Long non-coding RNAs: A new frontier in the study of Amyotrophic Lateral Sclerosis

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Introduction: The importance of various classes of regulatory non-coding RNAs (ncRNAs) in diseases is increasingly being recognized [1, 2]. We propose to perform a systematically profile, by RNA-Seq approaches, of the lncRNAs and mRNAs in human ALS lymphocytes mutated, unmutated and controls with the aim of extending our knowledge on molecular alterations of transcriptome and obtaining new data about its regulation.

Materials and Methods: three cohort of ALS mutated patients (FUS, SOD1 and TARDBP) have been recruited and have been compared with healthy subjects and ALS sporadic (non mutated) patients. RNA was extracted from Lymphoblast Cell Lines (LCLs) and Peripheral Blood Mononuclear Cells (PBMC), RNA libraries have been prepared by TruSeq Stranded Total RNA with Ribo-Zero Gold kit (illumina).

Results: Whole transcriptome analysis showed a general down-regulation in genes expression in all the studied groups in LCLs (Fig. 1). We hypothesized that the important detected down-regulation may be related to LCLs. In fact we have performed the same experiments on PBMC from the same patients and controls and the new data showed that in PBMCs the percentage of down-regulated genes is significantly lower than in LCLs (Fig. 2).

RNA-seq analysis in PBMCs clearly showed different profiles between patient groups: we have detected 94 altered genes in SALS patients, 130 genes in FUS group, 35 genes in TARDBP and only 20 genes in SOD1 patients. No genes have been found in common between the different groups. (Fig. 2).

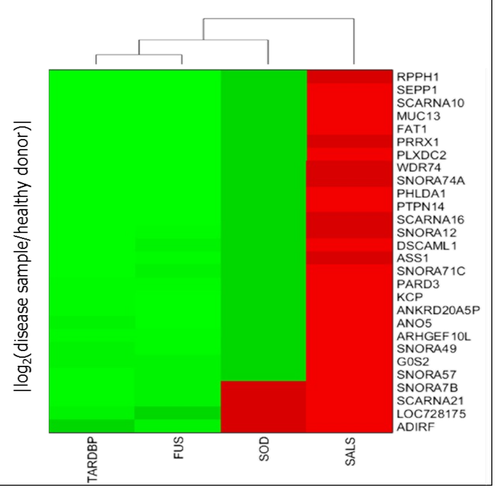
Next, we have analyzed the ncRNA in PBMC of each group (Fig. 3a).

SOD1: 2 ncRNA (1 AS, 1 unnoted); FUS: 16 unknown, 1 AS, 3 non coding RNA.;TARDBP: 1 LINC, 1 AS, 1, lncRNA and 13 unknown; SALS: We have been detected 65 annoted ncRNA (22 AS, 26 intergenici, 9 LINC, 7 GENES-LINKED) and 258 unnoted (Fig. 3b).

LCLs, mRNAs

Number of **mRNA** differentially expressed genes	UP	DOWN
FUS (N=2)	262	484
TARDBP (N=3)	23	220
SOD (N=4)	116	294
SALS (N=4)	18	109

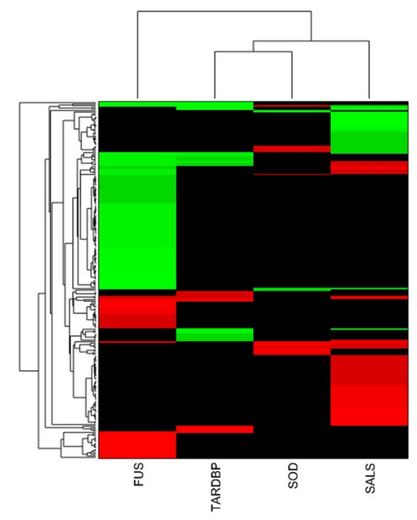
Fig. 1. 28 common genes were differentially expressed in all the groups compared to controls. Up- and down-regulated genes are represented in red and green, respectively



PBMCs, mRNA

Number of **mRNA** differentially expressed genes	UP	DOWN
FUS (N=2)	38	92
TARDBP (N=2)	15	5
SOD (N=4)	12	23
SALS (N=12)	61	33

Fig. 2. mRNA expression in PBMCs. Up- and down-regulated genes are represented in red and green, respectively.



PBMCs, lncRNAs

	AS	LINC	NCRNA	UNKNOWN
SALS	22	9	4	258
SOD1	1			1
FUS	1		3	16
TARDBP	1	1		13

a.

Fig. 3. lncRNA DE in PBMCs from ALS patients (a) List of AS, ALS-linked, Intergenic, LINC and unknown genes resulting from a pilot RNA-seq experiment on 12 sALS patients and 12 healthy controls (b).

AS (22)	ATG10-AS1	***INTERGENIC (26)***	ENST00000412436.1
	CDC42-IT1		ENST00000413818.2
	DLGAP1-AS2		ENST00000414157.1
	DPYD-AS2		ENST00000415437.1
	EAF1-AS1		ENST00000417346.1
	EHD4-AS1		ENST00000418699.1
	GCC2-AS1		ENST00000423715.1
	VIM-AS1		ENST00000423942.1
	KMT2E-AS1		ENST00000425110.1
	MAP3K14		ENST00000425493.1
	MBNL1-AS1		ENST00000429661.1
	MCM3AP-AS1		ENST00000432064.1
	RAI1-AS1		ENST00000436429.1
	RARA-AS1		ENST00000440629.1
	ZNF503-AS1		ENST00000440689.1
	RBM26-AS1		ENST00000443897.1
	SDCBP2-AS1		ENST00000446301.1
	TAPT1-AS1		ENST00000450917.1
	TMPO-AS1		ENST00000450996.1
	YEATS2-AS1		ENST00000454367.1
	HLA-F-AS1		ENST00000454411.1
	ZEB1-AS1		ENST00000478468.1
	RASAL2-AS1		ENST00000607047.1
ALS-LINKED (7)	FUS-AS		ENST00000607967.1
	ANG-AS		ENST00000608367.1
	ATXN2-AS		ENST00000608809.1
	UNC13A-AS		ENST00000609619.1
	DCTN1-AS	***LINC (10)***	LINC00174
	PRPH-AS		LINC00484
	LMNB1-AS		LINC00528
	PAXBP1-AS1		LINC00641
	MYC-AS		LINC00854
UNKNOWN	228		LINC00943
			LINC00963
			LINC01011
			LINC01044
			LINC01596

b

Discussion: Our preliminary data showed a comparable regulation in ncRNA between two groups positive for mutations in TARDBP and FUS, both involved in the same pathways, in fact we have detected some ncRNA common in both groups while a different profile arises from SOD1 and SALS groups. Moreover we have detected two interesting AS-genes involved in mitochondrial dysfunction and autophagy, known pathways in ALS that will be subject of work in the functional studies.
This preliminary analysis seems to indicate that it is not possible, with this set of ncRNAs, to discriminate between the different mutation states.

[1] Gagliardi et al. (2012). Neurol Res Int; 2012:27872.
[2] Fenoglio et al. (2013). Int J Mol Sci. 2013 Oct; 14(10): 20427–20442.

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