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ORIGINAL ARTICLE

Downregulation of *miR-185* is a common pathogenic event in 22q11.2 deletion syndrome-related and idiopathic schizophrenia

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Abstract

Schizophrenia (SCZ) is known as a complicated mental disease with an unknown etiology. The microdeletion of 22q11.2 is the most potent genetic risk factor. Researchers are still trying to find which genes in the deletion region are linked to SCZ. *MIR185*, encoding microRNA (miR)-185, is present in the minimal 1.5 megabase deletion. Nonetheless, the *miR-185* expression profile and its corresponding target genes in animal models and patients with 22q11.2 deletion syndrome (22q11.2DS) imply that more study is required about *miR-185* and its corresponding downstream pathways within idiopathic SCZ. The expression of *hsa-miR-185-5p* and its corresponding target gene, shisa family member 7 (*SHISA7*), sometimes called *CKAMP59*, were evaluated in the peripheral blood (PB) samples of Iranian Azeri patients with idiopathic SCZ and healthy subjects, matched by gender and age as control groups by quantitative polymerase chain reaction (qPCR). Fifty SCZ patients (male/female: 22/28, age (mean ± standard deviation (SD)): 35.9 ± 5.6) and 50 matched healthy controls (male/female: 23/27, age (mean ± SD): 34.7 ± 5.4) were enrolled. The expression of *hsa-miR-185-5p* in the PB samples from subjects with idiopathic SCZ was substantially lower than in that of control groups (posterior beta = -0.985, adjusted *P*-value < 0.0001). There was also a difference within the expression profile between female and male subgroups (posterior beta = -0.86, adjusted *P*-value = 0.046 and posterior beta = -1.015, adjusted *P*-value = 0.004, in turn). Nevertheless, no significant difference was present in the expression level of *CKAMP59* between PB samples from patients and control groups (adjusted *P*-value > 0.999). The analysis of the receiver operating characteristic (ROC) curve suggested that *hsa-miR-185-5p* may correctly distinguish subjects with idiopathic SCZ from healthy people (the area under curve (AUC) value: 0.722). Furthermore, there was a strong positive correlation between the expression pattern of the abovementioned genes in patients with SCZ and healthy subjects (r = 0.870, *P* < 0.001 and r = 0.812, *P* < 0.001, respectively), indicating that this miR works as an enhancer. More research is needed to determine if the *hsa-miR-185-5p* has an enhancer activity. In summary, this is the first research to highlight the expression of the *miR-185* and *CKAMP59* genes in the PB from subjects with idiopathic SCZ. Our findings suggest that gene expression alterations mediated by *miR-185* may play a role in the pathogenesis of idiopathic and 22q11.2DS SCZ. It is worth noting that, despite a substantial and clear relationship between *CKAMP59* and *hsa-miR-185-5p*, indicating an interactive network, their involvement in the development of SCZ should be reconsidered based on the whole blood sample since the changed expression level of *CKAMP59* was not significant. Further research with greater sample sizes and particular leukocyte subsets can greatly make these results stronger.

Keywords 22q11.2 deletion syndrome · *CKAMP59* · *miR-185* · Schizophrenia · *SHISA7*

Introduction

Schizophrenia (SCZ) is a psychiatric disorder that impacts a person believes, senses, and acts (Marder and Cannon, 2019). The symptoms fall into three categories: psychotic, negative, and cognitive. Psychotic symptoms include hallucinations, delusions, and thought disorders. Negative symptoms such as reduced motivation, reduced feelings, and

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reduced speaking. Cognitive symptoms including difficulties in attention, focus, and memory (Marder and Cannon 2019; Patel et al. 2014). SCZ affects about 1% of people around the globe. It is one of the leading 10 worldwide reasons for disability. According to twin and family studies, 80% of the risk of SCZ can be clarified by genetic factors. Nevertheless, just a few of these heritable factors are related to common single-nucleotide variants connected with SCZ; each has a negligible impact on risk. However, rare mutations can have a greater influence on risk (Marder and Cannon 2019; Purcell et al. 2014; Ripke et al. 2014). The most potent recognized genetic risk factor is chromosomal region 22q11.2 deletion, which triggers 22q11.2 deletion syndrome (22q11.2DS). Since the symptoms of patients with idiopathic SCZ are indistinguishable from 22q11.2DS-related SCZ, risk genes for 22q11.2DS-related SCZ may also be entangled in idiopathic cases (Forstner et al. 2014; Earls et al. 2012). Roughly one-third of 22q11.2DS patients will have SCZ in their adulthood (Karayiorgou et al. 2010). 22q11.2 deletion differs in size. Most are 1.5 megabases (Mb) or 3 Mb and span between 35 to 60 recognized genes, respectively (Edelmann et al. 1999; Shaikh et al. 2000). Deletion size and severity of 22q11.2DS phenotype are not correlated. This information may indicate the substantial etiological significance of the 1.5 Mb small deletion region. Various attempts were administered by the genetic association to determine the absent genes in the 1.5 Mb area that are responsible for the higher risk for SCZ. *MIR185* is one of the recognized genes in this deletion region, which encodes *microRNA (miR)-185*. (Karayiorgou et al. 2010).

MiR-185 is known as a thymus-expressed miR that responds to stress (Belkaya et al. 2011). Patients of 22q11.2DS are somewhat diagnosed with hypoparathyroidism, cardiac anomalies, thymic hypoplasia, and/or disabilities related to learning (Kobrynski and Sullivan 2007). In some patients, the T helper cell may become altered, and autoimmune disorders may become more recurring (Piliero et al. 2004; Kanaya et al. 2006). Nonetheless, previous literature outlined that T cell development is regulated by transgenic expression of *miR-185* when it targets various mRNAs, including marginal zone B and B1 cell specific Protein (*Mzb1*) (Belkaya et al. 2013). Shisa family member 7 (*SHISA7*), also known as *CKAMP59*, is a validated target of *hsa-miR-185-5p* (Marques et al. 2012). *CKAMP59* regulates long-term synaptic potentiation. This gene is a major player in gamma-aminobutyric acid (GABA) neurotransmitter regulation (Castellano et al. 2021). GABA is considered the main inhibitory neurotransmitter inside the central nervous system (CNS). GABA acts via two subclasses of receptors, $GABA_A$ receptors ($GABA_A$Rs) and $GABA_B$Rs (Sparrow et al. 2021). $GABA_A$Rs are ligand-gated ion channels, also called ionotropic receptors; whereas $GABA_B$Rs are G protein-coupled receptors (also known as metabotropic receptors) (Sparrow et al. 2021). The GABA is now considered an actor beyond the realm of CNS, in contrast with the previously supposed role of neuronal behavior regulator. Several studies also indicated the existence of some elements of GABAergic signaling within immune system cells. Aside from cell lines, the presence of $GABA_A$Rs was detected in several cells, including B cells, T cells, macrophages, and dendritic in rodents and humans alike. According to the mentioned data, the same mechanism in neurons is responsible for the modification of lymphocyte $GABA_A$R (Tian et al. 2004, 1999; Alam et al. 2006; Dionisio et al. 2011, 2013; Mendu et al. 2012). Due to the direct role in regulating $GABA_A$R at inhibitory synapses, *CKAMP59* has arisen as an intriguing constituent of the family of Shisa while contrasting its CKAMP counterparts (Castellano et al. 2021). However, by considering the expression patterns of *miR-185* and its target genes in 22q11.2DS patients and animal models, further investigation into the involvement of *miR-185* and the associated downstream pathways in idiopathic SCZ is warranted.

The purpose of this study was to investigate the role of *hsa-miR-185-5p* and its target gene *CKAMP59* in SCZ utilizing gene expression analysis in patients diagnosed with idiopathic SCZ and controls.

Materials and methods

Participants and samples

This study is conducted within the framework of the Azeri recent-onset acute phase psychosis survey (ARAS) (Farhang et al. 2021). The study protocol was approved by the ethical committee of Tabriz University of Medical Sciences (IR.TBZMED.REC.1398.1232). Fifty first-episode antipsychotic-naïve adult SCZ patients and fifty age and gender-matched healthy controls were enrolled. An experienced psychiatrist diagnosed first-episode SCZ patients based on the Diagnostic and Statistical Manual of Mental Disorders, 5th edition (DSM-5) criteria (Association 2013). 22q11.2 deletion syndrome, intellectual disability, and substance use (except cigarette) were considered as exclusion criteria. The Mini-International Neuropsychiatric Interview (Sheehan et al. 1998) was utilized to assess control subjects. For the control group, the existence of pregnancy, psychiatric conditions, or systemic disorders was considered as exclusion criteria. Furthermore, individuals who reported a significant mental disorder in a first-degree family were excluded. Ten ml of PB was taken after obtaining written informed consent from all participants and/or their caregivers.

Expression assays

Total RNA was extracted from whole blood based on the manufacturer's procedure using the Hybrid-R™ Blood RNA purification kit (GeneALL, Seoul, South Korea) and treated with DNase I to remove DNA contamination. NanoDrop was used to determine the amount and quality of isolated RNA (Thermo Scientific, Wilmington, DE). The synthesis of cDNA was done by the cDNA synthesis Kit (GeneALL) according to the manufacturer's instructions. The cDNA was kept at -20 °C for further investigation. Table 1 contains the primer sequences utilized in reverse transcription and quantitative polymerase chain reaction (qPCR) reactions. Internal controls hypoxanthine phosphoribosyl transferase 1 (*HPRT1*) and *U6* were used to normalize mRNA and miR levels, respectively. *HPRT1* was chosen because it has been demonstrated as a suitable reference gene for SCZ studies (Hwang et al. 2013; Chaumette et al. 2019; Asadzadeh Manjili et al. 2018). The qPCR was performed using the Step OnePlus™ Real-Time PCR and the RealQ Plus2x Master Mix (Ampliqon, Odense, Denmark).

Statistical analysis

The data analysis was performed using the R v.4 software packages brms, stan, pROC, and GGally. We examined the expression level of *hsa-miR-185-5p* and *CKAMP59* in the PB sample of healthy and diseased individuals.

The multiple Bayesian quantile regression model was used to compare the relative expressions of *hsa-miR-185-5p* and *CKAMP59* between SCZ patients and healthy controls. As relative expressions had a non-normal pattern of distribution, to parametrization of log-transformed dependent variable (relative expressions) we used asymmetric Laplace distribution. We used the asymmetric Laplace distribution corresponding to quantile regression with location (mu = 0), scale (sigma = 1) and asymmetry parameter quantile (q = 0.5). The default brms prior on sigma was considered (student t). Variables and Sex*Group interaction effects that were found to have a significant or borderline p-value ($p < 0.1$) in univariate data analysis were entered in the multiple regression model. The final model was adjusted for age and sex. Model with low value in Pareto smoothed importance-sampling leave-one-out cross-validation (PSIS-LOO) metric was preferred (Vehtari et al. 2015).

Gender and age effects were adjusted. The adjusted *P*-values < 0.05 were taken as significant. Additionally, the expressions of the aforementioned genes were examined across age groups and between males and females. Spearman correlation coefficients were used to assess the relationships between the study variables in both patients of SCZ and healthy controls participants. A receiver operating characteristic (ROC) curve analysis was used to determine the diagnostic power of genes. The simulation was used to examine the power for n, likelihood, and priors. Statistical analyses were conducted using the RStan, loo, and brms packages in the R 4.2 environment (Bürkner 2017).

Table 1 Sequences of primers used in reverse transcription (RT) and qPCR reactions

Gene name	Primer sequences
hsa-miR-185-5p	RT primer: GTCGTATCCAGTGCAGGGTCC GAGGTATTCGCACTGGATACGACTCAG GAA Forward primer: AATCGGCGTGGAGAGAAA GGC Reverse primer: GTCGTATCCAGTGCAGGGTCC
U6	RT primer: GTCGTATCCAGTGCAGGGTCC GAGGTATTCGCACTGGATACGACAAAA ATAT Forward primer: GCTTCGGCAGCACATATACTA AAAT Reverse primer: CGCTTCACGAATTTGCGT GTCAT
CKAMP59	Forward primer: TGAAGACCCCCAACCTCG ACTG Reverse primer: TCCTTCTCGGCCAGCCTCTTG
HPRT1	Forward primer: AGCCTAAGATGAGAGTTC Reverse primer: CACAGAACTAGAACATTGATA

Results

General demographic data

Fifty SCZ patients (male/female: 22/28) with age (mean ± standard deviation (SD)) of 35.9 ± 5.6 and 50 healthy controls (male/female: 23/27) with age (mean ± SD) of 34.7 ± 5.4, all with Turkish Azeri ethnic backgrounds, were investigated.

Expression assays

The relative expression of *hsa-miR-185-5p* and *CKAMP59* genes in SCZ patients and controls is depicted in Fig. 1.

The expression of *hsa-miR-185-5p* was suggestively reduced in PB samples of SCZ cases compared with controls (posterior beta = -0.985, adjusted *P*-value < 0.0001). Such decreased expression was found between male and female subgroups too (posterior beta = -0.86, adjusted *P*-value = 0.046 and posterior beta = -1.015, adjusted *P*-value = 0.004, respectively Nonetheless, we did not find a significant difference in the expression of *CKAMP59* (adjusted *P*-value > 0.999) in PB samples from the cases and controls. The detailed data of relative expression of *hsa-miR-185-5p* and *CKAMP59* are indicated in Tables 2 and 3, respectively.

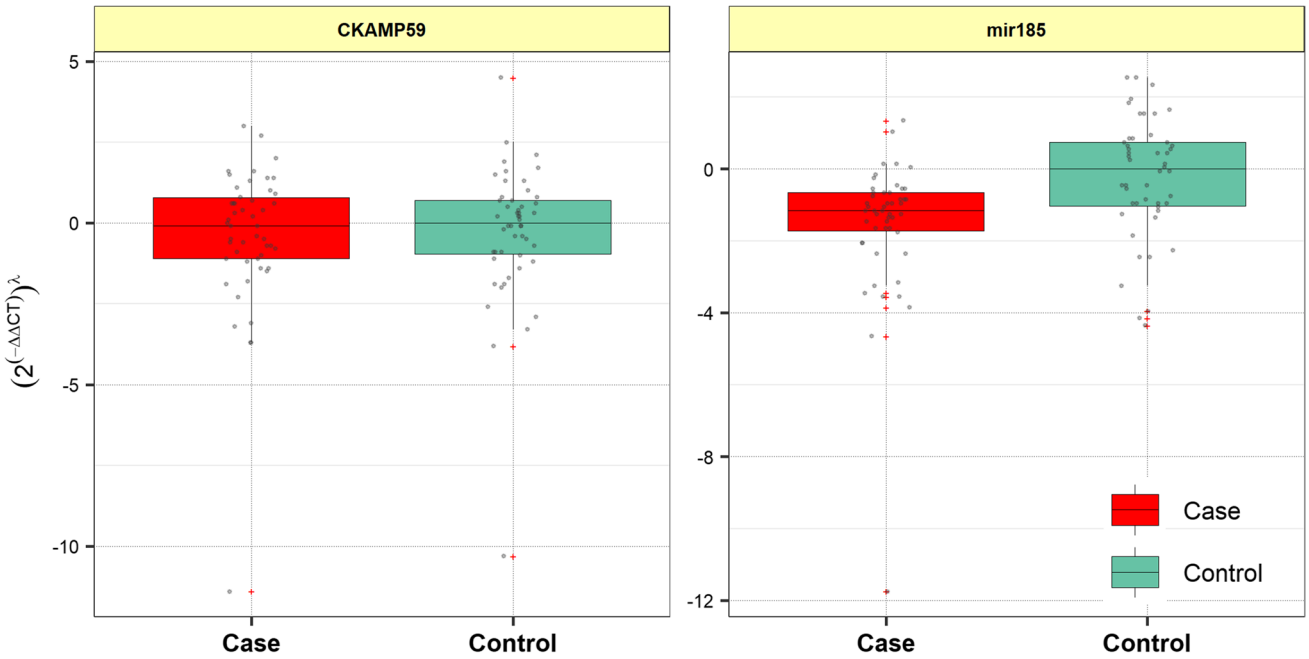

Fig. 1 Expression of *hsa-miR-185-5p* and *CKAMP59* in cases and controls' peripheral blood samples. Values are shown by gray dots. The means of expression and the interquartile range are shown. Internal controls *U6* and *HPRT1* were used to normalize *hsa-miR-185-5p* and *CKAMP59* levels, respectively

Table 2 Relative levels of hsa-*miR-185-5p* in idiopathic schizophrenia cases and controls according to the Bayesian quantile regression model

	hsa-miR-185-5p	Posterior Beta of $(2^{(-ddct)})^{\lambda}$	SE	Adjusted *P*-Value*	95% CrI for Beta
Total	Group, Case vs. control	-0.985	0.21	< 0.0001	[-1.42, -0.57]
	Sex, Male vs. Female	-0.022	0.2	0.628	[-0.42, 0.35]
	Age (years)	-0.013	0.01	0.831	[-0.04, 0.02]
	Group * Sex	0.02	0.35	0.611	[-0.67, 0.7]
Male	Case vs. control	-0.86	0.28	0.046	[-1.45, -0.34]
	Age	-0.01	0.02	0.893	[-0.05, 0.03]
Female	Case vs. control	-1.015	0.25	0.004	[-1.53, -0.54]
	Age	-0.021	0.02	0.874	[-0.06, 0.02]

*Estimated from frequentist methods; CrI: Credible interval, λ: Power transformation value estimated from Box-cox or Yeo-Johnson methods

Table 3 Relative levels of *CKAMP59* in idiopathic schizophrenia cases and controls according to the Bayesian quantile regression model

	CKAMP59	Posterior Beta of $(2^{(-ddct)})^{\lambda}$	SE	Adjusted *P*-Value*	95% CrI for Beta
Total	Group, Case vs. control	0.139	0.28	> 0.999	[-0.45, 0.67]
	Sex, Male vs. Female	0.145	0.3	0.942	[-0.48, 0.72]
	Age (years)	-0.007	0.02	0.577	[-0.05, 0.03]
	Group * Sex	-0.269	0.44	0.663	[-1.13, 0.57]
Male	Case vs. control	-0.171	0.3	> 0.999	[-0.78, 0.4]
	Age	0.003	0.03	0.989	[-0.04, 0.05]
Female	Case vs. control	0.198	0.24	0.604	[-0.31, 0.67]
	Age	-0.021	0.02	0.204	[-0.06, 0.02]

*Estimated from frequentist methods; CrI: Credible interval, λ: Power transformation value estimated from Box-cox or Yeo-Johnson methods

According the power analysis, the simulation results and 95% CrI`s indicated the consistency of results with n = 50 and n = 100 per group, while the estimated minimum power was about 68%.

Correlation analysis

The age of the participants was not correlated with the expressions of *hsa-miR-185-5p* and *CKAMP59*. In both patients of SCZ and healthy controls participants, the expressed levels of the examined genes were correlated significantly (r = 0.870, $P < 0.001$ and r = 0.812, $P < 0.001$, respectively) (Fig. 2).

ROC curve analysis

We assessed the diagnostic power of *hsa-miR-185-5p* in order to differentiate SCZ patients from healthy controls. By considering the area under curve (AUC), we attained the diagnostic power of 0.722 from the transcript levels of *hsa-miR-185-5p* (Fig. 3).

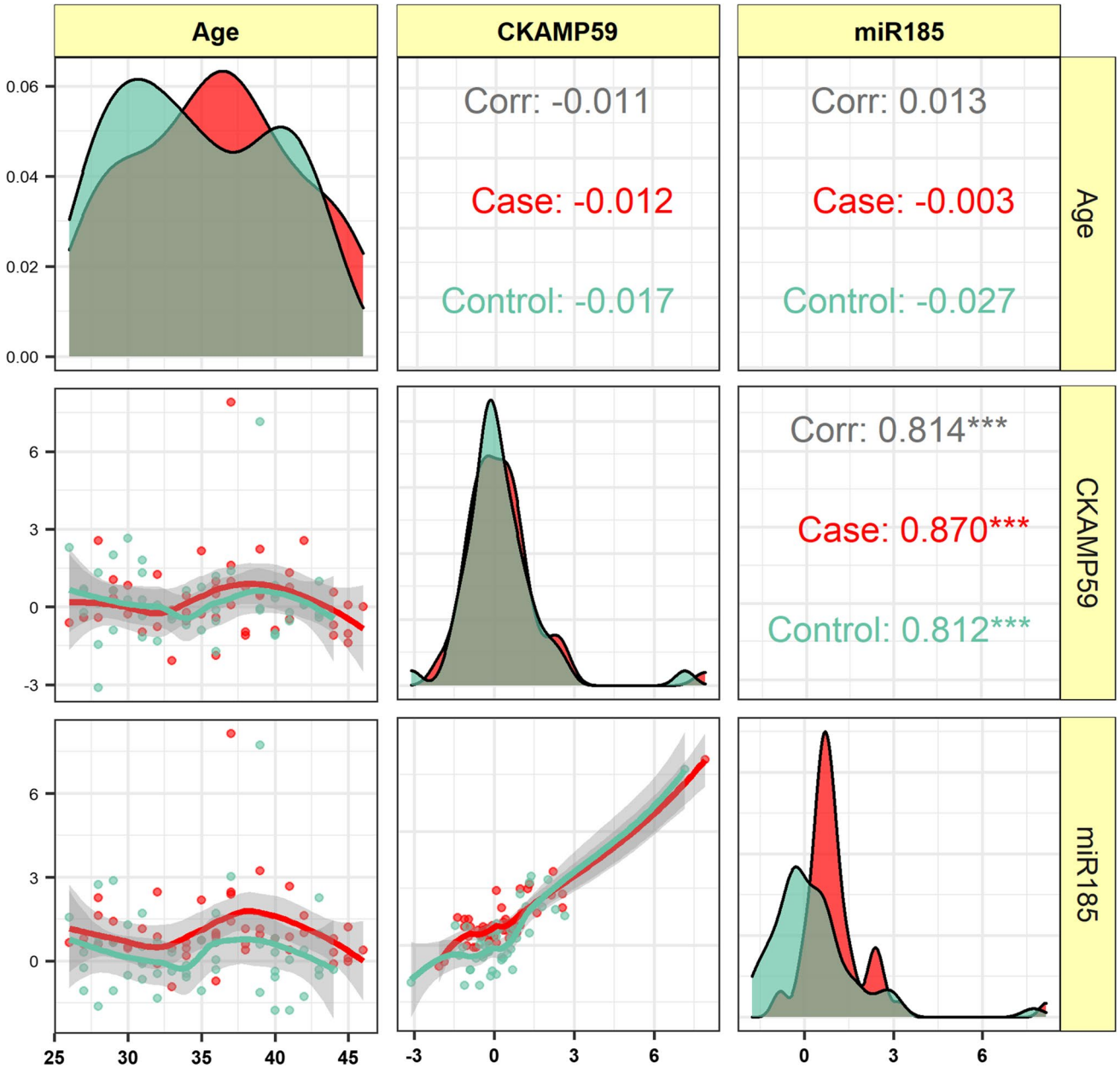

Fig. 2 The distribution of variables is depicted on the diagonal. The correlation coefficients plus the significance level as stars are shown. *** is significant correlation at *P*-value < 0.001

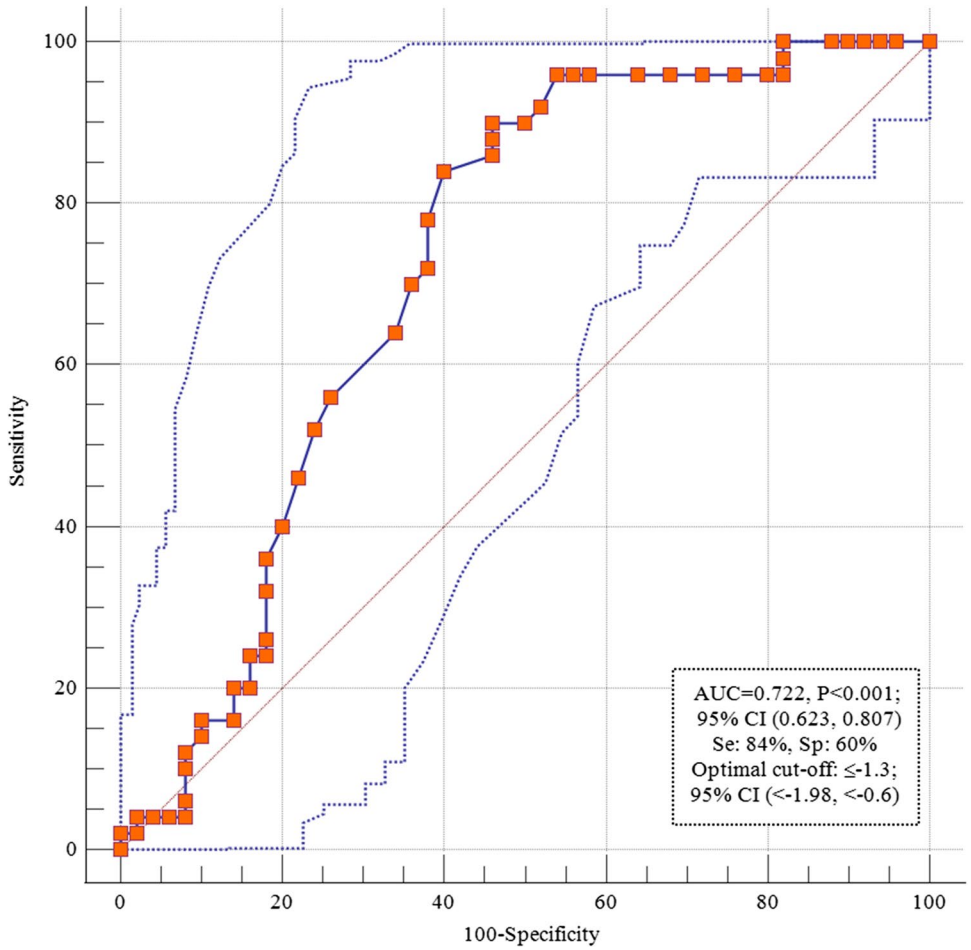

Fig. 3 Receiver operating characteristic (ROC) curve analysis. *Hsa-miR-185-5p* transcript levels displayed diagnostic power of 0.722

Discussion

As idiopathic SCZ is considered a multivariable disorder; hence, environmental and genetic factors influence the susceptibility of this disease. Investigations on the post-mortem brain tissues of humans have uncovered an altered expression of miRs in SCZ patients (Forstner et al. 2013). Particularly, a substantial overlapping of dysregulated miRs in post-mortem brain tissues of humans and the altered miRs in prefrontal cortex (PFC) of a 22q11.2DS mouse specimen have been found in previous studies (Stark et al. 2008; Moreau et al. 2011). As a result, the assumption that suggests the relevancy of the findings in 22q11.2DS with idiopathic SCZ is somewhat supported by this discovery. Furthermore, identifying the target genes that are affected by miRs dysregulation and the associated pathways will expand our knowledge of alteration in genetic networks regulated by miRs and their contribution to the pathophysiology of idiopathic SCZ and 22q11.2DS (Forstner et al. 2013). In the present study, we evaluated *hsa-miR-185-5p* and its target gene *CKAMP59* expression in the PB of idiopathic SCZ patients and healthy participants.

Decreased levels of *hsa-miR-185-5p* were detected in patients with SCZ compared to controls. The studies on mouse models suggested that *miR-185* in SCZ-related brain regions is deemed as the top-scoring down-regulated miR (Stark et al. 2008; Benetti et al. 2009; Forstner et al. 2014). To the best of our knowledge, the present study is the first investigation of the *miR-185* expression in PB of patients with idiopathic SCZ. A drastic decrease in levels of *miR-185* expression in hippocampus and PFC regions of D*f*(16)A+/− mice were corroborated in a previous study. It was suggested that this decrease contributed to deficits in dendritic complexity and spine development in hippocampal neurons. Additionally, an approximate 20% decrease of *miR-185* expression within the hippocampus occurs due to the *Dgcr8* deficit, which encodes an RNA-binding moiety of the 'microprocessor' complex and promotes behavioral and neurological impairments related to the 22q11.2 microdeletion. A previously unknown inhibitor, *Mirta22* (miRNA target of the 22q11.2 microdeletion) which its expression was higher in the prenatal brain and is situated in the Golgi apparatus, is shown to be repressed by *miR-185*. The lowered expression of *miR-185* in D*f*(16)A(+/−) mice brain regions leads to a sustained de-repression of *Mirta22*

upon birth. This matter will give rise to structural alterations in cognitive function as well as the hippocampus (Xu et al. 2013). An age-associated increase in long-term potentiation has been declared in the hippocampus of *Dgcr8*+/− mice. The loss of two miRs (*miR-25* and *miR-185*), which target the sarco (endo) plasmic reticulum Ca2+ATPase (*SERCA2*), are responsible for this elevation. Increased expression of *SERCA2* was identified in the post-mortem brain of SCZ patients (Earls et al. 2012). The reduced expression of *miR-185* was also shown in PB of individuals with 22q11DS (Sellier et al. 2014; de la Morena et al. 2013). Our result is in line with these findings. Additional evidence supporting *MIR185*'s involvement in SCZ comes from the observation that two of its validated targets, *Cdc42* and *RhoA* (Liu et al. 2011), have altered expression levels in individuals with SCZ (Hill et al. 2006; Ide and Lewis 2010).

We discovered no significant changes in *CKAMP59* expression levels between patients with SCZ and healthy controls in PB samples. Shisa family members are single-pass transmembrane proteins with an N- and C-terminal cysteine and proline-rich domain, respectively (Pei and Grishin 2012). Shisa6-9 are known as Cystine-knot AMPAR membrane proteins (CKAMP) (Farrow et al. 2015) on account of the C-terminal existence of an AMPAR interacting domain (von Engelhardt 2019). While other CKAMPs are localized to glutamatergic synapses (von Engelhardt et al. 2010; Klaassen et al. 2016; Peter et al. 2020), Shisa7 (CKAMP59) colocalizes exclusively with gephyrin and $GABA_A$Rs in hippocampal neurons (Han et al. 2019), not at excitatory synapses as previously reported (Schmitz et al. 2017). Shisa7 regulated the inhibitory transmission and trafficking of $GABA_A$Rs without impairing excitatory synaptic transmission (Han et al. 2019, 2021; Wu et al. 2021). Surprisingly, Shisa7 also affects the kinetics and pharmacological characteristics of $GABA_A$Rs. Indeed, CKAMP59 reduced the deactivation time constants of α1β2γ2 and α2β3γ2 receptors in heterologous cells, while Shisa7 KO increased the decay time constant of GABAergic transmission in hippocampal neurons. Finally, Shisa7 enhanced diazepam-induced potentiation of $GABA_A$Rs in heterologous cells, while Shisa7 KO substantially decreased diazepam effects in vivo (Han et al. 2019).

In contrast to the notion that miRs are repressive, our correlation data between miR and mRNA revealed a substantial positive correlation rather than a negative correlation. Though unconventional, the coexistence of negative and positive miR-mRNA correlations has been observed in several studies, implying the existence of a complex network involving inhibition of miR targets (leading to negative miR-mRNA correlations), as well as feed-forward regulation triggered by common transcription factors (leading to positive miR-mRNA correlations) (Chen et al. 2011; Chien et al. 2014; Friard et al. 2010; Diaz et al. 2015). As previously stated, *hsa-miR-185-5p* inhibits the *SHISA7* expression in human and murine neuroblastoma cells (Marques et al. 2012). This finding is inconsistent with our result. On the other hand, in line with our result, another study showed that *hsa-miR-3681-5p* behaves as a super-enhancer by recruiting alternative enhancer and promoter, mediators, transcription factors, activators, and RNA Pol II and that its enhancing function behaves as an inhibitor of variable number tandem repeats (VNTRs) activity in the 3′ UTR of *SHISA7* (Lee et al. 2020). Additional research is required to determine if *hsa-miR-185-5p* acts as an enhancer.

Additionally, we assessed the diagnostic power for *hsa-miR-185-5p* to differentiate idiopathic SCZ patients from healthy participants and determined that it had a diagnostic power of 0.722. Due to the study's limited sample size, these results should be used with care. If future research confirms the current study's results, the amount of *hsa-miR-185-5p* transcription may be used as a marker for idiopathic SCZ disease.

Conclusion

In conclusion, our study is the first to demonstrate the expression of *hsa-miR-185-5p* and *CKAMP59* in the PB of idiopathic SCZ patients. Our findings suggest that alterations in gene expression mediated by *miR-185* may be a mechanistic connection between idiopathic SCZ and 22q11.2DS. It is worth noting that, despite a significant positive correlation between *hsa-miR-185-5p* and *CKAMP59*, suggesting an interaction network, their role in SCZ development should be reconsidered using whole blood samples since the changed expression of *CKAMP59* was not strong enough to be significant. Additional research with bigger sample sizes and particular leukocyte subsets may strengthen these results. Moreover, evaluation of protein levels of *CKAMP59* in patients and controls would give better insight into the *miR-185* mediated function in SCZ.

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Authors' contributions HS, MR, MT, SB, and SD wrote the manuscript and revised it. MRA, HS and JG supervised the study and performed the experiment. SAJ analyzed the data. SF and NKA was the clinical consultant and assessed patients for inclusion in the study. All authors read and approved the final version of manuscript.

Data availability The analyzed data sets generated during the study are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participant All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. Informed consent forms were obtained from all study participants. The study protocol was approved by the ethical committee of Tabriz University of Medical Sciences. All methods were performed in accordance with the relevant guidelines and regulations.

Consent of publication Not applicable.

Competing interest The authors declare they have no conflict of interest.

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