Long non coding RNA (LncRNAs) expression analysis in patients with Multiple Sclerosis: potential biomarkers of disease susceptibility and progression

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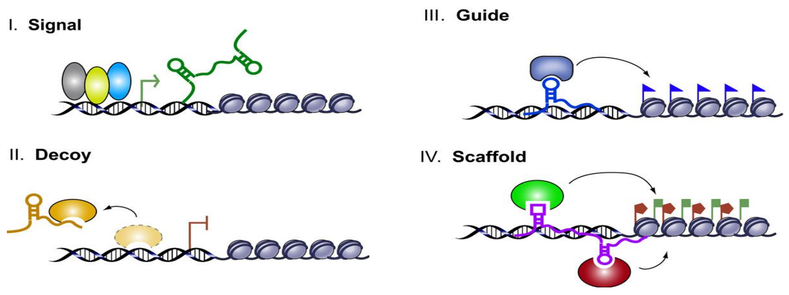
BACKGROUND

- Long non coding RNAs (lncRNAs) represent a novel class of transcripts that are >200 nucleotides in length and do not have the potential to encode for proteins exceeding lenghts of 30 amino acids.
- They are involved in neurological disorders (***Fenoglio et al., IJMS 2013***)
- they exert a role in:

  regulation of gene expression : signaling, molecular decoys, mRNA stability



Wang KC and Chang HY (2011), Mol. Cell.

- epigenetic: molecular scaffolds regulating histone modification
- architectural roles in nuclear paraspeckles

PLAN OF THE STUDY

- Wide analysis in PBMC cells through arrays
- Validation of the best hits via Real Time PCR in a larger population
- Correlation with clinical data

Discovery population	Controls	Total MS	RR-MS	MS-Progressive
N	6	10	5	5
gender (M:F)	1 : 5	2 : 8	0 : 5	2 : 3
Mean age yrs ± S.E.M.	40.67 ± 2.34	43.3 ± 4.94	41.33 ± 2.45	46.25 ± 6.78
Mean age at onset yrs ± S.E.M.	NA	36.4±2.47	34.21±3.58	40.39±2.57
Mean disease duration yrs ± S.E.M.	NA	4.77 ± 1.99	5.1 ± 2.54	4.37 ± 2.28
Mean EDSS (range)	NA	2.6 (1.0-5.0)	1.33 (1.0-2.5)	4.5 (2.5-5.0)

Validation population	Controls	Total MS	RR MS	MS-Progressive
N	25	30	21	9
Gender (M:F)	13:12	12:18	7:14	5:4
Età media anni ± S.E.M.	33.7 ± 2.6	47.1 ± 2.2	32.6 ± 2.0	56.3 ± 3.5
Età media all'esordio anni ± S.E.M.	NA	38.1 ± 2.2	30.7± 2.5	41.2 ± 3.3
Durata media malattia anni ± S.E.M.	NA	12.2 ± 1.5	10 0± 2.1	11.2 ± 2.0
Media EDSS (range)	NA	2.7 (0-7.0)	1.7 (0-5.0)	5.1 (3.0-7.0)

Human LncProfiler qPCR Array

	1	2	3	4	5	6	7	8	9	10	11	12
A	21A	7SK	7SL	Air	AK023948	Alpha 280	Alpha 250	ANRIL	anti-NOS2A	antiPeg11	BACE1AS	BC200
B	CAR Intergenic	DHFR upstream	Dio3os	DISC2	DLG2AS	E2F4 antisense	EgoA	EGO B	Emx2os	Evf1 and EVF2	GAS5	Gomafu
C	H19	H19 antisense	H19 upstream	HAR1A	HAR1B	HOTAIR	HOTAIRM1	HOTTIP	Hoxa11as	HOXA3as	HOXA6as	HULC
D	IGF2AS	IPW	Jpx	Kcnq1ot1	KRASP1	L1PA16	p21	RoR	SFMBT2	VLDLR	LOC 285194	LUST
E	Malat1	mascRNA	MEG3	MEG9	MER11C	ncR-uPAR	NDM29	NEAT1	Nespas	NRON	NTT	p53mRNA
F	PCGEM1	PR antisense	PRINS	PSF inhibiting	PTENP1	RNCR3	SAF	SCA8	snaR	SNHG1	SNHG3	SNHG4
G	SNHG5	SNHG6	Sox2ot	SRA	ST7OT	TEA ncRNAs	Tmevpg1	TncRNA	Tsix	TUG1	UCA1	UM9-5
H	WT1-AS	Xist	Y RNA-1	Zeb2NAT	Zfas1	Zfhx2as	18S rRNA	RNU43	GAPDH	LAMIN A/C	U6	No assay control

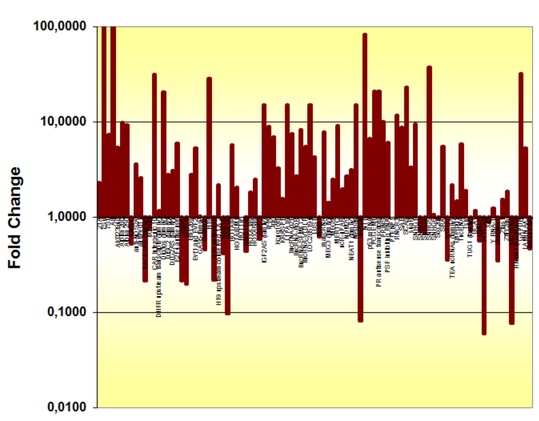
RT PCR with human LncProfiler qPCR Array from SBI , that analyzed the expression of the 90 lncRNAs most investigated and related to a disease status

AIM

To investigate the role of cellular LncRNA in Multiple Sclerosis pathogenesis by investigating their expression in patients compared with controls

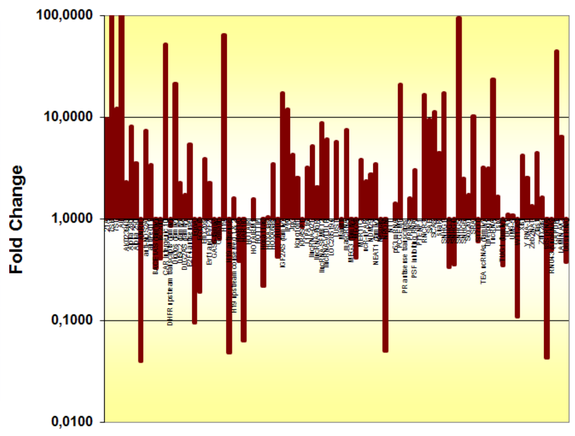
RESULTS: DISCOVERY PHASE:

lncRNAs expression levels in total MS patients compared with ct



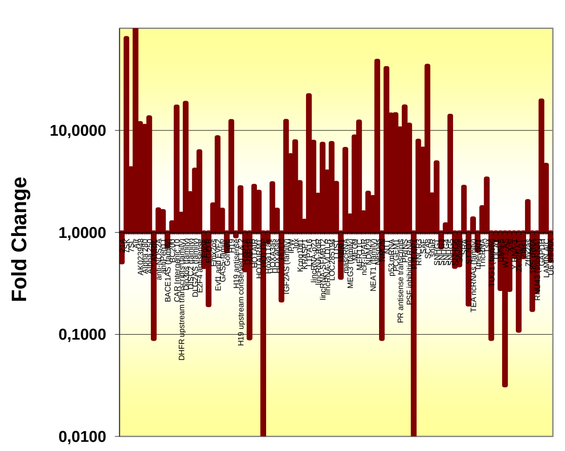
total MS population vs controls	
lncRNA	fold change SM
Anril	0,52
Bace-1AS	0,215
Gomafu	0,457
H19	28,2
HULC	0,587
MALAT-1	0,625
MEG9	2,5
NRON	0,082
Nespas	14,962
Sox2ot	0,941
TUG1	0,723
XIST	0,875
HOTAIR	5,73
AIR	803,52

lncRNAs expression levels in RR MS patients compared with ct



RR MS vs controls	
lncRNA	fold change SM-RR
Anril	0,4
Bace-1AS	0,3
Gomafu	0,6
H19	64,5
HULC	0,421
MALAT-1	0,832
MEG9	0,411
NRON	0,05
Nespas	0,68
Sox2ot	1,728
TUG1	0,349
XIST	4,219
HOTAIR	1,557
AIR	825,7

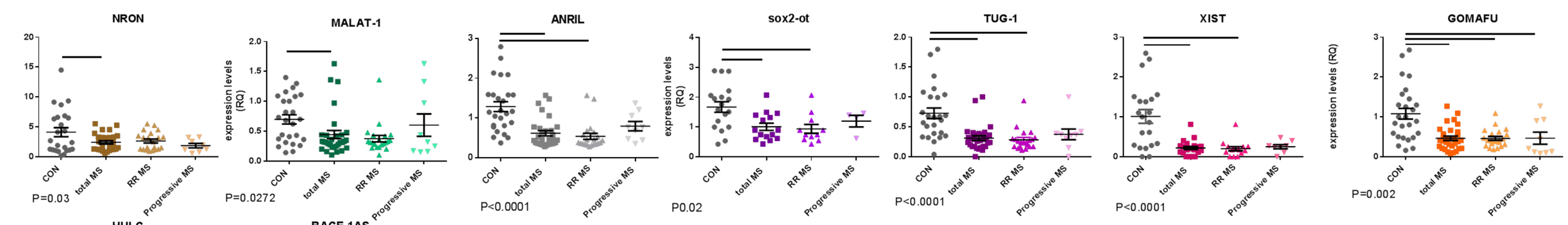
lncRNAs expression levels in Progressive MS patients compared with ct

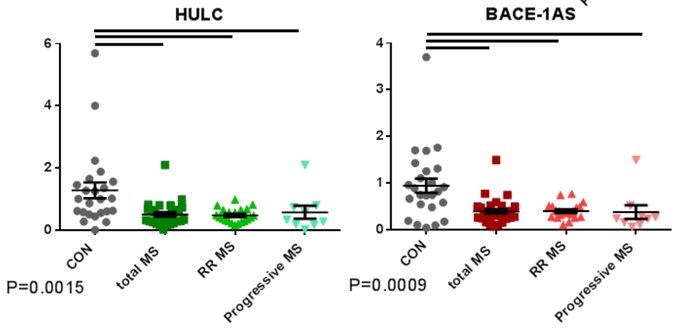


Progressive MS vs controls	
lncRNA	fold change Progressive MS
Anril	0,09
Bace-1AS	0,724
Gomafu	0,652
H19	12,346
HULC	0,215
MALAT-1	0,358
MEG9	8,716
NRON	0,09
Nespas	48,089
Sox2ot	0,474
TUG1	0,09
XIST	0,272
HOTAIR	2,502
AIR	575,2

- ❖ Results showed robust dysegulation of 14 lncRNAs in MS patients compared with controls
- ❖ Some of these dysregulation showed opposite trend in Progressive MS and RRMS

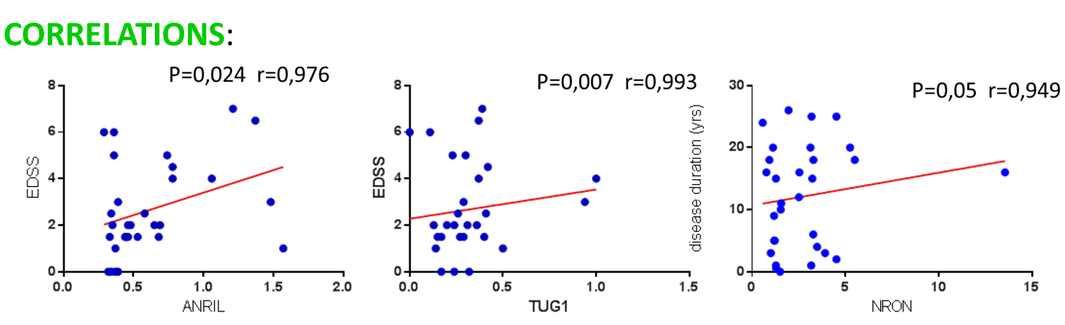
VALIDATION PHASE





- 9 out of 14 lncRNAs were validated
- NRON and MALAT-1 were downregulated total MS patients vs controls

CORRELATIONS:



- ANRIL, sox2-ot, TUG-1 and XIST were downregulated also in RR MS
- GOMAFU, HULC and BACE-1AS were dowregulated both RR and Progressive MS
- ANRIL and TUG1 correlate with EDSS whereas NRON correlates with disease duration

CONCLUSION

- LncRNAs investigated were found to be significantly downregulated in patients compared with controls.
- NRON is involved in inflammatory process (**Nguyen et al., Int J. Devl. Neursci,2008**) whereas TUG1, MALAT-1, Sox2-OT and GOMAFU are involved in brain development (**Wu et al., Brain Research Bullettin 2013**) and oligodendrocyte differentiation (***Mercer et al., BMC Neuroscience 2010***) and they could thus exert a role in MS pathogenesis.
- lncRNAs profiling may represent a new challenge in the research of biomarkers of disease susceptibility and progression.

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