Expression Analysis of lncRNAs in Refractory and Non-Refractory Epileptic Patients

Sara Mirzajani[1] · Soudeh Ghafouri-Fard[1] · Jafar Mehvari Habibabadi[2] · Shahram Arsang-Jang[3] · Mir Davood Omrani[1] · Seyed Sohrab Hashemi Fesharaki[4] · Arezou Sayad[1] · Mohammad Taheri[5]

Abstract

Long non-coding RNAs (lncRNAs) have been demonstrated to be involved in the pathogenesis of neuropsychiatric disorders such as epilepsy. In the current study, we evaluated expression of eight lncRNAs in 80 epileptic patients (40 refractory and 40 non-refractory ones) and 40 normal individual using quantitative real-time PCR. Bayesian regression model showed significant higher expression of UCA1 in both refractory and non-refractory groups compared with controls (posterior beta of relative expression (RE) = 2.03, P value = 0.003, and posterior beta of RE = 4.05, P value < 0.0001, respectively). Besides, expression of UCA1 was higher in non-refractory patients compared with refractory ones (posterior beta of RE = 2.008, P value = 0.019). When repeating statistical analyses in a gender-based manner, differences in expression of UCA1 were significant in all subgroup analyses except for male non-refractory vs. refractory subgroups analysis. Expression levels of NKILA and ANRIL were higher in both refractory and non-refractory groups compared with controls (posterior beta of RE = 1.565, P value = 0.018, and posterior beta of RE = 1.902, P value = 0.006 for NKILA; posterior beta of RE = 1.304, P value < 0.0001, and posterior beta of RE = 1.603, P value = 0.019 for ANRIL, respectively). However, expression levels of these two lncRNAs were not different between refractory and non-refractory groups. Gender-based analysis for these two lncRNAs revealed similar results except for lack of difference in ANRIL expression between male refractory group and controls. Expression of THRIL was significantly lower in both refractory and non-refractory groups compared with controls (posterior beta of RE = − 0.842, P value = 0.044 and posterior beta of RE = − 1.969, P value < 0.0001, respectively). Furthermore, expression of this lncRNA was lower in non-refractory patients compared with refractory ones (posterior beta of RE = − 1.129, P value = 0.002). However, no significant difference was detected between non-refractory and refractory patients either in males or females. The interactions between gender and relative expressions of PACER, DILC, and MALAT1 were significant, so the results were assessed in gender-based manner. In females, expression of DILC was higher in non-refractory patients compared with refractory ones (posterior beta of RE = 0.959, P value = 0.044). Expression of MALAT1 was lower in female non-refractory patients compared with controls and in female non-refractory patients compared with refractory ones (posterior beta of RE = − 1.35, P value = 0.002, and posterior beta of RE = − 0.942, P value = 0.045, respectively). Finally, expression of PACER was higher in refractory patients vs. controls and non-refractory patients vs. controls in both male and female subgroups. However, comparison between non-refractory and refractory patients revealed significant results only among females. Expression of none of the assessed lncRNAs was correlated with age of study participants. There were robust correlations between expression levels of lncRNAs. The most robust correlations were detected between UCA1 and PACER (r = 0.84, P < 0.0001) and between UCA1 and ANRIL (r = 0.75, P < 0.0001). Taken together, our study demonstrated dysregulation of lncRNAs in peripheral blood of epileptic patients and potentiated them as biomarkers for this neurologic condition.

✉ Arezou Sayad
ar.sayad@yahoo.com

✉ Mohammad Taheri
mohammad_823@yahoo.com

1 Department of Medical Genetics, Shahid Beheshti University of Medical Sciences, Tehran, Iran

2 Isfahan Neuroscience Research Center, Isfahan University of Medical Sciences, Isfahan, Iran

3 Department of Epidemiology and Biostatistics, Cellular and Molecular Research Center, Faculty of Health, Qom University of Medical Sciences, Qom, Iran

4 Pars Advanced and Minimally Invasive Medical Manners Research Center, Pars Hospital, Iran University of Medical Sciences, Tehran, Iran

5 Urogenital Stem Cell Research Center, Shahid Beheshti University of Medical Sciences, Tehran, Iran

Introduction

Several studies have shown that long non-coding RNAs (lncRNAs) are expressed in the brain, and are involved in the functional processes of this tissue such as neurodevelopment and differentiation as well as brain disorders such as degenerative conditions, ischemia, brain tumors, and epilepsy (Chen and Zhou, 2017). A previous microarray assessment of lncRNAs signature in pilocarpine and kainate epilepsy models has shown dysregulation of hundreds of lncRNAs (Lee et al., 2015). Human studies also revealed aberrant expression of lncRNAs in epileptic patients (Mazdeh et al., 2019, Hashemian et al., 2019, Mazdeh et al., 2018b, Mirzajani et al., 2019). For instance, a single study in hippocampus tissues excised from patients with temporal lobe epilepsy (TLE) has shown abnormal methylation of the lncRNA *urothelial cancer associated 1* (*UCA1*) (Miller-Delaney et al., 2015). Another functional study in animal models showed higher levels of *UCA1* in brain tissues and peripheral blood of epileptic rats compared with control group. The observed dynamic alterations in the expression of this lncRNA throughout the process of epilepsy development have implied the role of *UCA1* in the pathogenesis of this neurologic condition (Wang et al., 2017). Clues for participations of other lncRNAs in the pathogenesis of epilepsy are not so directive as *UCA1*. However, evidences have emerged that these transcripts are associated with epilepsy. For instance, metastasis-associated lung adenocarcinoma transcript 1 (*MALAT1*) is highly expressed in neurons. Its silencing has resulted in lower synaptic density, while its overexpression has led to an uncontrolled upsurge in synaptogenesis (Bernard et al., 2010). Moreover, some lncRNAs are involved in the regulation of the nuclear factor-kappa B (NF-κB) (Liu et al., 2015) which controls seizure threshold and gene expression after convulsant incentives thus participating in the susceptibility to seizure (Lubin et al., 2007). Among NF-κB-associated lncRNAs are *NF-κB interacting lncRNA* (*NKILA*) (Liu et al., 2015), *antisense noncoding RNA in the INK4 locus* (*ANRIL*) (Zhou et al., 2016), *downregulated in liver cancer stem cells* (*DILC*) (Wang et al., 2016), and *p50-associated COX-2 extragenic RNA* (*PACER*) (Krawczyk and Emerson, 2014). Finally, *TNF and HNRNPL related immunoregulatory lncRNA* (*THRIL*) regulates TNFα expression through its interaction with hnRNPL (Li et al., 2014). Based on the functional relation between TNF and NF-κB (Hayden and Ghosh, 2014), *THRIL* is expected to modulate NF-κB signaling. Based on the importance of NF-κB signaling in epilepsy, we assessed expression of NF-κB-related lncRNAs in peripheral blood of epileptic patients and healthy subjects to clarify their role in this neurologic condition.

Materials and Methods

Enrolled Individuals

The current study was conducted on blood samples obtained from 80 epileptic patients (40 refractory and 40 non-refractory ones) and 40 normal individual. Non-refractory individuals had no seizure attack throughout 6 months before sampling. Those with refractory seizures took appropriate doses of at least three antiepileptic drugs, but had seizures during this period. All patients were taking antiepileptic drugs prior to sampling (Mazdeh et al., 2018a). None of them had history of febrile seizures. Electroencephalogram (EEG) and brain magnetic resonance imaging (MRI) (diffusion weighted (DW), T1, T2, and gradient eco images) were assessed for diagnosis of patients. The study was approved by the ethics committee of Shahid Beheshti University of Medical Sciences. Informed consent forms were signed by all participants. Individuals recruited for control group had no neurological, psychiatric, or systemic disorder.

Expression Studies

Three milliliter of peripheral blood was collected from all study participants. Total RNA was isolated from all samples using Hybrid-RTM blood RNA extraction Kit (GeneAll, Seoul, South Korea). After assessment of the quality of RNA by using NanoDrop equipment (Thermo Scientific, MA, USA), first strand cDNA was synthetized using the OneStep RT-PCR Series Kit (BioFact™, Seoul, South Korea). Expression assays were performed in StepOnePlus™ RealTime PCR System (Applied Biosystems, Foster city, CA, USA). The RealQ Plus 2x PCR Master Mix Green without ROX™ PCR Master Mix (Ampliqon, Odense, Denmark) was used for preparation of reactions. *B2M* gene was used as normalizer. Table 1 shows the sequences of primers.

Statistical Analyses

The Bayesian regression model was used to examine the differences in means of relative expression values between different study groups. The effects of age and gender were adjusted. The Laplace prior distribution was assumed for parameters with 5000 iteration and 1000 warm up. The Bayesian model (Hamiltonian method) was used as an alternative to the ordinary quantile linear regression to reach more wealthy information about the samples and the association between variables. *P* values were estimated from frequentist method using

Table 1 Detailed information of primers

lncRNA	Primer	Sequence	Product length
ANRIL	Forward	tgctctatccgccaatcagg	108 bp
	Reverse	gcgtgcagcggtttagttt	
NKILA	Forward	aaccactatcattttattttccatt	100 bp
	Reverse	caaagcaattctcctttccta	
MALAT1	Forward	gacggaggttgagatgaagc	84 bp
	Reverse	attcggggctctgtagtcct	
UCA1	Forward	cttaggctggcaaccatcagatcc	129 bp
	Reverse	gtgttgtcctggatgctggtctg	
THRIL	Forward	aaggaggacacaacagat	100 bp
	Reverse	tagcagcaataagcaagc	
DILC	Forward	ggaaaggagagaagaatgg	144 bp
	Reverse	gtaagatgtggttgtcgg	
PACER	Forward	tggtcctaagcagttaccctgta	177 bp
	Reverse	accaaaataatccacgcatcagg	
B2M	Forward	agatgagtatgcctgccgtg	105 bp
	Reverse	gcggcatcttcaaacctcca	

quantile regression model or bootstrapped method. The quantile regression, Stan, LOO, and Shynistan packages were used in the R 3.6.1 environment. The R-hat and Gelman-Rubin were used to check the convergence of the models. The statistical significance was assessed by 95% credible interval (95% CrI).

Results

General Demographic Data of Enrolled Individuals

Table 2 shows the general demographic data of enrolled individuals. Mean (± standard deviation) values of age were 33.92 (± 9.03), 36.76 (± 7.73), and 32.8 (± 11.91) in control, refractory, and non-refractory groups, respectively.

Expression Studies

Significant differences in expression of all assessed lncRNAs except for DILC and MALAT1 were detected between study groups (Fig. 1).

Bayesian regression model showed significant higher expression of UCA1 in both refractory and non-refractory groups compared with controls (posterior beta of RE = 2.03, P value = 0.003, and posterior beta of RE = 4.05, P value <

0.0001, respectively). Besides, expression of UCA1 was higher in non-refractory patients compared with refractory ones (posterior beta of RE = 2.008, P value = 0.019). When repeating statistical analyses in a gender-based manner, differences in expression of UCA1 were significant in all subgroups analyses except for male non-refractory vs. refractory subgroups analysis.

Expression levels of NKILA and ANRIL were higher in both refractory and non-refractory groups compared with controls (posterior beta of RE = 1.565, P value = 0.018, and posterior beta of RE = 1.902, P value = 0.006 for NKILA; posterior beta of RE = 1.304, P value < 0.0001, and posterior beta of RE = 1.603, P value = 0.019 for ANRIL, respectively). However, expression levels of these two lncRNAs were not different between refractory and non-refractory groups. Gender-based analysis for these two lncRNAs revealed similar results except for lack of difference in ANRIL expression between male refractory group and controls.

Expression of THRIL was significantly lower in both refractory and non-refractory groups compared with controls (posterior beta of RE = − 0.842, P value = 0.044, and posterior beta of RE = − 1.969, P value < 0.0001, respectively). Furthermore, expression of this lncRNA was lower in non-refractory patients compared with refractory ones (posterior beta of RE = − 1.129, P value = 0.002). However, no significant difference was detected between non-refractory and refractory patients either in males or females. Table 3 shows results of Bayesian regression model for comparison of expression of these lncRNAs between study groups.

The interactions between gender and relative expressions of PACER, DILC, and MALAT1 were significant, so the results were assessed in gender-based manner. In females, expression of DILC was higher in non-refractory patients compared with refractory ones (posterior beta of RE = 0.959, P value = 0.044). Expression of MALAT1 was lower in female non-refractory patients compared with controls and in female non-refractory patients compared with refractory ones (posterior beta of RE = − 1.35, P value = 0.002, and posterior beta of RE = − 0.942, P value = 0.045, respectively). Finally, expression of PACER was higher in refractory patients vs. controls and non-refractory patients vs. controls in both male and female subgroups. However, comparison between non-refractory and refractory patients revealed significant results only among females. Table 4 shows results of Bayesian

Table 2 Characteristics of study participants

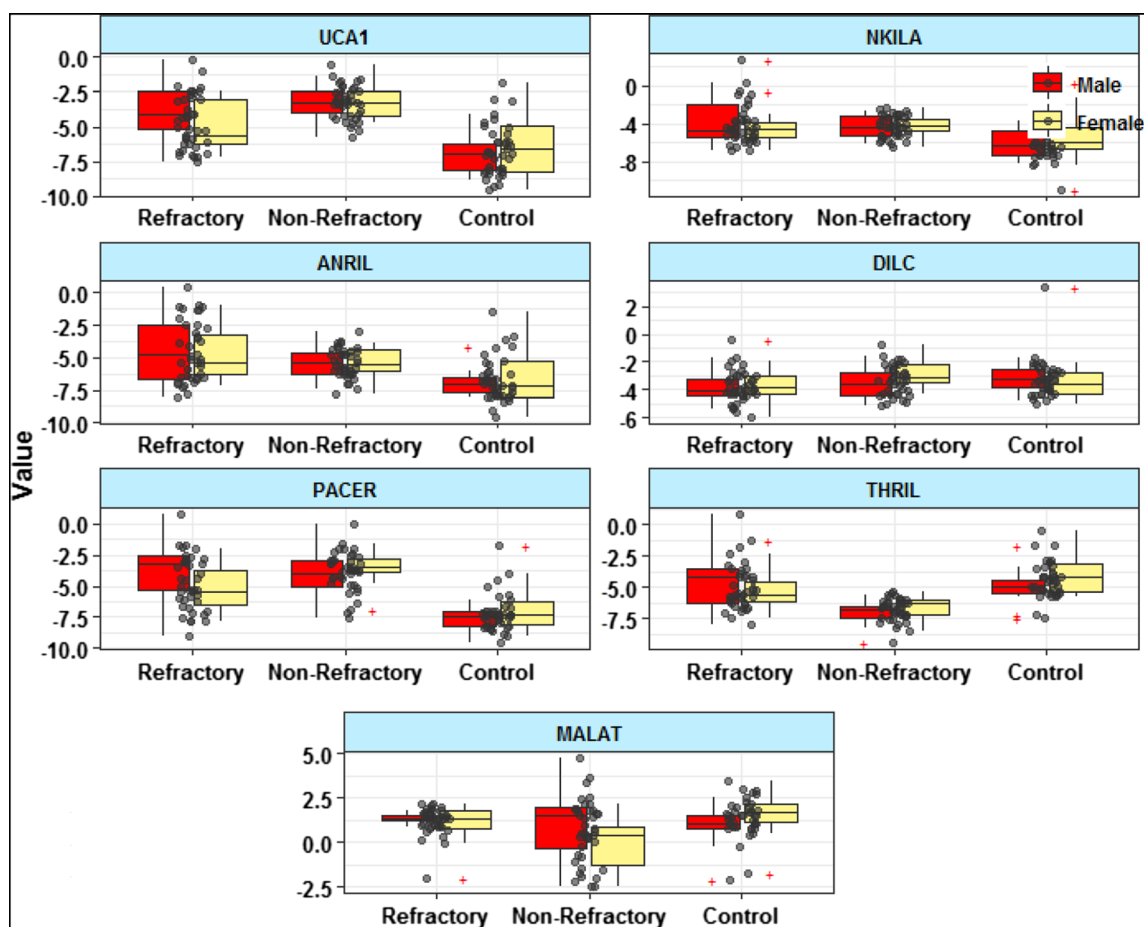| Groups	Control group	Refractory epilepsy	Non-refractory epilepsy
Total, n (%)	40 (33.33)	40 (33.33)	40 (33.33)
Male, n (%)	17 (29.31)	17(29.31)	24 (41.38)
Female, n (%)	23 (37.1)	23 (37.1)	16 (25.8)

Fig. 1 Relative expression of lncRNAs in refractory epilepsy, non-refractory epilepsy, and control groups

regression model for comparison of *PACER*, *DILC*, and *MALAT1* relative expressions.

Correlation Analysis

Expression of none of the assessed lncRNAs was correlated with age of study participants. There were robust correlations between expression levels of lncRNAs. The most robust correlations were detected between *UCA1* and *PACER* ($r = 0.84$, $P < 0.0001$) and between *UCA1* and *ANRIL* ($r = 0.75$, $P < 0.0001$). Figure 2 shows the correlations between expressions of lncRNAs and between their expressions and age.

ROC Curves

Based on the area under curve (AUC) values, *THRIL*, *PACER*, and *UCA1* had outstanding power to differentiate non-refractory group from controls (AUC values of 0.949, 0.933, and 0.929, respectively). *PACER* could also differentiate refractory group from controls with excellent power (AUC = 0.826). Finally, *THRIL* could differentiate non-refractory from refractory patients with diagnostic power of 0.825. Table 5 shows detailed data of ROC curve analysis.

Discussion

In the present study, we compared expression of seven lncRNAs between epileptic patients and normal controls. We detected significant higher expression of *UCA1* in both refractory and non-refractory groups compared with controls. Besides, expression of *UCA1* was higher in non-refractory patients compared with refractory ones. Consistent with our study, a previous study in lithium chloride-pilocarpine-induced model of epilepsy has shown constant upregulation of *UCA1* and *NF-κB* in brain tissues in epileptic animals compared with control group. Based on their results, authors concluded that *UCA1* provoked epilepsy through interaction with NF-κB (Wang et al., 2017). However, a more recent study in pilocarpine-induced epileptic rats demonstrated downregulation of *UCA1* in epileptiform hippocampal tissues and neurons of epileptic rats. Notably, overexpression of this lncRNA inhibited pilocarpine-induced epilepsy in experimental models (Geng et al., 2018). The discrepancy between these studies might be explained by the difference in study design. The former study has evaluated expression of *UCA1* at different time points after status

Table 3 Results of Bayesian regression model for comparison of lncRNAs relative expressions between study groups with adjusting the effects of age and gender (RE relative expression, SE standard error, CrI credible interval; P values are estimated from Frequentist method)

		UCA1				NKILA				ANRIL				THRIL			
Groups	Variables	Posterior beta of RE	SE	P value	95% CrI for RE	Posterior beta of RE	SE	P value	95% CrI for RE	Posterior beta of RE	SE	P value	95% CrI for ER	Posterior beta of RE	SE	P value	95% CrI for ER
Total	Refractory vs. control	2.03	0.42	0.003	(1.22, 2.84)	1.565	0.34	0.018	(0.91, 2.23)	1.304	0.32	<0.0001	(0.71, 1.93)	−0.842	0.3	0.044	(−1.43, −0.26)
	Non-refractory vs. control	4.05	0.35	<0.0001	(3.36, 4.68)	1.902	0.3	0.006	(1.31, 2.49)	1.603	0.27	0.019	(1.04, 2.12)	−1.969	0.28	<0.0001	(−2.53, −1.41)
	Non-refractory vs. refractory	2.008	0.41	0.019	(1.21, 2.77)	0.333	0.32	0.562	(−0.28, 0.99)	0.312	0.29	0.241	(−0.32, 0.83)	−1.129	0.3	0.002	(−1.7, −0.55)
	Gender	0.244	0.38	0.684	(−0.44, 0.96)	−0.439	0.27	0.517	(−0.94, 0.14)	−0.096	0.23	0.642	(−0.52, 0.38)	−0.184	0.25	0.457	(−0.69, 0.31)
	Age	0.006	0.02	0.903	(−0.03, 0.04)	0.002	0.01	0.601	(−0.03, 0.03)	−0.005	0.01	0.324	(−0.03, 0.02)	−0.003	0.01	0.355	(−0.02, 0.02)
Male	Refractory vs. control	3.17	0.62	<0.0001	(2.01, 4.39)	1.61	0.52	0.047	(0.65, 2.61)	0.9	0.8	0.057	(−0.19, 3.15)	0.54	0.58	0.343	(−0.76, 1.54)
	Non-refractory vs. control	4.04	0.47	<0.0001	(3.11, 4.95)	1.84	0.36	0.0001	(1.16, 2.55)	1.19	0.41	0.006	(0.42, 2.02)	−1.92	0.31	<0.0001	(−2.52, −1.27)
	Non-refractory vs. refractory	0.882	0.59	0.222	(−0.38, 1.99)	0.195	0.49	0.685	(−0.75, 1.08)	0.259	0.71	0.263	(−1.7, 1.31)	−2.44	0.6	<0.0001	(−3.39, −1.09)
	Age	−0.01	0.02	0.341	(−0.05, 0.02)	0	0.02	0.692	(−0.04, 0.03)	−0.01	0.02	0.365	(−0.04, 0.02)	−0.02	0.01	0.287	(−0.05, 0)
Female	Refractory vs. control	1.94	0.26	0.037	(1.4, 2.47)	1.25	0.53	0.014	(0.25, 2.3)	1.77	0.41	0.001	(1.02, 2.63)	−1.34	0.4	0.04	(−2.1, −0.55)
	Non-refractory vs. control	4.31	0.29	<0.0001	(3.7, 4.87)	1.76	0.52	0.003	(0.68, 2.73)	1.95	0.49	0.005	(1.1, 2.7)	−2.02	0.4	0.005	(−2.82, −1.28)
	Non-refractory vs. refractory	2.358	0.28	0.013	(1.78, 2.91)	0.512	0.49	0.462	(−0.44, 1.47)	0.201	0.42	0.666	(−0.66, 0.96)	−0.684	0.39	0.593	(−1.47, 0.08)
	Age	0.06	0.02	0.048	(0.02, 0.09)	0.01	0.02	0.764	(−0.04, 0.06)	0.000001	0.02	0.649	(−0.05, 0.04)	0.04	0.02	0.24	(0, 0.07)

Table 4 Results of Bayesian regression model for comparison of *PACER*, *DILC*, and *MALAT1* relative expressions between study groups with adjusting the effects of age and gender (RE relative expression, SE standard error, CrI credible interval; P values are estimated from Frequentist method)

| lncRNAs		DILC				PACER				MALAT1			
Groups	Variables	Posterior beta of RE	SE	P value	95% CrI for RE	Posterior beta of RE	SE	P value	95% CrI for RE	Posterior beta of RE	SE	P value	95% CrI for ER
Total	Refractory vs. control	−0.443	0.24	0.078	(−0.89, 0.04)	2.237	0.48	<0.0001	(1.35, 3.26)	−0.143	0.18	0.561	(−0.52, 0.2)
	Non-refractory vs. control	0.003	0.27	0.349	(−0.55, 0.52)	3.726	0.3	<0.0001	(3.13, 4.34)	−0.335	0.36	0.288	(−1.12, 0.26)
	Non-refractory vs. refractory	0.438	0.28	0.056	(−0.12, 0.99)	1.471	0.45	0.002	(0.56, 2.32)	−0.198	0.39	0.668	(−1.06, 0.42)
	Gender	−0.127	0.19	0.335	(−0.5, 0.25)	−0.005	0.29	0.97	(−0.6, 0.55)	−0.068	0.17	0.146	(−0.42, 0.26)
	Age	0.006	0.01	0.512	(−0.01, 0.02)	0	0.01	0.536	(−0.03, 0.03)	−0.004	0.01	0.894	(−0.02, 0.01)
	Refractory *gender	−0.567	0.44	0.368	(−1.43, 0.25)	2.044	0.78	0.006	(0.51, 3.45)	0.588	0.36	0.311	(−0.12, 1.29)
	Non-refractory *gender	−1.223	0.48	0.135	(−2.13, −0.27)	−0.444	0.6	0.701	(−1.67, 0.74)	1.858	0.42	0.004	(0.98, 2.69)
Male	Refractory vs. control	−0.72	0.33	0.021	(−1.36, −0.07)	4.43	0.53	<0.0001	(3.27, 5.4)	0.14	0.25	0.54	(−0.36, 0.64)
	Non-refractory vs. control	−0.62	0.35	0.459	(−1.29, 0.1)	3.86	0.45	<0.0001	(2.99, 4.76)	0.48	0.31	0.315	(−0.17, 1.08)
	Non-refractory vs. refractory	0.098	0.37	0.325	(−0.54, 0.87)	−0.58	0.55	0.246	(−1.64, 0.49)	0.346	0.3	0.736	(−0.24, 0.91)
	Age	−0.01	0.01	0.81	(−0.03, 0.02)	0.01	0.02	0.636	(−0.03, 0.04)	0	0.01	0.976	(−0.02, 0.02)
Female	Refractory vs. control	−0.28	0.33	0.481	(−0.92, 0.39)	1.66	0.4	<0.0001	(0.92, 2.46)	−0.43	0.28	0.369	(−1, 0.09)
	Non-refractory vs. control	0.59	0.36	0.35	(−0.13, 1.27)	3.96	0.38	<0.0001	(3.23, 4.7)	−1.35	0.3	0.002	(−1.93, −0.75)
	Non-refractory vs. refractory	0.859	0.36	0.044	(0.1, 1.52)	2.297	0.43	0.019	(1.46, 3.15)	−0.942	0.31	0.045	(−1.49, −0.27)
	Age	0.02	0.02	0.258	(−0.01, 0.05)	0	0.02	0.96	(−0.06, 0.04)	−0.01	0.01	0.237	(−0.04, 0.02)
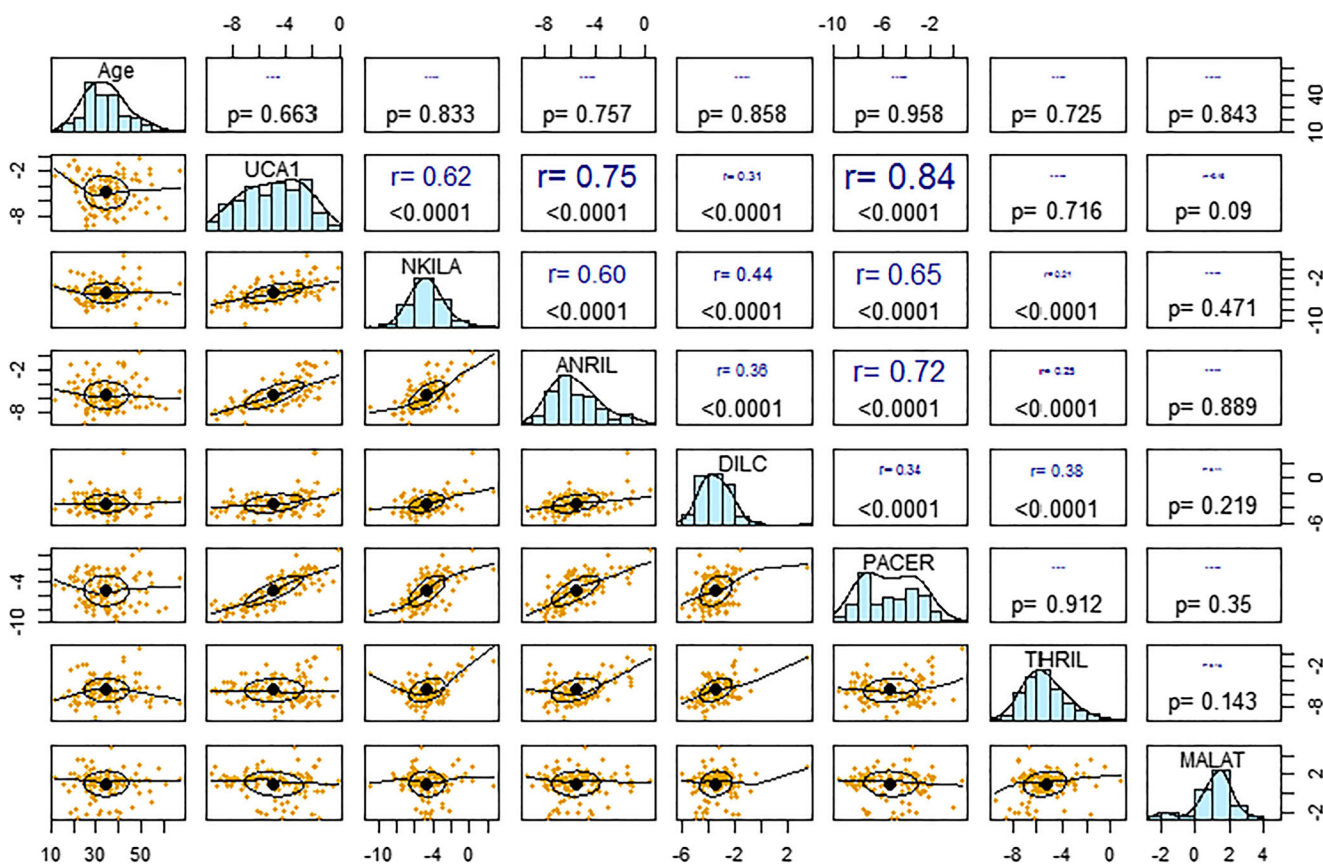
Fig. 2 Correlations between expressions of lncRNAs and between their expressions and age

epilepticus (Wang et al., 2017), but the latter studied *UCA1* expression just once (1 h after status epilepticus onset) (Geng et al., 2018). As stated by the authors, the results of the latter study might be limited by the relative small sample size (Geng et al., 2018). In line with the former study, the hypoxia-inducible factor-1α (HIF1A), which is over-expressed in epileptic brain tissues (Jiang et al., 2016), can enhance expression of *UCA1* through direct interaction with its promoter (Xue et al., 2014). We also reported higher expression of *ANRIL* in both refractory and non-refractory groups compared with controls. Notably, both *UCA1* and *ANRIL* have been reported to upregulate the multidrug resistance protein ATP binding cassette subfamily B member 1 (ABCB1) (Wang et al., 2018, Lan et al., 2016). Based on the role of ABCB proteins in induction of refractoriness in epileptic patients (Lazarowski et al., 2007), higher level of *UCA1* and *ANRIL* in epileptic patients is expected to confer refractoriness. However, we could not find significant difference in expression of *ANRIL* between refractory and non-refractory groups. Moreover, we reported lower levels of *UCA1* in refractory patients compared with non-refractory ones. This observation might imply the presence of a negative feedback loop between ABCB1 and *UCA1* in which high ABCB1 levels in refractory

patients try to downregulate *UCA1* expression. In line with this hypothesis, *UCA1* has been shown to be hypermethylated in temporal lobe epilepsy, one of the most frequent intractable epilepsies (Huang et al., 2017). Such speculation should be verified through functional studies.

We also demonstrated higher expression of *NKILA* in both refractory and non-refractory groups compared with controls. However, expression level of this lncRNAs was not different between refractory and non-refractory groups. Expression of *NKILA* is induced by NF-κB. However, the interaction between this lncRNA and NF-κB/ inhibitor κB (IκB) leads to suppression of IκB phosphorylation and NF-κB activation (Liu et al., 2015). Higher expression of *NKILA* in epileptic patients might be due to over-activation of NF-κB signaling in epileptic patients.

Besides, we reported lower expression of *THRIL* in both refractory and non-refractory groups compared with controls. Furthermore, expression of this lncRNA was lower in non-refractory patients compared with refractory ones. *THRIL* has a crucial role in induction of TNF-α gene expression (Li et al., 2014). Previous studies have shown contribution of TNF-α in epileptogenesis in an animal model of epilepsy (Patel et al., 2017). Moreover, this cytokine has been among over-expressed cytokines in the brain cortex, amygdala, and

Table 5 Characteristics of ROC curves for differentiation of disease status by transcript levels of lncRNAs

Groups	lncRNAs	Estimate criterion	AUC	J	Sensitivity (%)	Specificity (%)	P value area = 0.5
Refractory vs. control	UCA1	> exp (− 6.154)	0.787	0.45	70	75	< 0.0001
	NKILA	> exp (− 6.017)	0.729	0.45	90	55	0.0001
	ANRIL	> exp (− 6.55)	0.790	0.45	77.5	67.5	< 0.0001
	DILC	≤ exp (− 3.95)	0.628	0.25	52.5	72.5	0.041
	PACER	> exp (− 6.735)	0.826	0.6	80	80	< 0.0001
	THRIL	≤ exp (− 5.77)	0.633	0.35	40	95	0.0039
	MALAT1	≤ exp (1.475)	0.559	0.175	65	52.5	0.371
Non-refractory vs. control	UCA1	> exp (− 5.501)	0.929	0.775	97.5	80	< 0.0001
	NKILA	> exp (− 5.885)	0.755	0.55	95	60	< 0.001
	ANRIL	> exp (− 6.557)	0.774	0.55	87.5	67.5	< 0.0001
	DILC	≤ exp (− 4.103)	0.502	0.1	32.5	77.5	0.977
	PACER	> exp (− 5.706)	0.933	0.8	90	90	< 0.0001
	THRIL	≤ exp (− 5.855)	0.949	0.825	87.5	95	< 0.0001
	MALAT1	≤ exp (0.467)	0.647	50%	90		0.0223
Non-refractory vs. refractory	UCA1	≤ exp (− 5.028)	0.714	0.45	50	95	0.0003
	NKILA	≤ exp (− 2.332)	0.535	0.2	80	0	0.597
	ANRIL	> exp (− 3.86)	0.573	0.35	40	95	0.282
	DILC	>exp (3.396)	0.613	0.225	67.5	55	0.0753
	PACER	≤ exp (− 5.32)	0.636	0.35	50	85	0.034
	THRIL	> exp (− 5.855)	0.825	0.55	67.5	87.5	< 0.0001
	MALAT1	> exp (0.536)	0.608	0.425	90	52.5	0.117

hippocampus after seizures (Plata-Salaman et al., 2000). The lower expression of THRIL in epileptic patients compared with controls might be explained by the inhibitory effects of TNF-α on its expression as demonstrated previously (Li et al., 2014). Alternatively, the effects of THRIL in epilepsy might be independent from its role in regulation of TNF-α expression as this lncRNA has been shown to modulate expression of further immune-associated genes which are involved in innate immune responses (Li et al., 2014). Based on the role of dysregulation of innate immunity in epilepsy (Cordero-Arreola et al., 2017), THRIL might affect several aspects of this neurological condition.

We detected significant interactions between gender and relative expressions of PACER, DILC, and MALAT1. In females, expression of DILC was higher in non-refractory patients compared with refractory ones. DILC is involved in the regulation of interaction between TNF-α/NF-κB signaling and IL-6/STAT3 cascade (Wang et al., 2016). Several lines of evidence have suggested involvement of these signaling pathways and cascades in the pathogenesis of epilepsy (Rana and Musto, 2018). The observed gender-based difference in DILC expression pattern might be explained by the role of gender in regulation of immune responses (Klein and Flanagan, 2016).

Expression of MALAT1 was lower in female non-refractory patients compared with controls and in female non-refractory

patients compared with refractory ones. This lncRNA can enhance the density of dendritic spines, thus altering synaptic plasticity and neuronal regeneration (Wu et al., 2013). A previous study has reported dendrite spine loss in a model of early-onset epilepsy (Jiang et al., 1998). Further studies also demonstrated alterations in dendrite channels at molecular level in epilepsy (Swann et al., 2000). Downregulation of MALAT1 in epileptic patients might be regarded as a protective mechanism to preserve neurons since a previous study in epileptic rats has shown that downregulation of this lncRNA guards hippocampal neurons against autophagy and apoptosis (Wu and Yi, 2018).

Finally, expression of PACER was higher in refractory patients vs. controls and non-refractory patients vs. controls in both male and female subgroups. However, comparison between non-refractory and refractory patients revealed significant results only among females. This lncRNA induces COX-2 gene expression through blocking repressive NF-κB complexes (Krawczyk and Emerson, 2014). Previous studies have shown overproduction of COX-2 during seizure (Rojas et al., 2014). Although the overproduction of COX-2 in certain regions of the epileptic brain is indicative of seizure-associated brain inflammation, therapeutic effects of COX-2 inhibition in epilepsy have not been acceptable (Rojas et al., 2014). Such failure might be related with overexpression of other COX-2-related genes such as PACER in epileptic patients.

Expression of none of the assessed lncRNAs was correlated with age of study participants. However, there were robust correlations between expression levels of lncRNAs. Among the most robust correlations was the correlation between *UCA1* and *ANRIL* which is in line with the presence of a network among these lncRNAs and ABCB1.

We also demonstrated outstanding diagnostic power for a number of lncRNAs especially in differentiation of non-refractory patients from controls. Taken together, our study demonstrated dysregulation of lncRNAs in peripheral blood of epileptic patients and potentiated them as biomarkers for this neurologic condition.

Compliance with Ethical Standards

Conflict of Interest The authors declare that they have no conflict of interest.

Ethical Approval The study was approved by the ethics committee of Shahid Beheshti University of Medical Sciences.

Statement of Informed Consent Informed consent forms were signed by all participants.

References

Bernard D, Prasanth KV, Tripathi V, Colasse S, Nakamura T, Xuan ZY, Zhang MQ, Sedel F, Jourdren L, Coulpier F, Triller A, Spector DL, Bessis A (2010) A long nuclear-retained non-coding RNA regulates synaptogenesis by modulating gene expression. EMBO J 29:3082–3093

Chen Y, Zhou J (2017) LncRNAs: macromolecules with big roles in neurobiology and neurological diseases. Metab Brain Dis 32:281–291

Cordero-Arreola J, West RM, Mendoza-Torreblanca J, Mendez-Hernandez E, Salas-Pacheco J, Menendez-Gonzalez M, Freire RC, Machado S, Murillo-Rodriguez E, Nardi AE, Arias-Carrion O (2017) The role of innate immune system receptors in epilepsy research. CNS Neurol Disord Drug Targets 16:749–762

Geng JF, Liu X, Zhao HB, Fan WF, Geng JJ, Liu XZ (2018) LncRNA UCA1 inhibits epilepsy and seizure-induced brain injury by regulating miR-495/Nrf2-ARE signal pathway. Int J Biochem Cell Biol 99: 133–139

Hashemian F, Ghafouri-Fard S, Arsang-Jang S, Mirzajani S, Fallah H, Mehvari Habibabadi J, Sayad A, Taheri M (2019) Epilepsy is associated with dysregulation of long non-coding RNAs in the peripheral blood. Front Mol Biosci 6:113

Hayden MS, Ghosh S (2014) Regulation of NF-kappaB by TNF family cytokines. Semin Immunol 26:253–266

Huang L-G, Wang X-X, Zou J, Li J-J, Lu Q-C (2017) Dysregulation of miR-1304-3p in hippocampus and serum of patients with intractable epilepsy. Int J Clin Exp Pathol 10:4263

Jiang M, Lee CL, Smith KL, Swann JW (1998) Spine loss and other persistent alterations of hippocampal pyramidal cell dendrites in a model of early-onset epilepsy. J Neurosci 18:8356–8368

Jiang G, Zhou R, He X, Shi Z, Huang M, Yu J, Wang X (2016) Expression levels of microRNA-199 and hypoxia-inducible factor-1 alpha in brain tissue of patients with intractable epilepsy. Int J Neurosci 126:326–334

Klein SL, Flanagan KL (2016) Sex differences in immune responses. Nat Rev Immunol 16:626

Krawczyk M, Emerson BM (2014) p50-associated COX-2 extragenic RNA (PACER) activates COX-2 gene expression by occluding repressive NF-kappaB complexes. Elife 3:e01776

Lan WG, Xu DH, Xu C, Ding CL, Ning FL, Zhou YL, Ma LB, Liu CM, Han X (2016) Silencing of long non-coding RNA ANRIL inhibits the development of multidrug resistance in gastric cancer cells. Oncol Rep 36:263–270

Lazarowski A, Czornyj L, Lubienieki F, Girardi E, Vazquez S, D'giano C (2007) ABC transporters during epilepsy and mechanisms underlying multidrug resistance in refractory epilepsy. Epilepsia 48(Suppl 5):140–149

Lee DY, Moon J, Lee ST, Jung KH, Park DK, Yoo JS, Sunwoo JS, Byun JI, Lim JA, Kim TJ, Jung KY, Kim M, Jeon D, Chu K, Lee SK (2015) Dysregulation of long non-coding RNAs in mouse models of localization-related epilepsy. Biochem Biophys Res Commun 462: 433–440

Li Z, Chao TC, Chang KY, Lin N, Patil VS, Shimizu C, Head SR, Burns JC, Rana TM (2014) The long noncoding RNA THRIL regulates TNFalpha expression through its interaction with hnRNPL. Proc Natl Acad Sci U S A 111:1002–1007

Liu B, Sun L, Liu Q, Gong C, Yao Y, Lv X, Lin L, Yao H, Su F, Li D, Zeng M, Song E (2015) A cytoplasmic NF-kappaB interacting long noncoding RNA blocks IkappaB phosphorylation and suppresses breast cancer metastasis. Cancer Cell 27:370–381

Lubin FD, Ren Y, Xu X, Anderson AE (2007) Nuclear factor-kappa B regulates seizure threshold and gene transcription following convulsant stimulation. J Neurochem 103:1381–1395

Mazdeh M, Ghafouri-Fard S, Hatami M, Eftekharian MM, Ganji M, Sayad A, Arsang-Jang S, Taheri M, Omrani MD (2018a) Expression analysis of vitamin D signaling pathway genes in epileptic patients. J Mol Neurosci 64:551–558

Mazdeh M, Komaki A, Omrani MD, Gharzi V, Sayad A, Taheri M, Ghafouri-Fard S (2018b) Expression analysis of beta-secretase 1 (BACE1) and its naturally occurring antisense (BACE1-AS) in blood of epileptic patients. Neurol Sci 39:1565–1569

Mazdeh M, Zamani M, Eftekharian MM, Komaki A, Arsang-Jang S, Taheri M, Ghafouri-Fard S (2019) Expression analysis of vitamin D receptor-associated lncRNAs in epileptic patients. Metab Brain Dis 34:1457–1465

Miller-Delaney SFC, Bryan K, Das S, Mckiernan RC, Bray IM, Reynolds JP, Gwinn R, Stallings RL, Henshall DC (2015) Differential DNA methylation profiles of coding and non-coding genes define hippocampal sclerosis in human temporal lobe epilepsy. Brain 138:616–631

Mirzajani S, Ghafouri-Fard S, Habibabadi JM, Arsang-Jang S, Omrani MD, Fesharaki SSH, Sayad A, Taheri M (2019) Peripheral expression of Rubicon like autophagy enhancer is reduced in epileptic patients. Gene Rep 17:100539

Patel, D. C., Wallis, G., Dahle, E. J., Mcelroy, P. B., Thomson, K. E., Tesi, R. J., Szymkowski, D. E., West, P. J., Smeal, R. M., Patel, M., Fujinami, R. S., White, H. S. & Wilcox, K. S. (2017) Hippocampal TNFalpha signaling contributes to seizure generation in an infection-induced mouse model of limbic epilepsy. eNeuro 4

Plata-Salaman CR, Ilyin SE, Turrin NP, Gayle D, Flynn MC, Romanovitch AE, Kelly ME, Bureau Y, Anisman H, Mcintyre DC (2000) Kindling modulates the IL-1beta system, TNF-alpha, TGF-

beta1, and neuropeptide mRNAs in specific brain regions. Brain Res Mol Brain Res 75:248–258

Rana A, Musto AE (2018) The role of inflammation in the development of epilepsy. J Neuroinflammation 15:144

Rojas A, Jiang J, Ganesh T, Yang MS, Lelutiu N, Gueorguieva P, Dingledine R (2014) Cyclooxygenase-2 in epilepsy. Epilepsia 55: 17–25

Swann JW, Al-Noori S, Jiang M, Lee CL (2000) Spine loss and other dendritic abnormalities in epilepsy. Hippocampus 10:617–625

Wang X, Sun W, Shen W, Xia M, Chen C, Xiang D, Ning B, Cui X, Li H, Li X, Ding J, Wang H (2016) Long non-coding RNA DILC regulates liver cancer stem cells via IL-6/STAT3 axis. J Hepatol 64: 1283–1294

Wang HK, Yan H, Wang K, Wang J (2017) Dynamic regulation effect of long non-coding RNA-UCA1 on NF-kB in hippocampus of epilepsy rats. Eur Rev Med Pharmacol Sci 21:3113–3119

Wang J, Ye C, Liu J, Hu Y (2018) UCA1 confers paclitaxel resistance to ovarian cancer through miR-129/ABCB1 axis. Biochem Biophys Res Commun 501:1034–1040

Wu Q, Yi X (2018) Down-regulation of long noncoding RNA MALAT1 protects hippocampal neurons against excessive autophagy and apoptosis via the PI3K/Akt signaling pathway in rats with epilepsy. J Mol Neurosci 65:234–245

Wu P, Zuo X, Deng H, Liu X, Liu L, Ji A (2013) Roles of long noncoding RNAs in brain development, functional diversification and neurodegenerative diseases. Brain Res Bull 97:69–80

Xue M, Li X, Li Z, Chen W (2014) Urothelial carcinoma associated 1 is a hypoxia-inducible factor-1alpha-targeted long noncoding RNA that enhances hypoxic bladder cancer cell proliferation, migration, and invasion. Tumour Biol 35:6901–6912

Zhou X, Han X, Wittfeldt A, Sun J, Liu C, Wang X, Gan LM, Cao H, Liang Z (2016) Long non-coding RNA ANRIL regulates inflammatory responses as a novel component of NF-kappaB pathway. RNA Biol 13:98–108