
به نام خالق
هستی

Expression pattern of long non-coding RNAs in Schizophrenic Patients

Mohammad Reza Safari

Alireza Komaki

Mohammad Taheri

Department of Laboratory Medicine, School of Paramedicine, Hamadan University of Medical Sciences, Hamadan, Iran

Email: **safari@umsha.ac.ir**

Introduction

Schizophrenia with a median lifetime prevalence of 4 per 1000 individuals is regarded as one of the most profoundly disabling psychiatric disorders. It is characterized by early adulthood onset of hallucinations, delusions, disorganized communication, decreased motivation, and reduced affect.

Contribution of several genetic, developmental and environmental factors in the pathogenesis of schizophrenia necessitate design of studies to explore the molecular underlying mechanisms of the disease onset and progression.

The biomarker finding investigations have been hindered by the extraordinarily complex nature of the disorder and the dissimilarities in patient populations

- Long non-coding RNAs (lncRNAs) are among putative biomarkers for a wide range of diseases including cancer and neuropsychiatric disorders.
- Few studies have addressed the role of lncRNAs in schizophrenia.
- Amann-Zalcenstein et al., Morelli et al., Tamura et al. have demonstrated associations between expression of some lncRNAs such as the antisense transcript *HAR1*, *C6UAS* and *LINC00271* and schizophrenia.

- In the present study, we compared expressions of *FAS-AS1*, *PVT1*, *TUG1*, *OIP5-AS1*, *THRIL*, *NEAT1* and *GAS5* lncRNAs between schizophrenic patients and healthy subjects.

Material & Methods

. Patients

- The current case-control study was conducted on blood samples obtained from 50 unrelated schizophrenic patients who were referred to psychology department of Hamadan University of Medical Sciences and 50 age and sex-matched healthy subjects. Patients were evaluated based on the Diagnostic and Statistical Manual of Mental Disorders, fifth edition (DSM-V) criteria for schizophrenia.

- Individuals suffered from any concomitant conditions such as malignancy, recent or persistent infectious disorder, autoimmune disease, nerve muscle coupling disorders and pregnancy were excluded from the study.

- The study protocol was approved by Ethical Committee of Hamadan University of Medical Sciences(IR.UMSHA.REC.1396.928).
- Informed written consents were obtained from all study participants (including patients and parents of some patients).

Expression analysis

- Experiments were performed on total RNA extracted from whole venous blood of study participants using Hybrid-RTM blood RNA extraction Kit (Gene all Biotechnology Co Ltd, South Korea).
- cDNA was synthesized using High-Capacity cDNA Reverse Transcription kit (Applied Biosystems).

- Expressions of lncRNAs were compared between cases and controls using TaqMan Universal PCR Master Mix (Takara. Bio, Shiga, Japan) on the rotor gene 6000 Corbett Real-Time PCR System.

HPRT1 gene was used as reference gene.

Statistical analysis

- Relative expressions of lncRNAs were compared between schizophrenic patients and healthy subjects using frequentist method. P values less than 0.05 were regarded as significant.
- The receiver operating characteristic (ROC) curve was depicted to appraise the properness of transcript levels of lncRNAs for defining disease status.-

Results

➢ Table1. The demographic data of study participants

No significant difference was found between age and sex ratio of cases and controls (P values of 0.65 and 0.68).

Variables	Patients	Controls
Female/Male [no. (%)]	15 (30%) / 35 (70%)	23 (46%) / 27 (54%)
Age (mean ± SD, Y)	50.7 ± 4.2	49.2 ± 3.6
Age range (Y)	30-69	29-63
Age at onset (Y)	35 ± 1.2	-
Years of illness	8 ± .04	-
Education	-	-
Preschool (%)	30	12
School (%)	48	28
University (%)	22	60

Table 2- Relative expression of lncRNAs in schizophrenic patients and control subject ([a]: Expression difference: Case-Control, [b] P-value estimated from Frequentist method, [c]: 95% Credible Intervals).

			FAS-AS1				GAS5				NEAT1				OIP5-AS1				PVT1				THRIL				TUG1			
	Control no.	Patient no.	Expression difference [a]	SE	P-value [b]	95% CrI [c]	Expression difference [a]	SE	P-value [b]	95% CrI [c]	Expression difference [a]	SE	P-value [b]	95% CrI [c]	Expression difference [a]	SE	P-value [b]	95% CrI [c]	Expression difference [a]	SE	P-value [b]	95% CrI [c]	Expression difference [a]	SE	P-value [b]	95% CrI [c]	Expression difference [a]	SE	P-value [b]	95% CrI [c]
Total	50	50	0.88	0.51	**0.083**	-0.11, 1.9	-0.15	0.03	**<0.0001**	-0.21, -0.08	1.75	0.6	**0.152**	0.58, 2.93	2.71	0.83	**0.062**	1.06, 4.32	-4.15	0.9	**0.015**	-5.93, -2.34	0.16	0.04	**0.003**	0.07, 0.25	-3.19	0.8	**0.006**	-4.82, -1.64
Male	27	35	-1.83	0.72	**0.001**	-3.23, -0.4	-0.07	0.04	**0.529**	-0.15, 0.01	0.42	0.79	**0.761**	-1.11, 2	0.65	1.04	**0.437**	-1.41, 2.69	-5.21	1.12	**0.009**	-7.4, -3.06	0.1	0.05	**0.047**	-0.01, 0.2	-4.44	0.99	**0.001**	-6.37, -2.53
Female	23	15	1.78	1.27	**0.365**	-0.77, 4.28	-0.3	0.05	**<0.0001**	-0.4, -0.19	4.32	0.95	**0.06**	2.42, 6.12	6.61	1.38	**0.06**	3.9, 9.37	-1.31	1.61	**0.67**	-4.53, 1.84	0.29	0.09	**0.01**	0.11, 0.47	-0.36	1.45	**0.786**	-3.14, 2.49

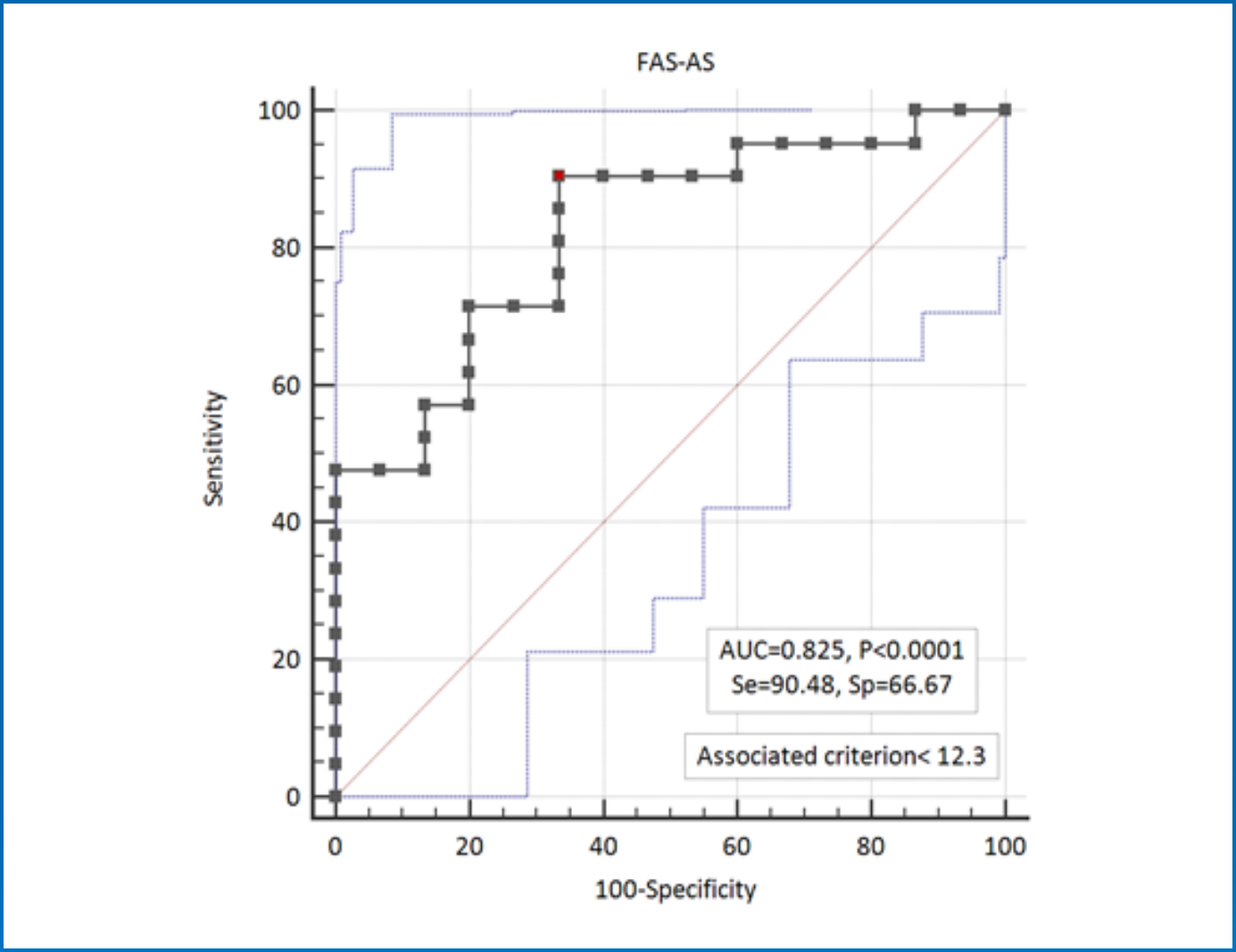

Fig. 1 The results of ROC curve analysis for assessment of diagnostic power of *FAS-AS1* in male subjects aged more than 50 years.

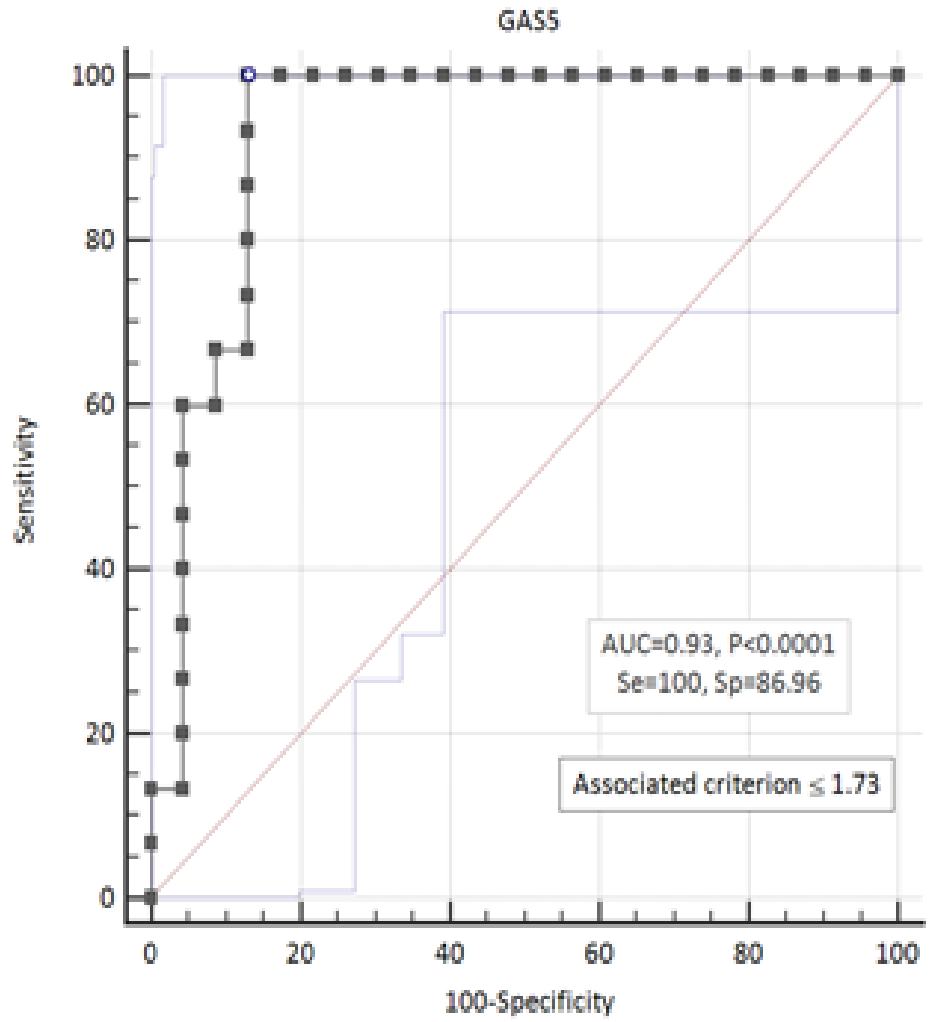

Fig. 2 The results of ROC curve analysis for assessment of diagnostic power of *GAS5* in female subjects.

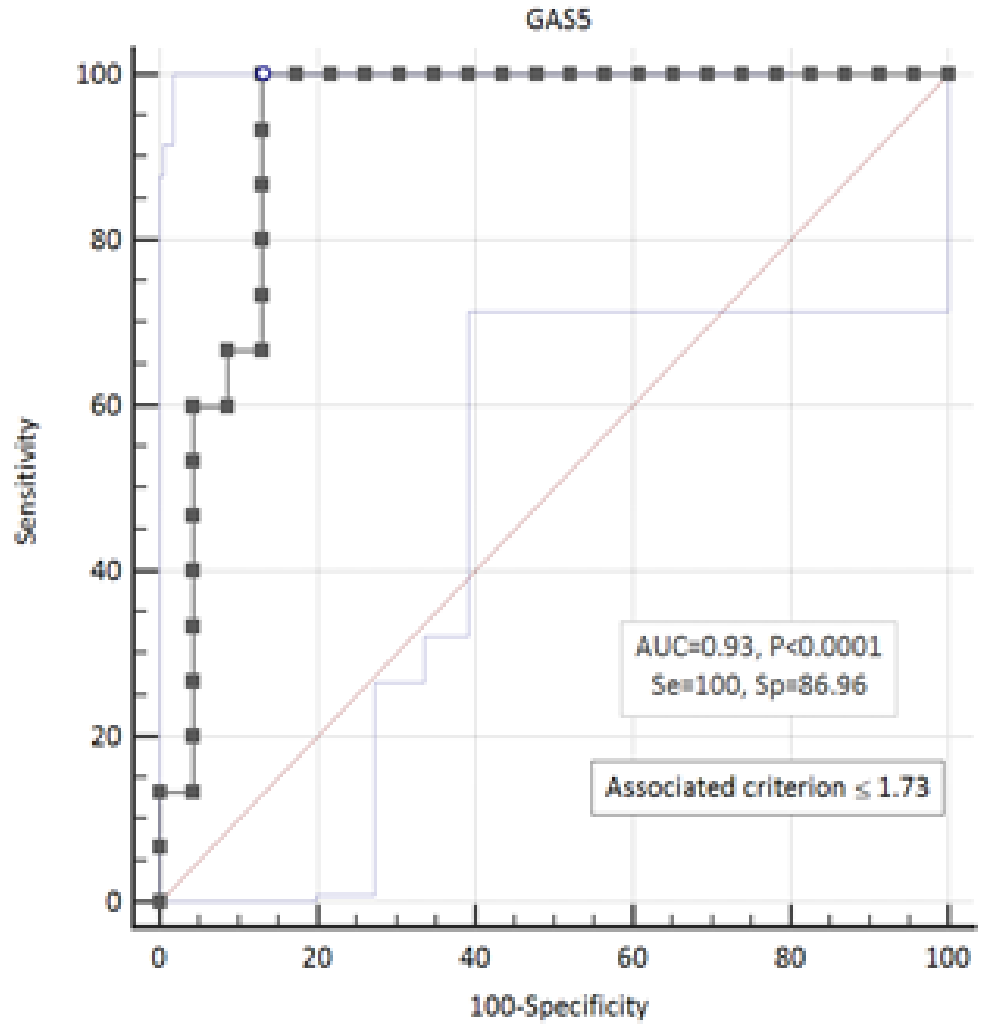

Fig. 3 The results of ROC curve analysis for assessment of diagnostic power of *GAS5* in female subjects aged less than 50 years

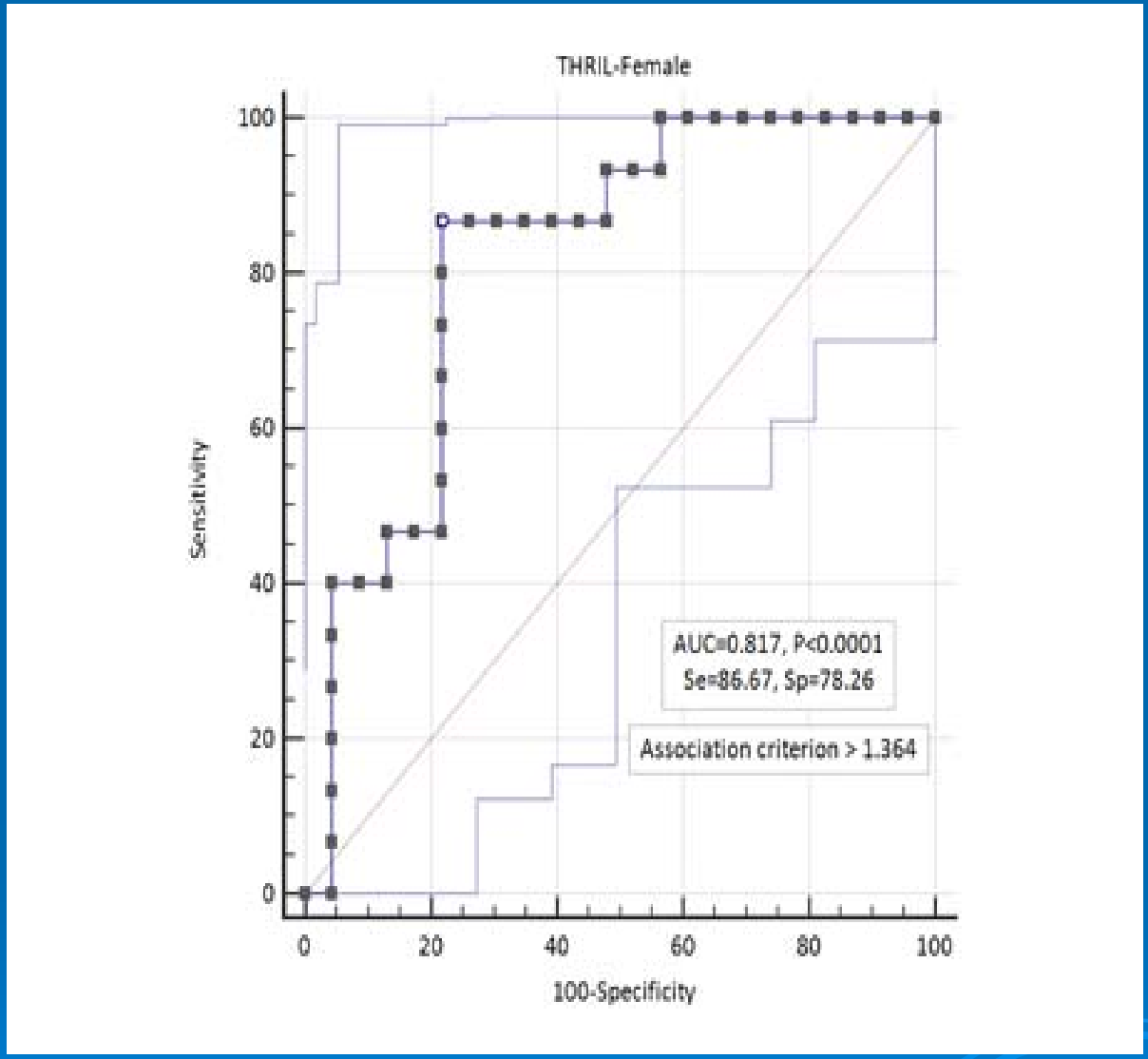

Fig. 4 The results of ROC curve analysis for assessment of diagnostic power of *THRIL* in female subjects

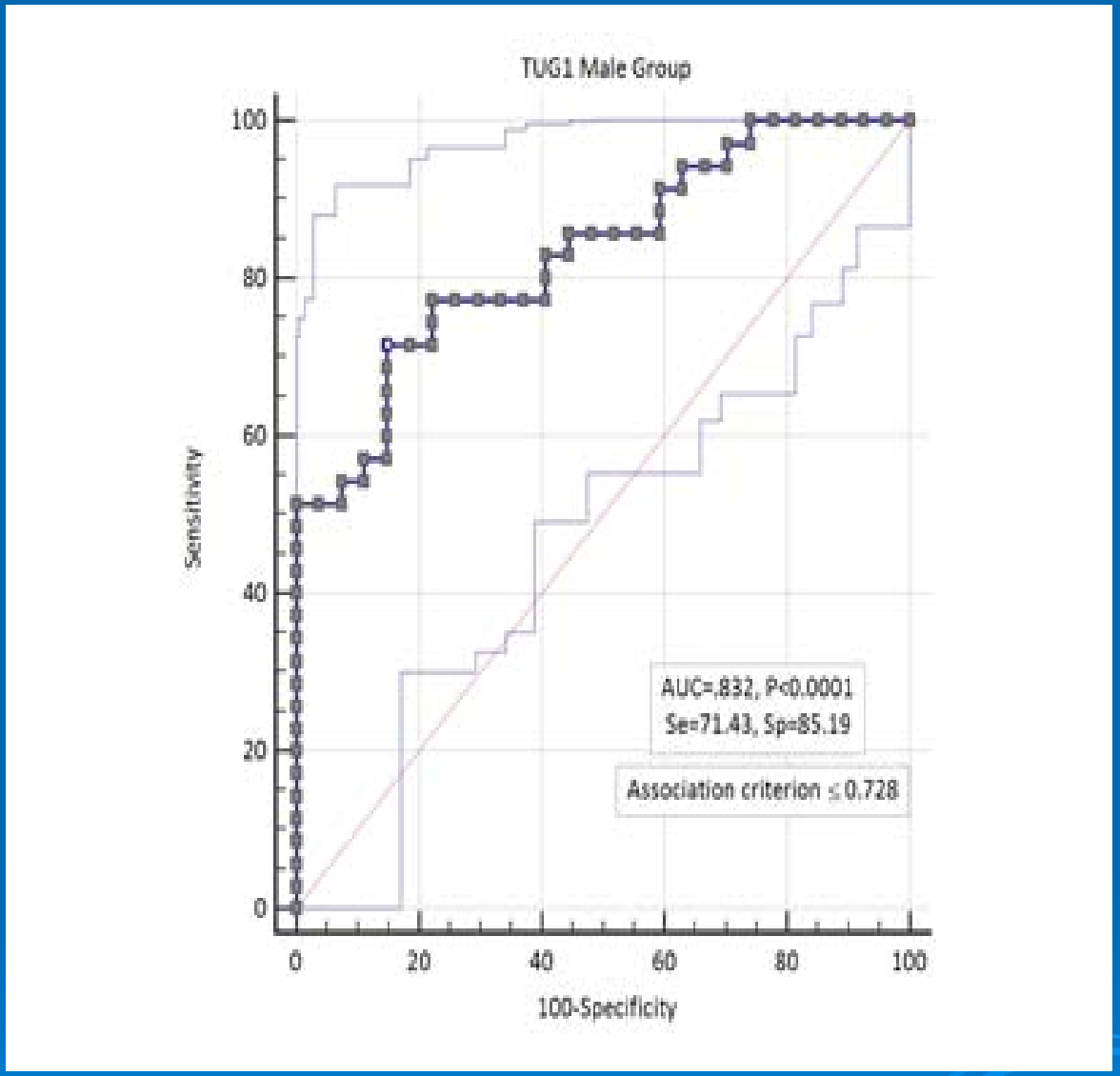

Fig. 5 The results of ROC curve analysis for assessment of diagnostic power of *TUG1* in male subjects

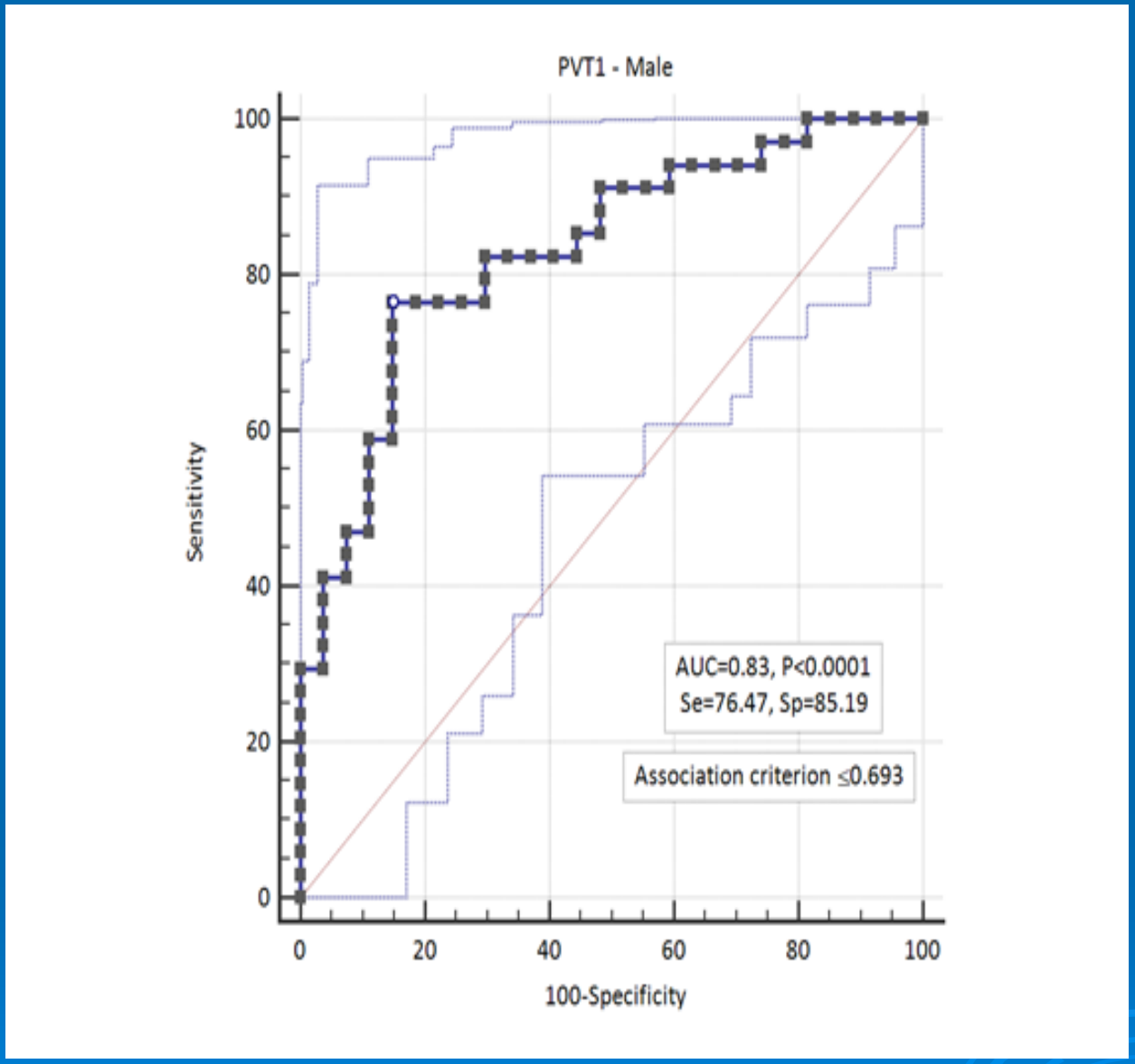

Fig. 6 The results of ROC curve analysis for assessment of diagnostic power of *PVT1* in male subjects.

Discussion

- In the present study, we compared expression of seven lncRNAs between schizophrenic patients and healthy subjects.
- I) We found significant down-regulation of *FAS-AS1*, *PVT1* and *TUG1* in patients.
- II) We also detected higher levels of *THRIL* in patients compared with healthy individuals.
- III) Although *GAS5*, *NEAT1* and *OIP5-AS1* expressions were not significantly different between patients and controls

- The results of ROC curve analysis showed 90.48% sensitivity and 66.67% specificity of *FAS-AS1* expression for diagnosis of schizophrenia in **male** subjects aged more than 50 years (AUC=0.825, P<0.0001).

- As we detected significant association between *GAS5* expression and schizophrenia in female subjects, we evaluated its diagnostic power in this subgroup of study participants.

- *GAS5* transcript levels had 100% sensitivity and 86.96% specificity (AUC=0.93, P<0.0001) for diagnosis of schizophrenia in **female** subjects. The sensitivity and specificity values were increased to 100% in female subjects aged less than 50 (n=10). These results show the appropriateness of *GAS5* expression levels for diagnosis of schizophrenia in female subjects.

- The observed down-regulation of *PVT1* in schizophrenic patients in the current study is consistent with the previously reported role for this lncRNA in neuronal protection. The *PVT1*-mediated autophagy may shield hippocampal neurons from synaptic plasticity damage and apoptosis.

- We found significant down-regulation of *TUG1* in patients. The surge in expression of *TUG1* is necessary for retinal development. *TUG1* has also been shown to interact with miR-9 and seclude it directly. On the other hand, abnormal levels and function of miR-9 have been regarded as one of the numerous elements that participate in the pathogenesis of schizophrenia.

- We found up-regulation of *THRIL* in schizophrenic patients compared with healthy subjects.
- This lncRNA regulates expression of tumor necrosis factor (TNF) in human monocytes through interactions with HNRNPL.

A previous study has shown association between increased TNF-alpha levels and acute exacerbations of schizophrenia. Consequently, we hypothesize that *THRIL* is involved in the pathogenesis of schizophrenia possibly through epigenetic regulation of TNF pathway.

- As we detected no significant correlation between expression of lncRNAs and age of study participants in any study subgroup, we propose these lncRNAs as age-independent disease markers.
- This independence implies that they are not affected by various phenotypic deficits during the disease.

- In brief, in the current study we demonstrated dysregulation of lncRNAs in peripheral blood of schizophrenic patients and suitability of their expression levels as diagnostic markers in certain subgroups of patients.
- While *FAS-AS1* was suitable marker in male subjects, *and GAS5* was more suitable for diagnosis of disease in female subjects compared with male subjects.

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Contributors

- Soudeh Ghafouri-Fard and Shahram Arsang-Jang analyzed the data.
- Mohammad Taheri and Alireza Komaki supervised the study.
- Mohammad Reza Safari performed the laboratory tests.

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ORIGINAL RESEARCH

Expression Pattern of Long Non-coding RNAs in Schizophrenic Patients

Mohammad Reza Safari[1] · Alireza Komaki[1] · Shahram Arsang-Jang[2] · Mohammad Taheri[3,4] · Soudeh Ghafouri-Fard[5]

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Abstract

The role of long non-coding RNAs (lncRNAs) in the pathogenesis of neurological disorders including schizophrenia has been highlighted by independent studies. In the present study, we compared peripheral blood expression of seven lncRNAs between schizophrenic patients and sex- and age-matched controls using quantitative real-time PCR technique. *FAS-AS1, PVT1* and *TUG1* were significantly down-regulated in schizophrenic patients compared with healthy individuals ($P = 0.007$, 0.003 and 0.001, respectively). The association between *FAS-AS1* expression and schizophrenia was significant in male subjects aged more than 50 but not in other subgroups. *GAS5, NEAT1* and *OIP5-AS1* expressions were not significantly different between patients and controls ($P = 0.523$, 0.739 and 0.267, respectively). The associations between *GAS5, NEAT1* and *OIP5-AS1* expressions and schizophrenia were significant in female subjects but not in male subjects. *THRIL* was up-regulated in schizophrenic patients compared with healthy subjects. Based on the results of bootstraped median regression, and after controlling for the effects of age and sex, the difference in its expression between cases and controls was significant


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