Role of CAMTA1 in cerebellar ataxia: a zebrafish study

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CAMTA proteins:

**CAMTAs: calmodulin-binding transcription activators**

- Evolutionary conserved transcription factors (from plants to humans)
- Characteristic set of domains
- In vertebrates: high expression in central nervous system

In humans CAMTA1 mutations have been associated with autosomal-dominant cerebellar ataxia (Thevenon et al., 2012)
Hereditary cerebellar ataxias

- Neurodegenerative disorders
- Motor incoordination, postural instability
- Cerebellar atrophy and Purkinje cells degeneration
- Genetic cause and pathophysiological mechanism for 40% of hereditary ataxias is still unclear
- No therapy available so far

Long et al., 2014
Aim of the Project:

A further understanding of the normal physiological function of CAMTA1 and how his altered function leads to hereditary cerebellar ataxia will be important for the development of therapeutic strategies.

- CAMTA1 KO

- Reflects the phenotype of CAMTA1 mutations in humans

BUT:

- Detailed analyses of regulated genes in mice is costly and slow to achieve

- Fast development

- Genetic manipulations

- High degree of conservation of the nervous system between zebrafish and mammals

- Drug screening

Aim of the project: Validate the zebrafish larvae as a model organism to study CAMTA1 dependent ataxia
Results: zebrafish CAMTA1 is strongly expressed in the nervous system

**Zebrasfish CAMTA1 orthologue: camta1a**

- 73% AA-sequence similarity with the human protein
- Expressed in the central nervous system

![In situ Hybridization](image-url)
Results: *camta1a* knock down experiments

*camta1a* knock down: Morpholino antisense oligos

Artificial oligonucleotides that block mRNA translation by binding to AUG

```
5' UTR  E1  E2  E3  E17
MO
Translation blocking
```

```
control MO

1 mm
5 dpf

camta1a MO

2 ng
5 dpf
```

```
control MO

Tg(huc:GCaMP5)
3 dpf

camta1a MO
```
Results: Purkinje cells markers are reduced in *camta1a* MO

**In situ Hybridization**

*In situ* Hybridization

**Immunofluorescence**

Immunofluorescence

*Aldocl*: aldolase c, fructose-biphosphate-like; *Pvalb*: parvalbumin 7
Behavioral effects of \textit{camta1aMO}: Movement pattern

Results: ataxia phenotype in zebrafish

Aspatwar A. \textit{et al.}, 2013; Mahmood F. \textit{et al.}, 2013
Results: camta1a morphants larvae have altered movement pattern

- Abnormal swimming behavior at 5 dpf
- Postural instability

Means of the distance swum in 5 minutes +/- SEM. P = 0.004 (unpaired t-test)

Aspatwar A. et al., 2013; Mahmood F. et al., 2013
Summary and conclusion

- Zebrafish *camta1a* displays a high similarity with the human protein
- Mainly expressed in the central nervous system
- Knock-down experiments showed a reduction in Purkinje cells, while the general morphology of the cerebellum remains unaltered
- Knock-down of *camta1a* reproduced in zebrafish larvae symptoms of ataxia, with shorter swimming path and postural instability.

Our results so far validate zebrafish as a valuable model organism for the study of CAMTA1-related ataxia
Generation of a zebrafish *camta1a* mutant line

CRISPR/Cas9 genome editing

- Analysis of adult phenotype will be possible
- Less off-target effects than MO

Reference sequence:

```
GGAAAAAAAAAGAAAGATGGGAAGACCACGCCGGAGGATCACA
```

Mutations in F1 fish:

```
GGAAAAAAAGAAAGATGGGA---------GGATCACA (-13 bp)
GGAAAAAAAGAAAGATGGGAAGACC--GCGGGAGGATCACA (-3 bp)
TTTACAATAGAAAGAG (-)x53GTGAAAGGATCACA (-53 bp)
GGAAAGA---------CGCGGGAGGATCACA (-11 bp)
```
Purkinje cells sorting

Candidate genes differentially expressed

Microarray data analysis

characterization of the functional role of CAMTA1 in the cerebellum

Camta1a CRISPR/Cas9 mutant

tagRFP-T:4xCa8:GCaMP5G

Development of potential therapeutic targets

Candidate genes differentially expressed

OUTLOOK:
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Our studies provides proof-of-principle that camta1a morphant zebrafish recapitulate salient features of ataxia and may represent ideal tools to address the pathogenic mechanisms underlying the disease phenotype in humans with CAMTA1 mutations.
Results: *kcnc3a* expression is decreased in *camta1a* morphants

- Strongly expressed in Purkinje cells
- Important for complex spike waveform of Purkinje cells
- *KCNC3* is mutated in spino-cerebellar ataxia type 13

**Kv3.3 voltage-gated potassium channel**

**In situ Hybridization**

**control MO**

**camta1a MO**

*kcnc3a*
Conclusion

- We generated and validated a model of ataxia using zebrafish larvae

- We showed that knockdown of the camta1 gene, responsible for ataxia in human patients, results in PC decrease and abnormal movement pattern in zebrafish, mimicking the ataxia phenotype in humans and mice

- Camta1a knock down zebrafish larvae can thus represent a good model system to address the role of CAMTA1 in HA

- Generate a zebrafish CAMTA1 mutant line with Crispr/Cas9 technique

- Find genes that might be transcriptionally regulated by camta1a