Zebrafish for the functional analysis of genetic variants of Amyotrophic Lateral Sclerosis

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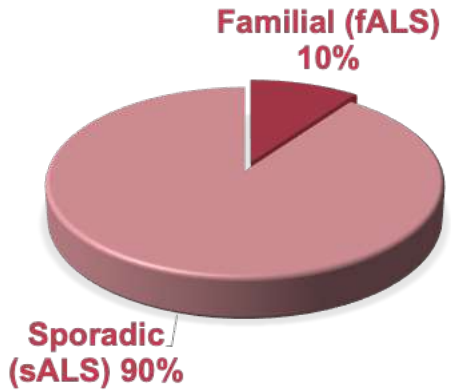
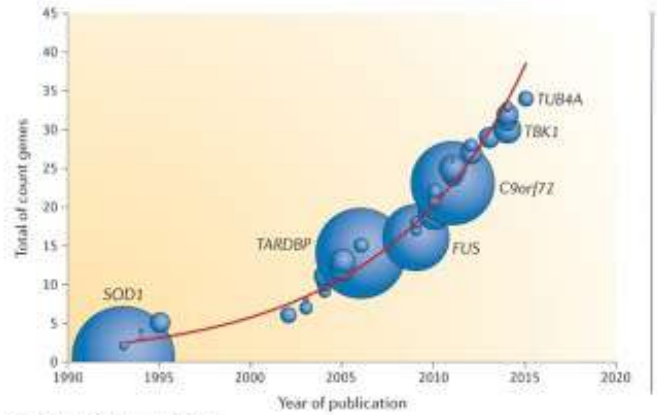
INTRODUCTION

The genetic architecture of ALS



ALS is a **complex disease**

Mendelian gene variations are found in **80% of fALS** and in **14% of sALS**



Al-Chalabi, A et al. (2017)

There are more than 30 causative ALS genes

The 4 major ALS genes are ***SOD1, C9ORF72, TARDBP*** and ***FUS***

They account for up to **70% of fALS** cases and **11% of sALS**

The *SOD1* gene

SOD1 : Cu/Zn superoxyde dismutase 1

$2O_2^- + 2H+ \longrightarrow O_2 + H_2O_2$

Mainly **Gain of Function** mutations

Forms **toxic protein aggregates**

There can be variant-specific clinical manifestation : **A5V, G94A, D91A**

SOD1 is one of the main targets **for antisense therapy in ALS**

Lack of functional data in variant pathogenicity interpretation

SEQUENCING

A lot of variants

…

VARIANT CLASSIFICATION

ACMG
American College of Medical Genetics and Genomics
Translating Genes into Health®

METHODS

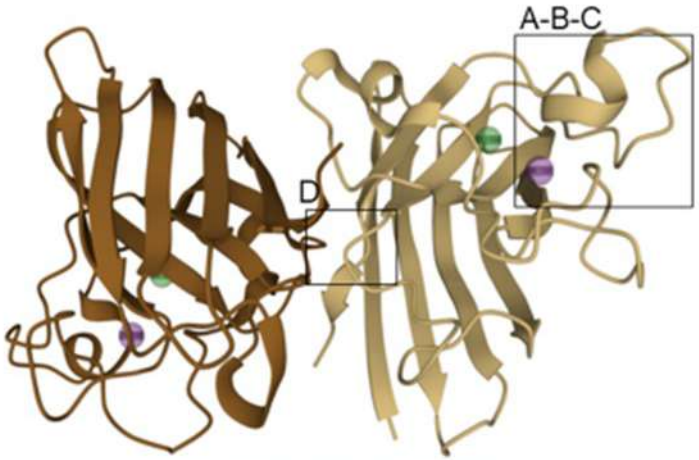
Candidate variants

SOD1 N126D (A)

SOD1 dE134 (B)

SOD1 K137* (C)

SOD1 I150M (D)



SOD1 : Cu/Zn superoxyde dismutase 1

Transient protein overexpression in zebrafish

1. mRNA injection into the zygote

hSOD1 WT : negative pathogenicity control

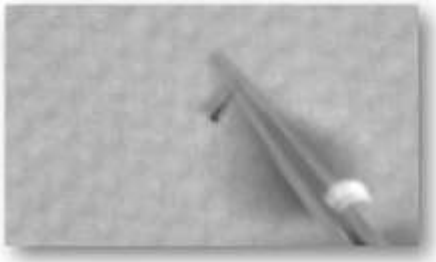
hSOD1 A5V : postitive pathogenicity control

hSOD1 ? : variant from sequencing

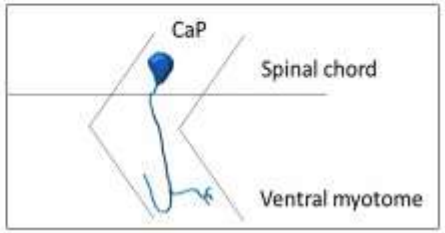
NI : not injected fish

(100µg/µL)

2. Touch Evoked Escape Response

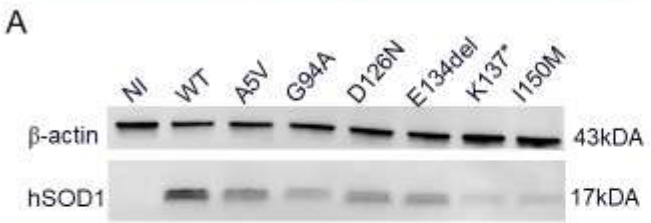


3. Motoneuron/NMJ analysis

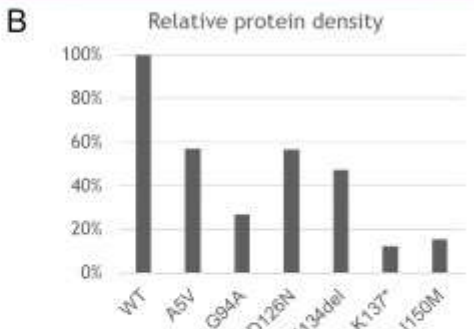


RESULTS

Differential expression of the different *SOD1* variants

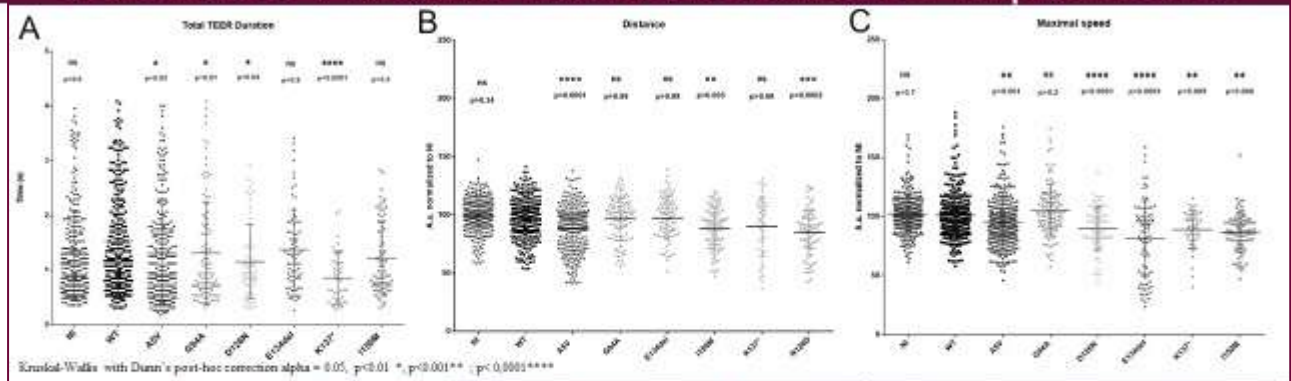


WB of proteins extracted from 2-day old larvae injected with different *hSOD1* variants shows that all variants are expressed in injected fish while not expressed in not injected larvae (NI). *hSOD1* monomer is noted as a band of approximately 17kDA, while β-actin is observed at 43kDa. We note that the WT variant is the most strongly expressed.



We calculated the relative protein densities compared to WT variant. A5V, D126N and E134 are expresses at around half of the WT quantity. G94A is expresses at less than 30% while K137* and I150M are expressed at less than 20% of the amont of WT.

Candidate v ariants induce locomotor impairments

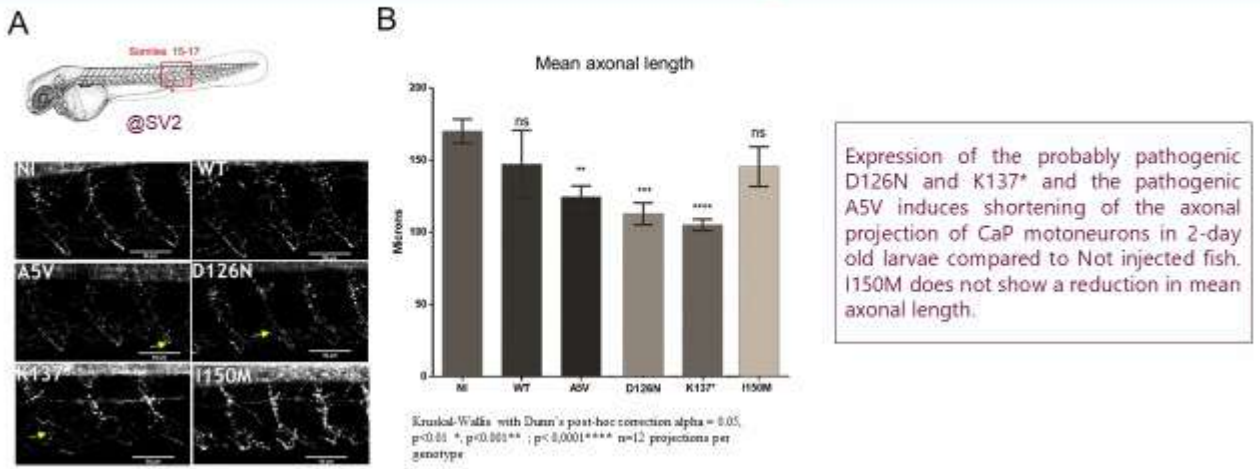


Significantly reduced total TEER duration for the groups expressing the probably pathogenic D126N and K137* and for the groups expressing the pathogenic A5V and G94A compared to group expressing WT variant.

Significantly reduced total distance for the groups expressing the probably pathogenic I150M and K137* and for the group expressing the pathogenic A5V compared to the group expressing WT variant.

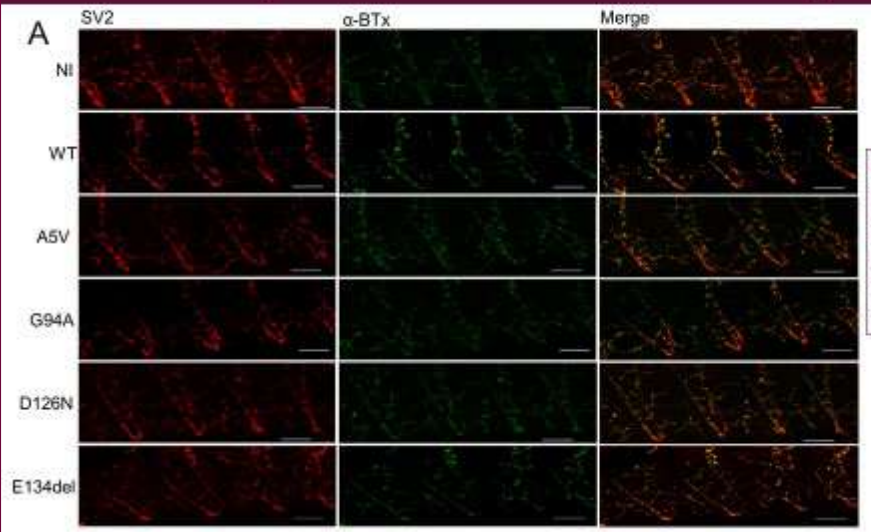
Significantly reduced maximal speed for all groups expressing the probably pathogenic variants D126N, E134del, K137* and I150M and for the group expressing the pathogenic A5V compared to the group expressing WT.

Candidate variants induce shortening of motoneuron axons



Expression of the probably pathogenic D126N and K137* and the pathogenic A5V induces shortening of the axonal projection of CaP motoneurons in 2-day old larvae compared to Not injected fish. I150M does not show a reduction in mean axonal length.

Possible impairments of NMJ in larvae expressing mutant *SOD1*



Preliminary data reveal impairments in the NMJ in all larvae expressing *SOD1* mutant variants, with presence of orphananed α-Btx clusters suggestive of loss of collocalisation with the neuronal maker SV2.

Conclusions

✓ We provide the first functional evidence in favor of a pathogenic effect for **4 *SOD1*** variants: **D126N, E134del, K137* and I150M**

✓ We show that **zebrafish** can be used for routine **variant** pathogenicity **testing** for the **molecular diagnosis of ALS**

Discussion and perspectives

- What part of the circuitry is differentially affected by the different variants?
- What is the molecular mechanism leading to the observed phenotypical heterogeneity?
- Screen for therapeutic compounds
- Apply to other ALS genes
- Develop complementary models *(Caenorhabditis elegans, Drosophila melanogaster)*

TAKE HOME MESSAGE

Zebrafish can be used as a rapid and efficient tool to help variant pathogenicity analysis in ALS molecular diagnosis