Conditioned Avoidance Learning:

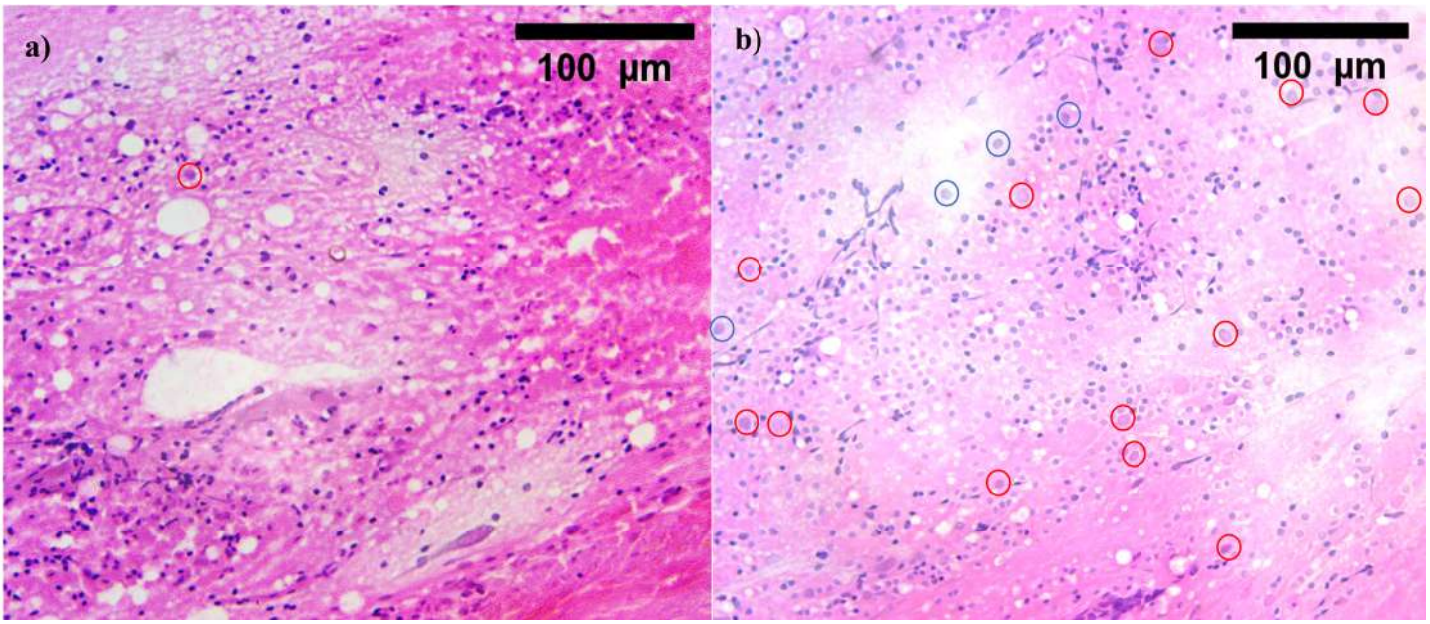
Larvae were exposed to tanks with a predatory fish placed in an enclosed chamber in one corner and conditioned learning ability to avoid predator exposure was measured. A score of one was provided for fish that learned to avoid predator fish and 0 if there was a lack of learning ability.

Depression:

Social isolation behaviour was calculated as to how much on an average per minute, the animal remained secluded from other fish in the tank.

Results:





3D smear and H&E stain, Midbrain of L483P (a) and L483P + Cell Debris insults (b) at brightfield magnification of 200x. Red circles indicate cells in pre-autophagy vacuolization stage that did not proceed to autophagy. Violet circles indicate monocytes.

Motor Co-ordination: Cell debris injected L483P larvae displayed severe deficits in motor co-ordination with a latency to fall within 3 seconds of exposure to opposing water flow whereas L483P larvae without cell debris insults performed better with a mean latency to fall of 8 seconds.

Conditioned Avoidance Learning: Cell debris injected larvae displayed more severe cognitive deficits in learning capabilities compared to L483P without cell debris

Depression: Social isolation behaviour was more pronounced in cell debris injected L483P larvae that spent a median 55 average seconds alone compared to L483P without cell debris that spent only a median of 26 average second alone per minute.

3D Smear Cells in vacuolation stage that has not proceeded to autophagy is higher in L483P injected with cell debris, indicating loss of autophagy as the one of the key driver for progress of PD.

Conclusion:

Modes of Drug Activity that can be Evaluated	
LRRK2 inhibitors	✓
SH3GL2 Dysfunction	✓
Autophagy Inducers	✓
Lysosomal damage Protection	✓

New therapeutic modalities that focus on cellular debris removal through macroautophagy and increased lysosome function have been evaluated for PD. However, measuring cell debris impact has been hampered by the progress of disease pathology that makes models too sensitive for long term screening of drugs. In this case L483P zebrafish model has a larger screening window and hence can be treated with higher and long-term dose of debris removal drugs.

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About
our company

Pentagrit CRO is an **AAALAC-accredited** lab offering In Vivo efficacy services, leveraging zebrafish for efficacy screening, phenotype-based drug discovery, and bio-marker endpoints.

We are Chennai-based Contract Research Organization (**CRO**) pioneering zebrafish-based preclinical research, offering zebrafish models that enable rapid and cost-effective drug screening, toxicity assessment, and target validation for various therapeutic areas, including oncology, neurology, immunology, eye, metabolic, and rare diseases.

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