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LETTER TO THE EDITOR

Expression of miR-132 in Down syndrome subjects

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Dear Sir,

Down syndrome (DS) is caused by the presence of three human chromosome 21 copies, this trisomy is the most frequent genetic etiology associated with a number of deleterious phenotypes in humans, where is included intellectual disability [1].

Gene expression plays a central role in neuronal plasticity and in dysfunction of the molecular events that can lead to severe neuronal disorders.

In addition, to coding transcripts (mRNAs), non-coding microRNAs (miRNAs) appear to play a role in these processes [2]. MiRNAs are short non-coding RNAs (~ 22 nucleotide) that mediate post-transcriptional gene silencing [3]. MiRNAs play a role in early neuronal development and in neurodegenerative diseases [4].

Previous studies on DS mainly focused on human chromosome 21-derived miRNAs, and few studies focused in total miRNAs expression profile from human blood samples [5].

Many miRNAs were detected to have significantly different expression, which may be involved in DS variable phenotypes [6].

MiR-132 (MIM 610016) maps to chromosome 17p13.3 and its role in central nervous system is not fully understood. MiR-132 was proposed to have a function in neurite outgrowth [6], in synaptic transmission, in dendritic growth and arborization, in learning, in memory and in apoptosis [7].

Hansen et al. showed a dysregulation/overexpression of miR-132 in cognitive deficiency mouse brain [8].

In this study, we describe a molecular evaluation of miR-132 leukocytes expression in DS patients compared to control subjects. A total of 46 subjects, including 23 DS patients (12 males and 11 females; age range 25–57 years) and 23 normal subjects (NS) (12 males and 11 females; age range 22–55 years), were recruited at the IRCCS Associazione Oasi of Troina (Italy). This study was approved by the Ethical Committee of the Research Institute "IRCCS Associazione Oasi Maria SS.", Troina (EN), Italy (2017/05/31/CE-IRCCS-OASI/9 of 3 June 2017). An informed consent was obtained from all study participants.

MiRNA quantification was performed using case–control modality in DS subjects and normal subjects coupled for sex and age ± 3 years.

RNA extraction from leukocytes of peripheral blood was performed using RNeasy Mini Handbook (QIAGEN Sciences, Germantown, PA), following the manufacturer's protocol.

RNA purity and quantity were confirmed by spectrophotometry and agarose gel electrophoresis. To avoid any genomic DNA contamination, a brief incubation of the samples at 42 °C with a specific Wipeout buffer (QuantiTect Reverse Transcription Kit, QIAGEN Sciences, Germantown, PA) was carried out.

Retro-transcription of about 50 ng of total RNA from each sample was then performed in a final volume of 100 µl of cDNA.

CDNA was used as a template for real-time quantitative PCR analysis with gene expression products. For each sample, qRT-PCRs were carried out in duplicate using 4 µl of cDNA and QuantiTect Probe PCR Master Mix Kit (QIAGEN Sciences, Germantown, PA) in a total volume of 25 µl. For qRT-PCR experiments, we used the Light Cycler 480 (Roche Diagnostics; Mannheim, Germany). The reference gene glyceraldehyde-3-phosphate dehydrogenase (GAPDH)

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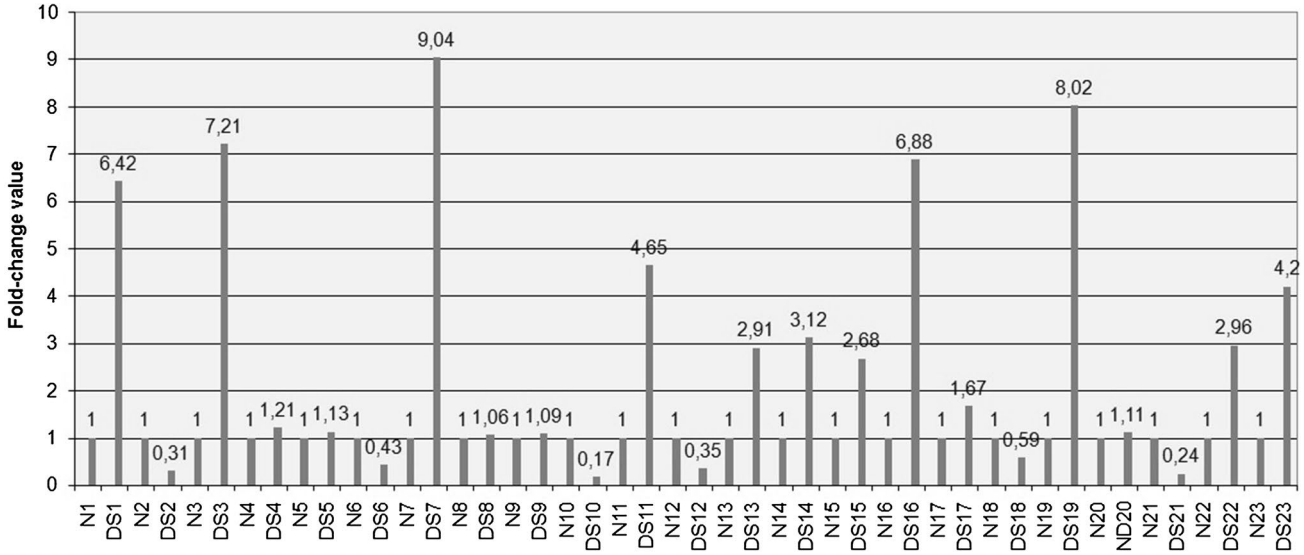

Fig. 1 miR-132 expression in DS subjects and normal controls. Data shown were obtained via qRT-PCR

and the target miR-132 assays were obtained from Applied Biosystems (Carlsbad, CA, USA).

The thermal cycling conditions consisted of one cycle for 2 min at 50 °C, one cycle of 15 min at 95 °C and 45 cycles for 15 s at 94 °C followed by 1 min at 60 °C. The qRT-PCR was quantified using comparative $\Delta\Delta C_t$ method and Light Cycler 1.5 software. Analysis of expression values was performed using Shapiro–Wilk test, statistical analysis of results was carried out using Wilcoxon rank-sum test and Graph Pad Prism 5 software. A p value < 0.05 was considered significant.

We have observed an increased expression of miR-132 in 17 DS samples, 11 of them have an expression value greater than 2.5-fold compared to relative control. Distribution of expression values was found to be not normal ($p < 0.01$). Statistical analysis reveals a significant difference between two groups, NS and DS ($p = 0.001$; Fig. 1). No significant difference was found by analyzing subgroups of sex and age groups ($p > 0.05$). DS subjects with an expression less than 1 are aged between 22 and 35.

Data obtained showed miR-132 expression levels to be significantly high in DS subjects compared to normal subjects.

These data agree with Hansen et al., which indicates a greater expression of miR-132 in mouse brain with cognitive deficiency coupled to significant decreases of methyl CpG-binding protein 2 (MeCP2) [8]. It is also interesting that miR132 transgenic mice exhibited a decrease in the expression of MeCP2, a protein implicated in Rett syndrome and other disorders of mental retardation [9, 10].

Furthermore, gene expression omnibus (GEO) profile studies have shown that overexpression of miR-132 is associated with Hodgkin lymphoma, type 2 diabetic and asthma (https://www.ncbi.nlm.nih.gov/geoprofiles/114679998; https://www.ncbi.nlm.nih.gov/geoprofiles/83478098; https://www.ncbi.nlm.nih.gov/geoprofiles/106709598); these pathologies belong to the phenotype of subjects with Down syndrome.

In conclusion, this preliminary study supports the possibility that miR-132 may be involved in various molecular aspects associated with the phenotype of subjects with Down syndrome.

Moreover, miR-132's role as a regulator of many genes' expression encourages us to expand our study on other tissue, to better understand molecular mechanisms in DS phenotype.

Compliance with ethical standards

Conflict of interest The authors report no declarations of interest.

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