Zebrafish as a new model organism for Parkinson's disease

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Parkinson's Disease

Parkinson's Disease (PD) is the second most common human neurodegenerative disorder. PD patients present aberrant motor activity with resting tremor, muscular rigidity, bradykinesia and postural imbalance [1, 2]. The cause of these symptoms is loss of dopaminergic neurons in the substantia nigra, pars compacta. Another pathological hallmark of PD is the frequent formation of Lewy bodies in surviving neurons. Lewy bodies are cytoplasmic aggregates of insoluble proteins. About 85-90% of the PD cases are sporadic with a complex aetiology. In 10-15% of the PD patients, a familial predisposition can be found. Linkage analysis in these families conclusively identified α-synuclein, parkin, *PINK1, DJ-1*, *LRRK2* and *ATP13A2* as PD-causing genes [1, 3, 4]. The normal cellular function of these genes suggests that several pathways can be involved in neurodegeneration, including oxidative stress, proteosomal and mitochondrial dysfunction and protein aggregation and misfolding [5]. Our research is focused on two PD-causing genes, that is, *LRRK2* and *ATP13A2*. *LRRK2* has been implicated in both familial and sporadic cases but the precise role of the LRRK2 protein in neurodegeneration is not clear. Clinical relevance is implicated by the similarity between the PD-phenotype of patients carrying mutations in this gene and that of sporadic PD patients. The relatively high mutation rate in sporadic PD in some populations also adds to the hypothesis that LRRK2 plays a key role in PD aetiology [6]. The other gene, ATP13A2, was identified in Kufor-Rakeb Syndrome. Patients are bound to bed before the age of 15 due to severe Levodopa-responsive Parkinsonism and pyramidal symptoms. This gene was selected because of its severe phenotype and autosomal recessive inheritance. In the human patients these mutations result in a loss of functional protein. The co-localization of ATP13A2 and β-Synuclein [7] in lysosomal inclusions also suggest a role for this gene in Lewy Body formation.

Zebrafish

Recently we have implemented a new simple vertebrate model with a richness of embryology/anatomy and genetics knowledges, creating a new vertebrate model system for PD, the zebrafish (*Danio rerio*).

The zebrafish, a fresh water tropical fish, is a premiere model organism to study vertebrate development. Fast external development and transparency during embryogenesis allow for visual screening at the macroscopical and microscopical level, including visualization of organogenesis. High fecundity and short generation times facilitate genetic analyses. Importantly, The Sanger Institute has recently released assembly version Zv7, an almost finished sequence, of the 1.5Gb zebrafish genome. Zebrafish may be a particular powerful model for the study of human disease because many cellular processes are conserved throughout vertebrate evolution, including the corresponding disease genes. Finally, the ability to generate

transgenic zebrafish allows for (over-) expression as well as for the suppression of gene expression during early development (using morpholino gene knockdown techniques) creates easy access to new animal models. Although the zebrafish brain does not contain a mesencephalic region comparable to the substantia nigra, treatment with MPTP (a PD-inducing drug in humans, apes and to a lesser extent mice) showed a direct effect on diencephalic dopaminergic neurons. This resulted in a loss of diencephalic dopaminergic neurons and an aberrant swimming pattern, illustrating that zebrafish can develop a phenotype comparable to PD [8]. Importantly, the homologues of the two selected PD-causing genes have been identified in zebrafish and morpholinos to study gene knockdown are available.

Aims

Our research project aims at accomplishing two objectives. The first objective is a fundamental research question: Understanding the cellular function of atp13a2 and lrrk2 during brain development and in adult brain.

The second objective is of translational nature: understanding the molecular mechanisms underlying PD by generating animal models of PD using zebrafish.

Methods

Manipulation of gene expression in zebrafish includes: gene knockdown and transgenic strategies. For gene knockdown, we will use antisense oligonucleotides with a synthetic backbone called morpholinos (MOs). MOs are 25 basepairs long and are designed to uniquely bind to our gene of interest, thereby disrupting translation initiation or pre-mRNA splicing by binding to the pre-mRNA. MOs are used for functional genomic applications and have been shown to be very effective in zebrafish. MOs are injected in the yolk sac of 1 or 2 cells-stage embryos and knockdown of gene function is transient (till 5 days post fertilization=dpf). For the generation of transgenic zebrafish, we will microinject plasmid DNA,

containing our gene of interest fused to EGFP under the control of a brain-specific promoter (GATA-2 or Tyrosine Hydroxylase (TH) promoter) into the cytoplasm of a 1-cell stage embryo.

Both knockdown morphants and transgenic zebrafish will be further characterized for the presence of a phenotype, including microscopical screening for morphological features, in situ hybridization (ISH) strategies using molecular probes to study gene expression patterns, immunohistochemical techniques (i.e. TH expression) and biochemical analyses (Western blot). Another important aspect of characterization of a phenotype is the use of behavioural tests. Recently, a system to monitor locomotor behaviour in zebrafish has been established (Noldus Information Technology bv; EthoVision). This system enables to record movement and swimming patterns of zebrafish embryos (6 dpf) in a highthroughput fashion. Currently, this system is implemented at Erasmus MC. Activity is monitored by a camera and the software detects the fish. This data visualizes the swimming pattern and allows for statistical analysis of locomotor activity (figure 1).

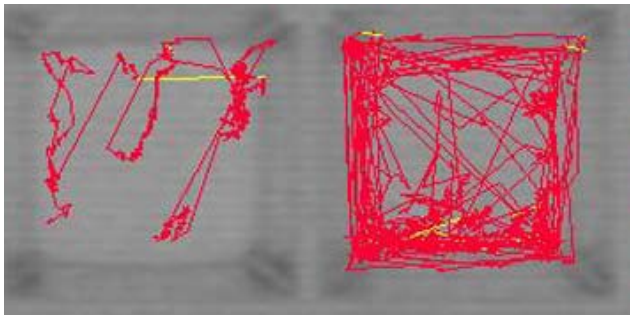

Figure 1. *Zoom in on 2 wells of a 96 well plate with plots of the swimming pattern of larvae at 6dpf during 5 minutes. The larva in the left well is treated with 10μM Diazepam, while the larva on the right is an untreated control*

References

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