SOD1 Enzymatic Activity in CSF of ALS patients

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1 The Background Story

Intrinsic SOD1 mutations can destabilize the native protein and lead to the formation of disease propagating aggregates, an assumed core pathological mechanism of SOD1-linked ALS. While the majority of mutations cause reduction or loss of enzymatic activity, some mutations have wild type like activity. Two other SOD isoenzymes, the mitochondrial matrix SOD2 and extracellular SOD3 are expressed in humans.

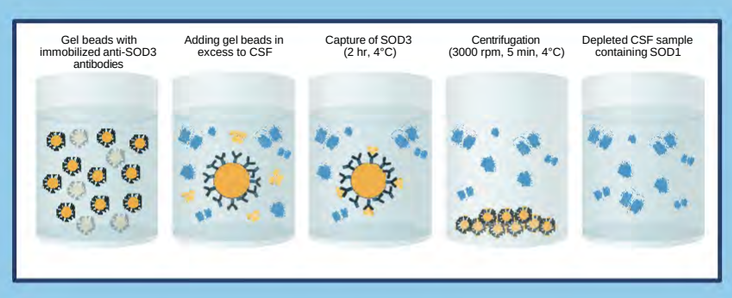
Novel method to measure SOD1 activity in CSF

2 Challenges in CSF

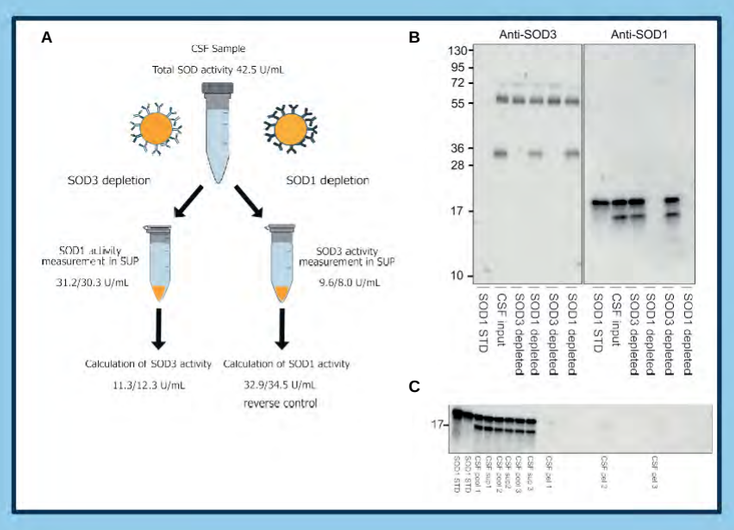
Since biopsies of the central nervous system are not available from ALS patients, CSF is the best proxy of SOD1 activity in living humans. However, to measure SOD1 activity in CSF is challenging due to the presence of the extracellular SOD3 isoenzyme and because SOD1 protein levels and activity are low in the CSF.

Hence, we developed a bead based method to deplete SOD3 from CSF using immobilized SOD3 specific antibodies.

3 Immunocapture with antibody beads



To evaluate our method, we tested the specificity and binding efficacy of our antibody beads in a reversed assay set-up. We further controlled for the possibility of SOD protein trapped in vesicles by ultracentrifugation and immunoblot.



Using the direct method to determine SOD1 activity (Marklund, 1976) after SOD3 depletion, allows us to measure low SOD activities in CSF with high accuracy and precision.

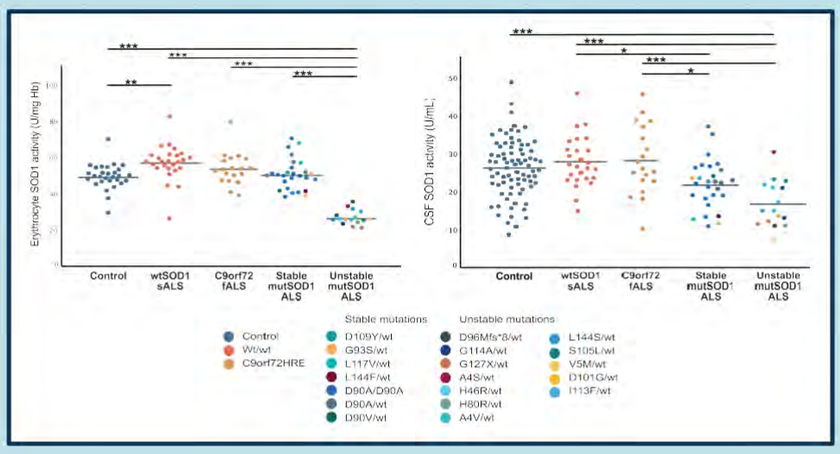
Why SOD1 activity is important

Children homozygous for inactivating SOD1 mutations develop the SOD1 Deficiency Syndrome (ISODDES), showing motor neuron injuries. This discovery suggest a possible deleterious effect of too low antixidant SOD1 activity.

Results

4 SOD1 activity in CSF

We founda large variation in CSF SOD1 activity within the groups as well as between individual patients with the same SOD1 mutation, besides the observed group level differences.

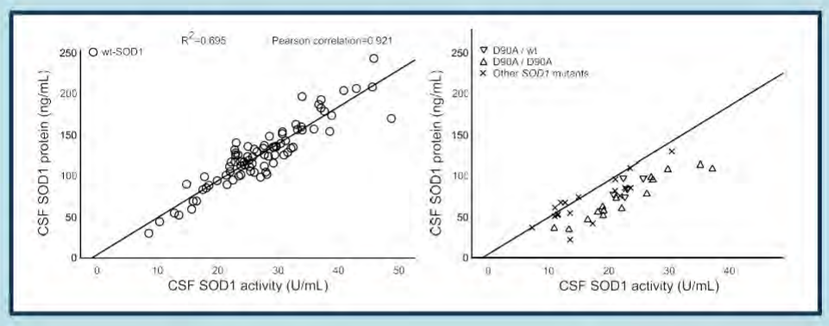


In ALS patients with wildtype SOD1, the SOD1 activity in CSF was similar to controls. However, patients with unstable mutant SOD1 had lower activity in CSF. Even for patients with mutants previously reported to have normal enzymatic activity in erythrocytes, the SOD1 activity was lower then in patients with wildtype SOD1 but not when compared to controls.

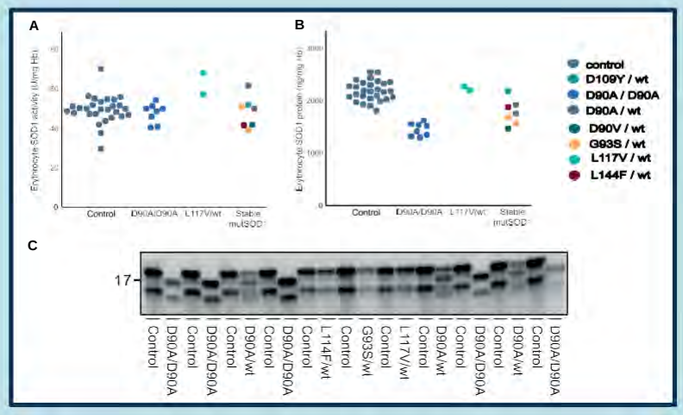
	Wildtype SOD1			Mutant SOD1		
	Control	sALS	C9orf72HRE fALS	Stable SOD1 ALS	Unstable SOD1 ALS	p
	(n=81)	(n=26)	(n=19)	(n=29)	(n=16)	
Age at sampling (years)	55.1 ± 15.6	60.7 ± 11.3	59.2 ± 11.2	55.6 ± 10.7	50.7 ± 13.7	0.127
Storage time (years)	9.1 ± 7.1	13.9 ± 6.1	9.7 ± 6.8	9.1 ± 9.1	6.0 ± 6.7	0.012
CSF total SOD activity (U/mL)	37.3 ± 8.2	40.1 ± 7.7	39.3 ± 9.3	33.3 ± 8.3	28.5 ± 6.8	>0.001
CSF SOD1 activity (U/mL)	26.1 ± 7.6	27.8 ± 6.7	28.2 ± 9.1	21.6 ± 6.6	16.7 ± 6.1	>0.001
CSF SOD3 activity (U/mL)	11.2 ± 3.0	12.3 ±4.1	11.0 ± 3.8	11.8 ± 3.6	11.8 ± 2.8	0.594
CSF SOD1 protein (ng/mL)	119.3 ± 39.8	136.7 ± 36.5 (n=25)	132.1 ± 36.5	74.9 ± 22.9 (n=27)	66.9 ± 29.1 (n=12)	>0.001
CSF total protein (mg/L)	413 ± 155 (n=77)	408 ± 119 (n=24)	421 ± 107	415 ± 170 (n=26)	393 ± 98 (n=12)	0.844
Erythrocyte SOD1 activity (U/mg Hb)	49.6 ± 6.9 (n=29)	57.1 ± 9.7	53.7 ± 8.8	51.1 ± 8.5 (n=28)	27.0 ± 4.0	>0.001
CSF SOD1 activity / CSF total protein	0.070 ± 0.032 (n=77)	0.076 ± 0.034 (n=24)	0.072 ± 0.029	0.055 ± 0.022 (n=26)	0.045 ± 0.021 (n=12)	0.002

5 Reduced ELISA reactivity of SOD1 mutants

We observeda discrepancy between SOD1 protein levels and activity in the mutants SOD1 groups. Commonly used ELISA antibodies for SOD1 quantification are often raised against native SOD1. Mutant SOD1s might therefore show a lower reactivity towards these antibodies.



Control experiments revealed that our ELISA antibodies have reduced reactivity towards SOD1 mutant protein and even wtSOD1.



6 Take home message and considerations

- We present a novel method to directly measure SOD1 activity in CSF samples without the interference of the SOD3 isoenzyme.
- The results revealed a discrepancy between the activity measurements in erythrocytes and CSF and a larger variability in activity levels between individuals in CSF.
- Monitoring the effect of SOD1 expression altering therapies solely by SOD1 protein quantification is problematic due to a potential reduced reactivity of used antibodies towards mutated SOD1 variants. Therefore we recommend additional SOD1 activity measurements in ALS patient follow ups.

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