Transcriptomic analysis of C9orf72 iPSC-derived motor neurons identifies a dysregulation in vesicle transport pathways

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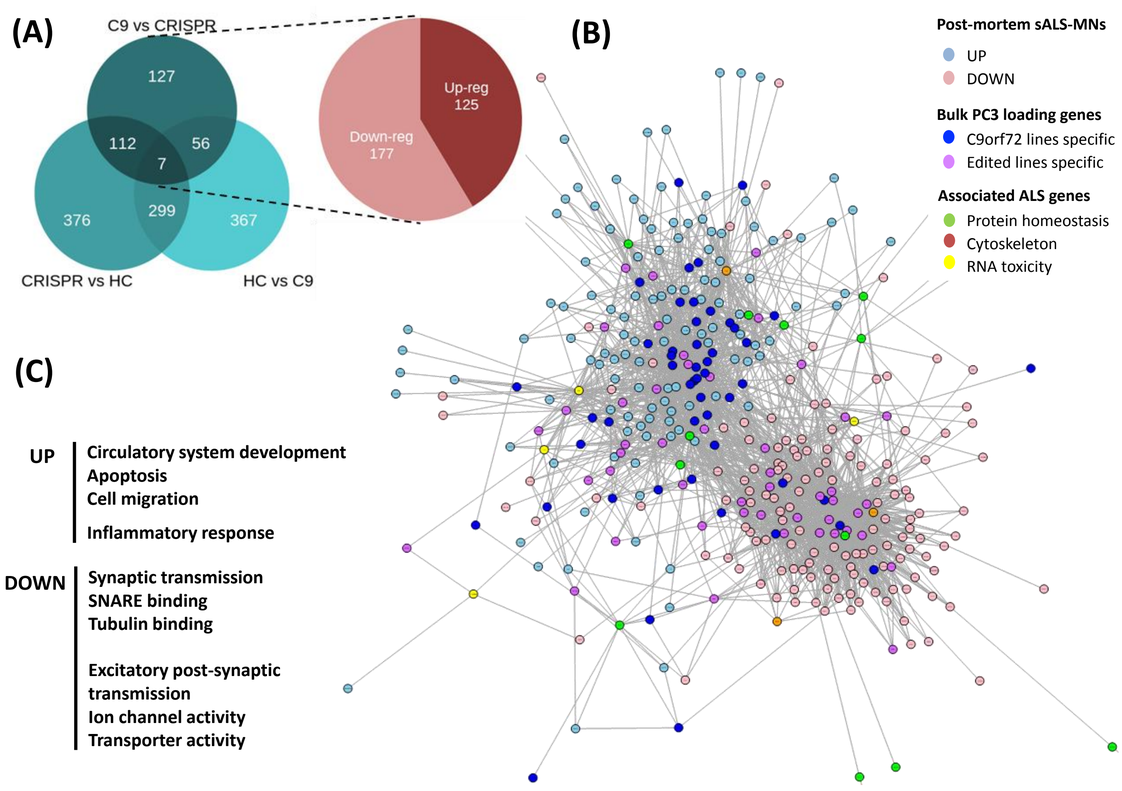
Background and aims

Induced pluripotent stem cell-derived motor neuron (iPSC-MN) cultures reproduce in vitro some of the typical features of C9-related ALS/FTD, such as the presence of RNA foci and dipeptide repeats (DPRs), activation of apoptosis, calcium dysregulation, stress granule formation and protein aggregation (Dafinca et al. 2016). The use of isogenic CRISPR/Cas9 edited controls and FAC sorting produces pure iPSC-MNs, minimising the intrinsic variability of these cultures, with the potential to unmask important biological alterations in the transcriptomic profile of C9 iPSCs-MNs.

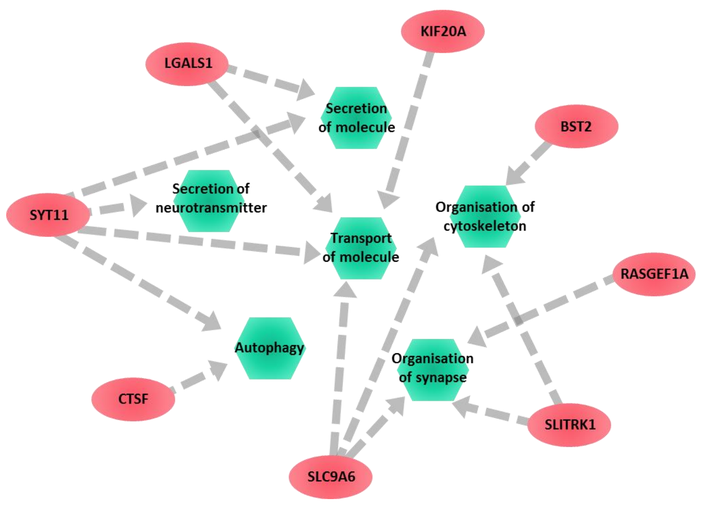
Aim: To identify differentially expressed genes in pure iPSC-MN cultures from C9orf72 patients (C9) compared to isogenic C9-corrected lines (Ed) and age-matched healthy controls (HC), and to determine whether these are effectors of G4C2-triggered neurodegeneration.

1. HB9^{+}/p75^{NTR+} MNs RNA sequencing



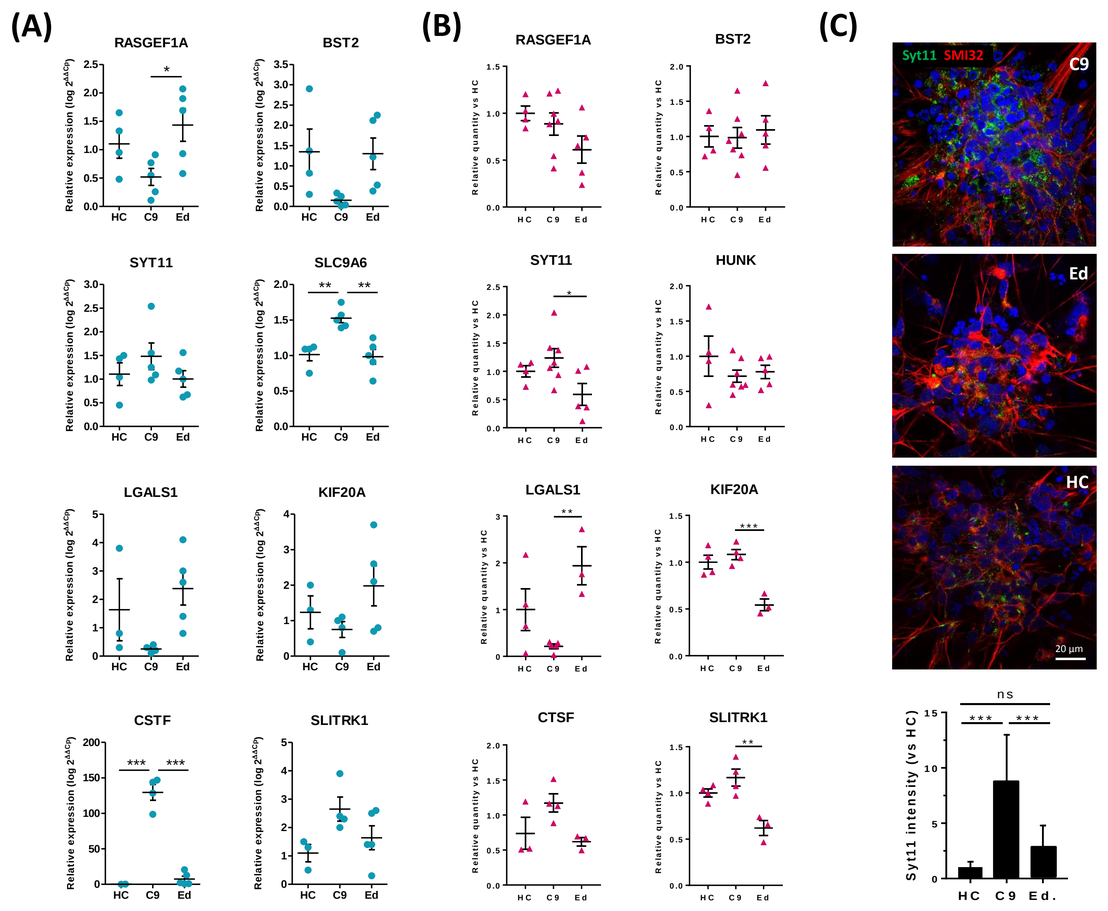
FACS double selection for MNs was performed using an antibody against p75^{NTR} coupled to FITC and an HB9::RFP lentivirus reporter (Toli et al., 2015). Two iPSC-MNs clones of a C9orf72 patient and 3 isogenic C9-edited clones, as well as 2 lines of age-matched HC, were used in this study. Samples were analysed in a HiSeq4000 system at 75bp paired end , using a Smart-Seq2 library preparation. **(A)** Venn's diagram for the differentially expressed genes (DEG) between the groups, identified using TopGO, and number of up and down regulated genes between the C9 and the isogenic lines (cutoff applied: TPM>1, FDR<0.1). **(B)** Phenotypic linkage network analysis for the DEGs using as external data post-mortem MNs from sALS (D'Erchia et al., 2017) and reported ALS-associated genes. Up-regulated genes in C9 lines co-cluster with the up-regulated genes in sALS-MNs. **(C)** GO terms enrichment for the different modules obtained.

2. Targets are related to vesicle transport pathways



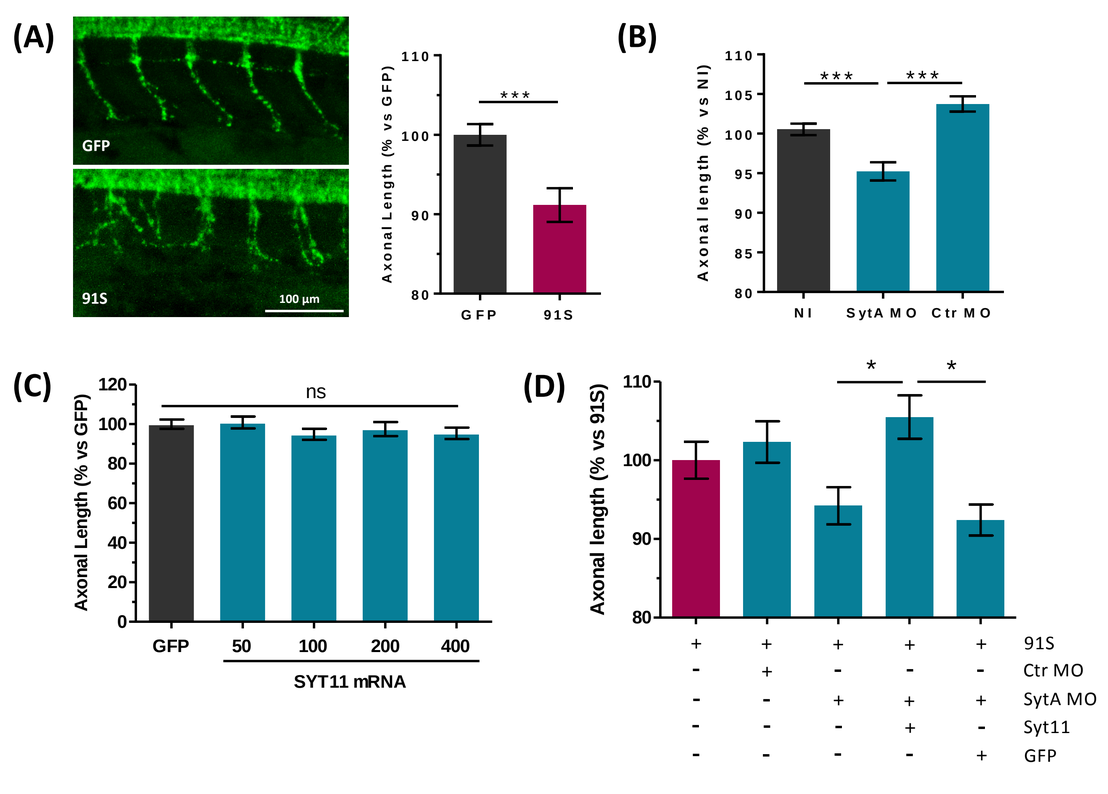
	Gene ID	Log2FC	FDR
Down regulated	RASGEF1A	-3.04	0.001
	BST2	-3.00	4E-5
	LGALS1	-2.27	0.018
	KIF20A	-1.21	0.037
Up regulated	SLC9A6	0.70	0.034
	SYT11	0.90	2E-6
	SLITRK1	1.01	0.036
	CTSF	6.68	0.001

3. Targets selection and validation



Validation of selected targets in 24DIV iPSC-MNs at mRNA level by RT-qPRC (n=3-5) **(A)** and protein levels by western blot (n=3) **(B)** (*, **, *** for p<0.05, 0.01, 0.001 one-way ANOVA followed by a Dunnet's post test). **(C)** C9 iPSC-MN at 24DIV show significant higher amounts of Syt11 than the edited or healthy controls (*** p<0.001 one-way ANOVA followed by Bonferroni's test).

4. SYT11 role in a C9orf72 zebrafish model



(A) Microinjection of 91 GGGGCC repeats RNA (91S) significantly decreases the length of the motor axons (stained with SV2 antibody) in 30 hours post fertilisation (hpf) zebrafish embryos when compared to a control GFP RNA (*** p<0.001 t test) (n=7). **(B)** Injection of a morpholino against the endogenous zebrafish Syt11 (SytA MO) significantly decreases the axonal length when compared to a control morpholino (Ctr MO) or non-injected embryos (NI) (*** p<0.001 one-way ANOVA followed by Bonferroni's test) (n=3). **(C)** Increasing concentrations (50 to 400 nM) of exogenous Syt11 RNA don't show any effect on axonal length when compared to GFP RNA injection (n=3). **(D)** The co-injection of the 91S repeat RNA with SytA MO causes a non significant decrease in axonal length, that is significantly reversed by the addition of exogenous Syt11 RNA, but not by the addition of the GFP control (*p<0.05 one-way ANOVA followed by Bonferroni's test) (n=2).

Conclusions

- RNA sequencing identified 183 differentially expressed genes between C9 and CRISPR edited lines, 56 of these are also differentially expressed compared to HC.
- Phenotypic linkage analysis reveals an enrichment of genes involved in vesicle dynamics pathways.
- mRNA levels for different targets involved in vesicle transport were confirmed by RT-qPCR.
- Syt11 is overexpressed in C9 patient lines. knock-down or overexpression in a zebrafish model expressing G4C2 repeats indicates a protective effect of Syt11 on axonal length.
- Further work will include the detailed study of vesicle trafficking pathways in C9-MNs and the effect of overexpression/knock-down of the key targets identified in this work.

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