Introduction: Autism spectrum disorders (ASD) are the most prevalent neurobiological disorders in children. The etiology comprises genetic, epigenetic, and environmental factors such as dysfunction of the immune system. Epigenetic mechanisms are mainly represented by DNA methylation, histone modifications, and microRNAs (miRNA). The major explored epigenetic mechanism is mediated by miRNAs which target genes known to be involved in ASD pathogenesis. Salivary poly-omic RNA measurements have been associated with ASD and are helpful to differentiate ASD endophenotypes. This study aims to comprehensively examine miRNA expression in children with ASD and to reveal potential biomarkers and possible disease mechanisms so that they can be used to improve faction between individuals by promoting more personalized therapeutic approaches.

Materials and methods: Saliva samples were collected from 10 subjects: 5 samples of children with ASD and 5 from healthy controls. miRNAs were analyzed using an Illumina Next-Generation-Sequencing (NGS) system.

Results: Preliminary data highlighted the presence of 365 differentially expressed miRNAs. Pathway analysis, molecular function, biological processes, and target genes of 41 dysregulated miRNAs were assessed.
of which 20 were upregulated, and 21 were downregulated in children with ASD compared to healthy controls.

**Conclusion:** The results of this study represent preliminary but promising data, as the identified miRNA pathways could represent useful biomarkers for the early non-invasive diagnosis of ASD.

**KEYWORDS**

autism spectrum disorder (ASD), miRNA, salivary poly-omic RNA, biomarkers, miRNAs targeted genes

---

**Introduction**

Autism spectrum disorder (ASD) is a neurological and developmental disorder characterized by altered social interaction, restricted/repetitive behavior, and medical and mental health conditions that result in lifelong functional and social impairments. It is estimated that ASD is associated with intellectual disability in 70% of cases, with seizures in 30% of cases (Minshew and Williams, 2007). Furthermore, ASD leads to alterations in the circadian cycle: it has been reported that people affected by ASD present a reduced total sleep, with an increased sleep latency. Studies have shown a higher frequency or absence of circadian variation of melatonin and cortisol in ASD patients (Tordjman et al., 2015).

Autism spectrum disorder presents a multifactorial etiology, comprising both genetic and environmental factors (Hallmayer et al., 2011; Singh et al., 2013; Sehovic et al., 2020; Wu et al., 2020). A metaanalysis published in 2016, including a cohort of 6,413 twin pairs, estimated that the heritability of ASD to be 74–93% (Tick et al., 2016). Similar values were recently reported by Wu et al. (2020), while moderate heritability values (37–38%) were obtained by Hallmayer et al. (2011) in a Californian study (2011). Both genetic and epigenetic factors may be involved, such as histone modification, DNA methylation, and non-coding sequences of RNA (miRNA) (Zhang et al., 2020). Parental age, in utero exposure to several substances including pollution and inflammation, and low birth weight, may also increase the risk (Hallmayer et al., 2011; Sehovic et al., 2020).

The diagnosis of ASD, according to DSM-5, is mainly based on the examination of behavioral characteristics. A person must present deficits in three areas of social interaction: social-emotional reciprocity, non-verbal communicative behaviors, and the ability to maintain and understand relationships (Hallmayer et al., 2011; Wu et al., 2020; Qin et al., 2022). Unfortunately, all these characteristics can be interpreted differently, especially in young patients, leading to delays in diagnosis. Objective hallmarks are therefore needed (Hicks et al., 2020).

The number of children with ASD has grown dramatically over the last 2 decades with a current estimated prevalence of approximately 1 in 60 worldwide, an increase of 154% and even higher figures in future (Sehovic et al., 2020; Salloum-Asfar et al., 2021; Qin et al., 2022). Although the etiology of ASD is still mainly unknown, major genetic aberrations have been recognized in only 25–30% of patients with ASD and are mainly related to pathological mechanisms that start during gestation (Wisniowiecka-Kovalnik and Nowakowska, 2019). Even if ASD could be diagnosed as early as 18 months of age, on average the diagnosis is done at ~60 months of age (van’t Hof et al., 2021). Furthermore, as early diagnosis of ASD is often unreliable, effective therapy is usually delayed. This might have negative consequences, considering that the effectiveness of therapy often depends on earliness of treatment beginning (Hicks et al., 2020; Salloum-Asfar et al., 2021). Therefore, recent research on early diagnosis of ASD has focused on large screening programs that can be efficient and easy to implement on a population scale (Hicks et al., 2018a).

Macroscopically, among other brain abnormalities observed in patients with ASD, alterations have been described in Broca and Wernicke’s areas, which are associated with language, and in the frontal lobe, superior temporal cortex, parietal cortex, and amygdala, which are strongly implicated in social behaviors (Ha et al., 2015). However, their use in the clinical practice and to monitor treatment is difficult and expensive as magnetic resonance is needed (Squarcina et al., 2021).

Numerous biomarkers have been studied, measured and analyzed in order to identify potential ADS indicators for both early diagnosis and pathogenesis (Ballini et al., 2020; Hicks et al., 2020; Isacco et al., 2021; Salloum-Asfar et al., 2021). ASD presents typical features of synaptic dysfunction and synaptopathy (Wu et al., 2020). Indeed, aberrant overexpression of the postsynaptic density 95 protein (PSD95), a member of the membrane-associated guanylate kinase that promotes synaptic stability in excitatory synapses, has been observed in patients with ASD. This alteration leads to increased dendritic spines and pathologic synaptic hyperconnectivity (Coley and Gao, 2018). Another important feature of synapses that have been found...
altered in ASD is SHANK (SH and multiple ankyrin repeat domains protein). SHANK proteins are scaffolding proteins that organize intermediate scaffold proteins. Their location is principally in the excitatory synapse, where they allow the synapse to develop and function properly. Alterations in the SHANK gene have been reported as a possible cause and predictive feature of ASD (Wu et al., 2020).

Among others, miRNA have been proposed as plausible new biomarkers with high predictivity. More than 91 miRNAs associated with ASD have been identified. For instance, alterations of miRNA related to cellular respiration have been found in the serum of patients with ASD. Furthermore, miRNA-500a-5p and miRNA-197-5p expressions in the serum have been suggested as useful tools to diagnose ASD in children (Kichukova et al., 2021). Through analysis of miRNAs, genetic correlations were observed between clinically unaffected parents of children with ASD and their siblings (Ozkul et al., 2020). A great number of miRNA are altered in ASD, and several are related to alteration in central nervous system (Salloum-Asfar et al., 2021; Qin et al., 2022). Preclinical and clinical models of ASD have demonstrated up- and down-regulations of several miRNAs. For instance, upregulation of miRNA-29b, miRNA329, miRNA-199b, miRNA-382, miRNA-296, miRNA-221 and miRNA-92 and downregulation of miRNA-146a, miRNA-146b, miRNA-130, miRNA-122a, miRNA-342 and miRNA-409 were described. Among these targets, for example, miRNA-199 is known to be able to regulate BDNF (Brain Derived Neurotrophic Factor), while miRNA-146a and miRNA-146b have been shown to be involved in the aetiopathogenesis of ASD (Urduinguio et al., 2010). Ivanov et al. (2022), developed a highly sensitive "silicon-on-insulator"-based nanosensor (SOI-NS) for the detection of ASD-associated miRNAs with a lower limit of 10^{-17}M.

Nowadays, among all body sampling tissues, saliva is gaining a pivotal role as it is extremely easy and non-invasive to collect and monitor. It should be noted that when choosing a body fluid for biomarker measurement, it is important to avoid stress as much as possible, as stressful procedures can alter the production of hormones such as cortisol that influence the secretion of other hormones through feedback-sideways interaction. Urine or blood collection is more stressful than saliva collection and this makes saliva an appropriate fluid for biological sampling in either patients or healthy subjects. Furthermore, its informative value is considered particularly high, as the salivary proteome is estimated to include more than 3,000 expressed proteins and peptides, comprising biomarkers of neurological diseases (Hicks et al., 2018b, 2020). After the first reports by Hicks and colleagues, several large-scale screening studies have focused on salivary poly-omic RNA measurements to identify children with ASD (Hicks et al., 2018a; Sehovic et al., 2020). The rise of the ASD epidemic and numerous hypothetical pathways to be elucidated both diagnostically and pathogenetically have given impetus to related research, resulting in an enrichment of recent evidence.

The complexity of ASD makes clinical diagnosis difficult, therefore, by identifying the biomarkers associated with ASD severity and combining them with the diagnosis, it is possible to better divide the factions within the spectrum and devise more targeted therapeutic strategies. To date, there are no reliable biomarkers to diagnose ASD or define its severity. This study aims to comprehensively examine miRNA expression in children with ASD and to reveal potential biomarkers and possible disease mechanisms so that they can be used to improve faction between individuals by promoting more personalized therapeutic approaches.

### Materials and methods

#### Samples collection

This prospective comparative and study included 10 children aged between 3 and 7 years. The ASD group comprised 4 males and 1 female (mean age 5.6 years) and the non-ASD group, healthy controls, comprised 3 males and 2 females (mean age 5.4 years) (Table 1). Date on birth weight and parental age were retrieved for all participants and reported on Table 2. For approval, written informed consent was obtained from the parents/caregivers of each participant for approval. Exclusion criteria for the ASD participants included global developmental delay (cognitive disability with IQ < 70), g-tube dependence, confirmed chromosomal deletion or duplication, epilepsy, psychiatric diagnosis and premature birth of more than 6 weeks. Exclusion criteria for the non-ASD groups included a family history of ASD in a first degree relative or a chronic medical condition requiring daily medical care by a specialist. Common exclusion criteria among groups included acute illness, sensory disorders such as hearing or visual impairments, upper respiratory tract and oral cavity infections and wards of the state. Saliva samples were collected from all children in a non-fasting state using Oracollect RNA swabs (DNA Genotek; Ottawa, ON, Canada) through a simple, non-invasive process that consists of applying a highly absorbent swab for a few seconds under the base of the tongue. Each sample collected at least 500 µl of saliva. Samples were immediately sent to the laboratory for analysis. The clinical investigation protocol for this study was submitted to the ethical committee of the Policlinico Fondazione Cà Granda Milano Review Board, and approval has been successfully obtained (727_2021).

#### miRNAs extraction

miRNAs were extracted from saliva samples using NucleoSpin® miRNA Plasma kit (MACHEREY-NAGEL,
TABLE 1 | Demographical and clinical parameters of ASD subjects.

| Patient ID | Sex | Age  | Comorbidities | ADOS-II* test score | WPPSI-IV** test score |
|------------|-----|------|---------------|---------------------|-----------------------|
| 1          | F   | 5.4  | None          | 7                   | 15                    |
| 2          | M   | 5.7  | None          | 8                   | 17                    |
| 3          | M   | 6.6  | None          | 8                   | 16                    |
| 4          | M   | 4.7  | None          | 9                   | 17                    |
| 5          | M   | 5.8  | None          | 9                   | 18                    |

*ADOS-II test for ASD diagnosis (ranging from 1 to 10). **WPPSI-IV test for cognitive evaluation (ranging from 1 to 19).

TABLE 2 | Birth weight and parental age.

| Participants | Birth weight (gr) | Parental age (years) |
|--------------|-------------------|----------------------|
|              |                   | Mother | Father |
| Healthy 1    | 2,650             | 25     | 28     |
| Healthy 2    | 2,975             | 32     | 39     |
| Healthy 3    | 3,640             | 28     | 27     |
| Healthy 4    | 3,365             | 29     | 35     |
| Healthy 5    | 2,897             | 27     | 32     |
| ADS 1        | 2,495             | 31     | 33     |
| ADS 2        | 2,990             | 28     | 35     |
| ADS 3        | 3,760             | 24     | 26     |
| ADS 4        | 3,805             | 26     | 25     |
| ADS 5        | 2,805             | 25     | 27     |

Library preparation and sequencing

QIAseq miRNA library kit (Qiagen, Hilden, Germany) has been used for library preparation following the manufacturer’s instructions.

RNA samples were quantified and quality tested by Agilent 2100 Bioanalyzer RNA assay. In addition, final libraries were checked with both Qubit 2.0 Fluorometer (Invitrogen, Carlsbad, CA, USA) and Agilent Bioanalyzer DNA assay. The libraries were sequenced on single-end 150 bp mode on NovaSeq 6000 (Illumina, San Diego, CA, USA).

Bioinformatic analysis

The bioinformatics analysis of small RNA-Seq includes:

- Base calling and demultiplexing. Processing of raw data for format conversion and demultiplexing by Bcl2Fastq 2.20 version of the Illumina pipeline.
- The sRNAbench tool, a sRNAtoolbox2,3 tool, gives expression profiling of small RNAs, prediction of novel microRNAs, analysis of isomiRas, genome mapping, and read length statistics.

Pathways analysis and gene ontology

To better understand the functional role of the 41 selected miRNAs, a pathway prediction analysis was performed. For this purpose, the bioinformatics tool DIANA-miPath (version 3) (Vlachos et al., 2015) and miRDB (Chen and Wang, 2020) were used. With this computational approach all target genes and related molecular pathways altered by the selected miRNAs were identified by using this computational approach. Furthermore, GO enrich-ment analysis was performed using the tool GO PANTHER version 14.0.1 GO PANTHER analysis was conducted for the lists of genes obtained from DIANA-miPath analyses and using the NPinter tool to evaluate the interactors of the top 10 dysregulated miRNAs.

Statistical analyses

Fold change values of miRNA expression levels were calculated through differential analysis. Student’s t-tests were performed to select the differentially expressed miRNAs with a statistical significance, as reported in Tables 3, 4. For the Kaplan–Meier survival analysis, the non-parametric log-rank test was used to compare the survival distributions of the patients with ASD according to the down-regulation or overexpression of selected miRNAs. Data with a \( P \)-adj \( \leq 0.05 \)

1. http://pantherdb.org/
were considered to indicative of statistically significant differences.

**Results**

**Identification of dysregulated miRNAs**

Saliva samples were collected from 10 subjects: 5 samples from children with ASD and 5 from healthy controls, respectively. Preliminary data highlighted the presence of 365 differentially expressed miRNAs. Applying a more stringent statistical analysis (padj < 0.05) to create hierarchical clustering, we found 41 dysregulated miRNAs were found, of which 20 were upregulated and 21 miRNAs were downregulated in saliva from children with ASD compared to from healthy controls (Table 3). Figure 1 shows the top five upregulated miRNAs in ASD patients compared to healthy control. Venn diagram, Volcano plot of the identified miRNAs, and heat map diagram showing cluster analysis of differently expressed miRNAs: hsa-miRNA-1246 (5-fold, p < 0.00000035), hsa-miRNA-199b-5p (3.59-fold, p < 0.000014), hsa-miRNA-4516 (3.5-fold, p < 0.000004), hsa-miRNA-199a-3p (3.4-fold, p < 0.000006), hsa-miRNA-199b-3p (3.36-fold, p < 0.000008), and the top five downregulated: hsa-miRNA-545-5p (3-fold, p < 0.001), hsa-miRNA-660-3p (2.5-fold, p < 0.000006), hsa-miRNA-4284 (2.28-fold, p < 0.00006), hsa-miRNA-199b-3p. IRDF1 is a predicted gene in ASD (hsa-miRNA-199b-3p). AUTS2 (hsa-miRNA-199b-5p; hsa-miRNA-199a-3p; hsa-miRNA-199b-3p); AUTS2 (hsa-miRNA-199b-5p; hsa-miRNA-199a-3p; hsa-miRNA-199b-3p). IRDF1 is a predicted gene in ASD (hsa-miRNA-199b-5p; hsa-miRNA-199a-3p; hsa-miRNA-199b-3p). Table 6. Using the same criteria, Table 7 lists only the top 10 dysregulated miRNAs: target genes are ZNF106, GFPT2, JUNB, VPS33A. The subsequent analysis evaluated the interactors of the miRNAs. Twenty-three interactors were revealed by the analysis of the top 10 dysregulated miRNAs (Table 8) (NPinter). Involved in ASD were: AASDHPPT (hsa-miRNA-1246; hsa-miRNA-199b-5p; hsa-miRNA-199a-3p; hsa-miRNA-199b-3p); AUTS2 (hsa-miRNA-199b-5p; hsa-miRNA-199a-3p; hsa-miRNA-199b-3p). IRDF1 is a predicted gene in ASD (hsa-miRNA-199b-5p; hsa-miRNA-199a-3p; hsa-miRNA-199b-3p) (Table 8). The last step of the analysis consisted of the GO enrichment analysis by PANTHER, listing the 15 genes. As shown in Figure 2, the selected genes were grouped according to molecular function, biological process, and protein class (Figure 2). As regards the molecular function, it was observed that the majority of genes were involved in catalytic activity (GO:0003824) functions (46% of genes) (Figure 2A). When considering the biological processes, 68.8–43.8% of the genes were involved in cellular processes (GO:00036) and metabolic processes (GO:0008152) (Figure 2B). The most represented protein classes were protein modifying enzyme (PC00260), transporter (PC00227), and metabolite interconversion enzyme (Figure 2C). Finally, pathway analysis revealed the association to molecular mechanisms for only a few genes. Indeed, only 4 genes out of the 15 recognized were assigned to a molecular pathway (Figure 2D). The most represented pathways were the Inflammation mediated by chemokine and cytokine signaling pathway (P00031), Interleukin signaling pathway (P00036), PDGF signaling pathway (P00047), Alzheimer’s disease-presenilin pathway (P00004), and EGF receptor signaling pathway (P00018).

**Discussion**

Autism spectrum disorders is a highly heritable neurodevelopmental disease with a sharply increasing incidence (Santini et al., 2014). The diagnosis of ASD is
mainly based on psychometric evaluations and is often difficult and subjective. Therefore, molecular detection for an early and objective ASD diagnosis is indispensable. This study is focused on the non-invasive evaluation of miRNA/gene biomarkers collected from salivary samples of children. As aforementioned, miRNAs are small non-coding regulatory RNAs that play an essential role in fundamental neurobiological processes and neurodevelopmental diseases. From preliminary data, dysregulated miRNAs in ASD are presented in several pathways. As expected in the canonical
| KEGG pathway                                         | p-value      | No. of genes | #miRNAs |
|-----------------------------------------------------|--------------|--------------|---------|
| Hippo signaling pathway (hsa04390)                  | 5.48E + 03   | 79           | 21      |
| Signaling pathways regulating pluripotency of stem cells (hsa04550) | 2.86E + 06   | 68           | 21      |
| Thyroid hormone signaling pathway (hsa04919)        | 3.96E + 06   | 58           | 21      |
| Ubiquitin mediated proteolysis (hsa04120)           | 0.01791      | 61           | 21      |
| Proteoglycans in cancer (hsa05205)                  | 5.28E + 04   | 94           | 20      |
| Axon guidance (hsa04360)                            | 3.76E + 06   | 58           | 20      |
| Wnt signaling pathway (hsa04310)                    | 9.89E + 06   | 68           | 20      |
| Glioma (hsa05214)                                   | 0.0008       | 32           | 20      |
| Pancreatic cancer (hsa05212)                        | 0.0008       | 36           | 20      |
| FoxO signaling pathway (hsa04068)                   | 0.0018       | 61           | 20      |
| Ras signaling pathway (hsa04014)                    | 0.0019       | 95           | 20      |
| PI3K-Akt signaling pathway (hsa04151)               | 0.0019       | 142          | 20      |
| Pathways in cancer (hsa05200)                       | 0.0020       | 166          | 20      |
| Regulation of actin cytoskeleton (hsa04810)         | 0.0042       | 97           | 20      |
| Focal adhesion (hsa04510)                           | 0.0045       | 91           | 20      |
| Neurotrophin signaling pathway (hsa04722)           | 0.0053       | 57           | 20      |
| Chronic myeloid leukemia (hsa05220)                 | 0.0111       | 36           | 20      |
| Oxytocin signaling pathway (hsa04921)               | 0.0151       | 66           | 20      |
| Glutamatergic synapse (hsa04724)                    | 0.0225       | 50           | 20      |
| Insulin signaling pathway (hsa04910)                | 0.0461       | 60           | 20      |
| ErbB signaling pathway (hsa04012)                   | 0.0007       | 46           | 19      |
| TGF-beta signaling pathway (hsa04350)               | 0.0019       | 33           | 19      |
| Adherens junction (hsa04520)                        | 0.0019       | 39           | 19      |
| Prostate cancer (hsa05215)                          | 0.0044       | 45           | 19      |
| Adrenergic signaling in cardiomyocytes (hsa04261)    | 0.0142       | 61           | 19      |
| Arrhythmogenic right ventricular cardiomyopathy (ARVC) (hsa05412) | 0.0161       | 35           | 19      |
| Choline metabolism in cancer (hsa05231)             | 0.0189       | 48           | 19      |
| Oocyte meiosis (hsa04114)                           | 0.0191       | 48           | 19      |
| Dilated cardiomyopathy (hsa05414)                   | 0.0195       | 41           | 19      |
| GABAergic synapse (hsa04727)                        | 0.0200       | 37           | 19      |
| Melanoma (hsa05218)                                 | 0.0223       | 34           | 19      |
| Cholinergic synapse (hsa04725)                      | 0.0461       | 52           | 19      |
| Insulin secretion (hsa04911)                        | 0.0470       | 38           | 19      |
| T cell receptor signaling pathway (hsa04660)        | 0.0480       | 46           | 19      |
| N-Glycan biosynthesis (hsa00510)                    | 0.0001       | 23           | 18      |
| Renal cell carcinoma (hsa05211)                     | 0.0022       | 37           | 18      |
| Non-small cell lung cancer (hsa05223)               | 0.0042       | 29           | 18      |
| Colorectal cancer (hsa05210)                        | 0.0046       | 32           | 18      |
| Hedgehog signaling pathway (hsa04340)               | 0.0069       | 28           | 18      |
| Sphingolipid signaling pathway (hsa04071)           | 0.0111       | 53           | 18      |
| AMPK signaling pathway (hsa04152)                   | 0.0224       | 55           | 18      |
| Phosphatidylinositol signaling system (hsa04070)     | 0.0471       | 36           | 18      |
| Endometrial cancer (hsa05213)                       | 0.0042       | 29           | 17      |
| Central carbon metabolism in cancer (hsa05230)      | 0.0179       | 32           | 17      |
| Type II diabetes mellitus (hsa04930)                | 0.0255       | 24           | 17      |
| Long-term depression (hsa04730)                     | 0.0260       | 30           | 17      |
| Lysine degradation (hsa00310)                       | 0.0138       | 21           | 16      |

(Continued)
TABLE 4 (Continued)

| KEGG pathway                        | p-value | No. of genes | #miRNAs |
|--------------------------------------|---------|--------------|---------|
| Circadian rhythm (hsa04710)          | 0.0282  | 17           | 16      |
| Dorso-ventral axis formation (hsa04320) | 0.0242  | 16           | 15      |
| Fatty acid metabolism (hsa01212)     | 0.0009  | 20           | 14      |
| Prion diseases (hsa05020)            | 5.28E + 04 | 11      | 9       |
| Fatty acid biosynthesis (hsa00061)   | 1.28E-01 | 6           | 6       |

**FIGURE 1**

(A) The top miRNAs dysregulated in ASD patients compared to healthy control. (B) Venn Diagram. (C) Volcano plot of the identified miRNAs. (D) Heat map diagram showing cluster analysis of differently expressed miRNAs. The miRNAs selected are differentially expressed between ASD and HC (Healthy Control) groups (p < 0.05). The color scale reflects the signal strength and goes from green (low intensity) to black (medium intensity) to red (high intensity).

Wnt pathway, axon guidance and GABAergic synapse pathways adversely affect neurodevelopment and lead to the pathogenesis of ASD (Giudice et al., 2017; Somekh, 2021). The data obtained show that many miRNAs are involved in the TGF-beta signaling pathway (Boccellino et al., 2017; Dana et al., 2020), involved in ASD development, justifying the presence of dysregulated miRNAs in these other pathways. The present findings show that the adherents junctions, FoxO, Hippo and the Hedgehog signaling pathway are altered.

Interestingly, miRNA-199b-5p increased significantly in saliva of children with ASD, which is involved in the regulation of the AUTS2 gene («miRDB» database). The miRNA-199a-3p, which was observed to be strongly increased in the present work, is considered to be involved in the regulation, among all the other targets, of the CB1 receptor (Cannabinoid receptor type 1) («miRDB» database). Indeed, CB1 receptor, responsible for the social reward, has been observed reduced in the brain of people with ASD postmortem (Shohami et al., 2011). The miRNA-4516 was also found upregulated in the serum of ASD patients (Huang et al., 2015), which is considered to be involved in the sodium voltage-gated channel («miRDB» database). Altered gene expression of this channel is considered to be a solid predictor of ASD (Yamakawa, 2016). As previously
| KEGG pathway                                | p-value     | No. of genes | No. of miRNAs |
|--------------------------------------------|-------------|--------------|---------------|
| Pathways in cancer (hsa05200)              | 6.15E + 05  | 175          | 18            |
| Endocytosis (hsa04144)                     | 0.00134     | 90           | 17            |
| Hippo signaling pathway (hsa04390)         | 0.00025     | 64           | 16            |
| Ras signaling pathway (hsa04014)           | 0.00064     | 94           | 16            |
| Transcriptional mis regulation in cancer (hsa05202) | 0.00148  | 79           | 16            |
| ErbB signaling pathway (hsa04012)          | 0.00745     | 41           | 16            |
| Axon guidance (hsa04360)                   | 0.00745     | 59           | 16            |
| Wnt signaling pathway (hsa04340)           | 0.04625     | 56           | 16            |
| Proteoglycans in cancer (hsa05205)         | 1.80E + 04  | 92           | 15            |
| PI3K-Akt signaling pathway (hsa04151)      | 1.47E + 06  | 148          | 15            |
| Morphine addiction (hsa05032)              | 3.38E + 06  | 41           | 15            |
| Renal cell carcinoma (hsa05211)            | 6.32E + 06  | 36           | 15            |
| FoxO signaling pathway (hsa04068)          | 8.37E + 06  | 64           | 15            |
| Chronic myeloid leukemia (hsa05220)        | 0.00024     | 39           | 15            |
| Prostate cancer (hsa05215)                 | 0.00074     | 45           | 15            |
| Acute myeloid leukemia (hsa05221)          | 0.00162     | 30           | 15            |
| Regulation of actin cytoskeleton (hsa04810) | 0.00423   | 90           | 15            |
| Colorectal cancer (hsa05210)               | 0.00745     | 31           | 15            |
| Pancreatic cancer (hsa05212)               | 0.00745     | 32           | 15            |
| Ubiquitin mediated proteolysis (hsa04120)   | 0.01635     | 56           | 15            |
| Jak-STAT signaling pathway (hsa04630)      | 0.01635     | 66           | 15            |
| MAPK signaling pathway (hsa04010)          | 0.03528     | 99           | 15            |
| Oxytocin signaling pathway (hsa04921)      | 0.04960     | 63           | 15            |
| GABAergic synapse (hsa04727)               | 3.27E + 05  | 38           | 14            |
| Glioma (hsa05214)                          | 1.65E + 06  | 34           | 14            |
| TGF-beta signaling pathway (hsa04350)      | 5.38E + 06  | 39           | 14            |
| Melanoma (hsa05218)                        | 5.38E + 06  | 40           | 14            |
| Choline metabolism in cancer (hsa05231)    | 0.00032     | 51           | 14            |
| Thyroid hormone signaling pathway (hsa04919) | 0.00148  | 52           | 14            |
| Rap1 signaling pathway (hsa04015)          | 0.00423     | 90           | 14            |
| Phosphatidylinositol signaling system (hsa04070) | 0.00505  | 36           | 14            |
| Estrogen signaling pathway (hsa04915)      | 0.01385     | 42           | 14            |
| Cholinergic synapse (hsa04725)             | 0.03249     | 48           | 14            |
| cAMP signaling pathway (hsa04024)          | 0.04960     | 79           | 14            |
| Signaling pathways regulating pluripotency of stem cells (hsa04550) | 0.00027  | 64           | 13            |
| Prolactin signaling pathway (hsa04917)      | 0.00423     | 34           | 13            |
| Lysine degradation (hsa03010)               | 0.00505     | 20           | 13            |
| Circadian entrainment (hsa04713)           | 0.01663     | 44           | 13            |
| mTOR signaling pathway (hsa04150)          | 0.04513     | 28           | 13            |
| Endometrial cancer (hsa05213)              | 0.03739     | 26           | 12            |
| Thyroid cancer (hsa05216)                  | 0.04513     | 13           | 12            |
| Non-small cell lung cancer (hsa05223)      | 0.04513     | 25           | 12            |
| HIF-1 signaling pathway (hsa04066)         | 0.04867     | 44           | 12            |
| Bacterial invasion of epithelial cells (hsa05100) | 0.04960  | 32           | 12            |
| Prion diseases (hsa05020)                  | 1.54E + 02  | 14           | 9             |
| Dorso-ventral axis formation (hsa04320)    | 0.045132    | 15           | 9             |
| Mucin type O-Glycan biosynthesis (hsa05012) | 9.65E + 04  | 14           | 8             |
| Fatty acid biosynthesis (hsa00061)         | 2.11E-01    | 6            | 4             |
| Glycosaminoglycan biosynthesis - chondroitin sulfate/dermatan sulfate (hsa00532) | 0.03317 | 7 | 3 |
observed in the blood of children with ASD (Huang et al., 2015), an increase of miRNA-1246 in saliva was also found. miRNA-1246 is predicted to be implicated in the regulation of SYN II (Synapsin II) (miRDB database), which was identified as an ASD predisposition gene (Corradi et al., 2014). Also, miRNA-1246 together with the miRNA-4516, is involved in circadian cycles, and metabolic processes (miRDB database). Furthermore, miRNA-1246 is predicted to regulate the expression of the voltage-gated sodium channel (miRDB database). In this sense, the discovery of an altered miRNA-1246 in the saliva, which follows the same trend as observed in the peripheral blood, suggests that it could be a suitable peripheral, non-invasive biomarker for the early diagnosis of ASD.

Furthermore, a decrease in miRNA-454-5p, which is among the other functions is involved in CREB (cAMP Response Element-Binding) regulation (miRDB database) was observed. miRNA-660-5p was also found decreased in saliva of children with ASD. Interestingly, this miRNA is implicated in regulating insulin receptor substrate 1 (miRDB database). miR-4284 was found to be decreased in patients with ASD. This miRNA is in fact implicated, as is miR-1246, in the regulation of SYN II gene (miRDB database). Miao et al. (2021), identified miR-4284 as an anticancer in colon cancer. It inhibits colon cancer tumorigenesis by reducing PLIN5 and inhibiting EMT. Thus, miR-4284 could be a potential therapeutic target in metastatic colon cancer. Another miRNA that resulted downregulated is miRNA-203a-5p. This miRNA is predicted to be implicated in the regulation of ERK1 (miRDB database) (Vanacore et al., 2017; Cossu et al., 2019). Lastly, a decrease of the miR-1973 was observed. This miRNA is associated, among other functions, with the regulation of the potassium gated voltage channel.

Furthermore, analyses performed on the genes regulated by the first 5 up- and downregulated miRNAs showed the main pathways related to these miRNAs. Among other pathways, it was found that the EGF (epidermal growth factor) receptor signaling was affected by two miRNAs. Consistent with the present findings, the EGFR signaling was observed to be altered in children with ASD (Vallés and Barrantes, 2021). Another interesting pathway observed is that of the nicotinic receptor’s pathway, which resulted regulated by three different miRNAs. Indeed this receptor was observed to be altered in people with ASD, and more in particular in the frontal and parietal cortex, as well as in the cerebellum, which is deeply implied in social cognition, which means that cerebellar alteration could be involved in eye avoidance and similar behaviors. PDGF (Platelet-Derived Growth Factor), was the single gene most regulated by the miRNA studied in the present work, with a total of four different miRNAs. Although there are only a few papers are available that demonstrate a connection between PDGF and ASD, it should be noted that PDGF was indeed observed increased in the plasma of children with ASD (Zakareia et al., 2012). Another noteworthy element that was found regulated by a miRNA from the studied pool, was the JAK/STAT signaling. The tumor suppressor p53 was also observed regulated by two different miRNAs of the first five analyzed. In fact, in a preclinical model of ASD, it was suggested that dysregulation of p53 was correlated with altered DNA repair capacity and genomic instability that might lead to ASD (Wong et al., 2016). Some of the miRNAs in our study are found to be associated with inflammatory and proliferative diseases (Table 9). In particular, hsa-miRNA-1246 has been reported as a biomarker secreted in different biological samples in many neoplastic diseases. In fact, studies in colorectal cancer cell lines have shown oncogenic role of miR-1246. In this cancer, the methyltransferase METTL3 oncogene has been shown to increase methylation of pri-miR-1246 to enhance maturation of pri-miR-1246. Notably, miR-1246 has been predicted to suppress expression of the Sprouty Related EVH1 Domain Containing 2 (SPRED2) tumor suppressor, by increasing MAPK pathway (Peng et al., 2019). In this study, we would like to report that this miRNA is upregulated with highest fold change.

Furthermore, our analysis showed that genes in significant pathways that are predicted to be targeted by deregulated miRNAs control apoptosis,
TABLE 8  Interactors of top 10 miRNAs dysregulated.

| Interactor | P-value   | P-adjusted | Observed miRNAs/precursors |
|------------|-----------|------------|----------------------------|
| LMO3       | 4.34e-4   | 0.0408158  | 4 hsa-miR-199b-5p; hsa-miR-199a-3p; hsa-miR-199b-3p; hsa-miR-4284 |
| AASDHPPPT  | 0.0019694 | 0.0498852  | 4 hsa-miR-1246; hsa-miR-199b-5p; hsa-miR-199a-3p; hsa-miR-199b-3p |
| VEGFA      | 0.0018716 | 0.0498852  | 4 hsa-miR-1246; hsa-miR-199b-5p; hsa-miR-199a-3p; hsa-miR-199b-3p |
| IFRD1      | 7.7e-5     |            | 3 hsa-miR-199b-5p; hsa-miR-199a-3p; hsa-miR-199b-3p |
| ZNF763     | 7.7e-5     |            | 3 hsa-miR-1246; hsa-miR-199a-3p; hsa-miR-199b-3p |
| FGFR1OP2   | 9.68e-5    | 0.0259363  | 3 hsa-miR-199a-3p; hsa-miR-199b-3p; hsa-miR-4284 |
| SEC61B     | 8.48e-5    | 0.0259363  | 3 hsa-miR-1246; hsa-miR-199a-3p; hsa-miR-199b-3p |
| TRIM71     | 7.39e-5    | 0.0259363  | 3 hsa-miR-199a-3p; hsa-miR-199b-3p; hsa-miR-4284 |
| AMZ2       | 5.71e-4    | 0.0408158  | 3 hsa-miR-199b-5p; hsa-miR-199a-3p; hsa-miR-199b-3p |
| EHD3       | 4.26e-4    | 0.0408158  | 3 hsa-miR-1246; hsa-miR-199a-3p; hsa-miR-199b-3p |
| MBD1       | 5.32e-4    | 0.0408158  | 3 hsa-miR-1246; hsa-miR-199a-3p; hsa-miR-199b-3p |
| TBC1D4     | 4.26e-4    | 0.0408158  | 3 hsa-miR-1246; hsa-miR-199a-3p; hsa-miR-199b-3p |
| WIP12      | 6.99e-4    | 0.0408158  | 3 hsa-miR-1246; hsa-miR-199a-3p; hsa-miR-199b-3p |
| AP2A2      | 8.44e-4    | 0.0437519  | 3 hsa-miR-199a-3p; hsa-miR-199b-3p; hsa-miR-4284 |
| MAP1A      | 8.96e-4    | 0.0437519  | 3 hsa-miR-199a-3p; hsa-miR-199b-3p; hsa-miR-4284 |
| MOG        | 0.0011897  | 0.0443354  | 3 hsa-miR-199a-3p; hsa-miR-199b-3p; hsa-miR-4284 |
| RPL37A     | 0.0010074  | 0.0443354  | 3 hsa-miR-199b-5p; hsa-miR-199a-3p; hsa-miR-199b-3p |
| USP33      | 0.0012549  | 0.0443354  | 3 hsa-miR-199a-3p; hsa-miR-199b-3p; hsa-miR-4284 |
| ZDHHC21    | 0.0010074  | 0.0443354  | 3 hsa-miR-1246; hsa-miR-199a-3p; hsa-miR-199b-3p |
| ZNF227     | 0.0011267  | 0.0443354  | 3 hsa-miR-1246; hsa-miR-199a-3p; hsa-miR-199b-3p |
| autosomal  | 0.0019469  | 0.0496852  | 3 hsa-miR-199b-5p; hsa-miR-199a-3p; hsa-miR-199b-3p |
| LIMD2      | 0.0015387  | 0.0496852  | 3 hsa-miR-199a-3p; hsa-miR-199b-3p; hsa-miR-4284 |
| PCYOX1     | 0.0018602  | 0.0496852  | 3 hsa-miR-199a-3p; hsa-miR-199b-3p; hsa-miR-4284 |
| SCARNA9    | 0.0021278  | 0.0496852  | 3 hsa-miR-199b-5p; hsa-miR-199a-3p; hsa-miR-199b-3p |

![Gene ontology enrichment analysis by PANTHER for the 15 genes identified through miEEA.](image-url)

**FIGURE 2**
Gene ontology enrichment analysis by PANTHER for the 15 genes identified through miEEA. (A) Distribution of genes according to molecular function. (B) Distribution of genes according to biological process. (C) Distribution of genes according to protein class. (D) Distribution of genes according to signaling pathway.
TABLE 9  Reports of disease association for the dysregulated miRNAs in current study.

| miRNA      | Biological sample | Disease                                        | References                  |
|------------|-------------------|------------------------------------------------|-----------------------------|
| hsa-mir-1246 | Plasma            | Systemic lupus erythematosus                   | Ishibe et al., 2018         |
| hsa-mir-1246 | Whole blood       | Heart failure                                  | Nair et al., 2013           |
| hsa-mir-1246 | Serum             | Esophageal neoplasms                           | Takeshita et al., 2013      |
| hsa-mir-1246 | Serum             | Early-stage cervical squamous cell carcinoma   | Chen et al., 2013           |
| hsa-mir-1246 | Plasma            | Melanoma                                       | Armand-Labit et al., 2016   |
| hsa-mir-1246 | Serum             | Acute myeloid leukemia                         | Hornick et al., 2015        |
| hsa-mir-1246 | Serum             | Breast neoplasms                               | Shimomura et al., 2016      |
| hsa-mir-1246 | Serum             | Carcinoma, ovarian, serous                     | Todeschini et al., 2017     |
| hsa-mir-1246 | Plasma exosome    | Pancreatic neoplasms                           | Xu et al., 2017             |
| hsa-mir-1246 | PBMC               | Dengue virus infection                         | Jiang and Sun, 2018         |
| hsa-mir-1246 | Serum             | Colorectal carcinoma                           | Guo et al., 2018            |
| hsa-mir-1246 | Whole blood       | Plasmodium falciparum malaria                  | Li et al., 2018             |
| hsa-mir-1246 | SiHa cells        | Cervical carcinoma                             | Du et al., 2018             |
| hsa-mir-1246 | Colorectal tumors | Colorctal carcinoma                            | Piepoli et al., 2012        |
| hsa-mir-1246 | HepG2 cells       | Hepatocellular carcinoma                      | Yan et al., 2013            |
| hsa-mir-1246 | Whole blood       | Hemophilia A                                   | Sarachana et al., 2015      |
| hsa-mir-1246 | HCC1588 cells     | Non-small-cell lung carcinoma                  | Kim et al., 2016            |
| hsa-mir-1246 | Saliva            | Biliary tract carcinoma                        | Machida et al., 2016        |
| hsa-mir-1246 | SiHa cervical cancer cell | Cervical squamous cell carcinoma            | Chen et al., 2014          |
| hsa-mir-1246 | Exosomes          | Alcoholic hepatitis                            | Momen-Heravi et al., 2015   |
| hsa-mir-1246 | HCC cells         | Hepatocellular carcinoma                      | Sun et al., 2014            |
| hsa-mir-1246 | Panc1 cells       | Pancreatic neoplasms                           | Hasegawa et al., 2014       |
| hsa-mir-1246 | Serum Exosomes    | Pancreatic neoplasms                           | Madhavan et al., 2015       |
| hsa-mir-1246 | BC tumor tissue   | Breast carcinoma                               | Chen et al., 2016           |
| hsa-mir-1246 | Serum exosome     | Gastrointestinal neoplasms                    | Wei et al., 2018            |
| hsa-mir-1246 | Plasma exosome    | Breast carcinoma                               | Zhai et al., 2018           |
| hsa-mir-1973 | K562 cell-derived exosomes | Chronic myeloid leukemia                    | Feng et al., 2013           |
| hsa-mir-1973 | Serum             | Hodgkin lymphoma                               | Jones et al., 2014          |
| hsa-mir-378d | Serum             | Lung neoplasms                                 | He et al., 2018             |
| hsa-mir-4284 | Colonic tissue    | Ulcerative colitis                             | Koukos et al., 2015         |
| hsa-mir-4284 | GBM specimen      | Glioblastoma                                   | Yang et al., 2014           |

cell death of immune cells, cell cycle progression, epilepsy and neuronal cell proliferation. Our results reinforce the findings of other groups regarding important pathways, such as the regulation of cell growth, the role of organs besides the brain in ASD, and overlap with other non-neurodevelopmental diseases, such as cancer.

Conclusion

In our pilot study, we screened differentially expressed miRNAs in the saliva sample of ASD children and healthy control. Our sequencing investigation identified the significant number of miRNAs that were up- or down-regulated in the ASD group. The targets of miRNAs under study have been observed through specific tools to predict associated pathways and its regulation. The dysregulated miRNAs and their target genes were found to be involved in developmental pathways already known as the cause of typical ASD and neurobiological dysfunctions. This pilot study can be improved on biostatistical grounds by increasing the size of ASD cohort. Also, the lack of proteomic and genetic validation by RT-PCR of the specific miRNAs, and target genes, remains an unfinished task to claim these miRNAs as biomarkers for ASD.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.
Ethics statement

The study was conducted in accordance with the Declaration of Helsinki and approved by Ethics Committee "DIAGNOSI PRECOCE DEI DISTURBI DELLO SVILUPPO NEUROLOGICO IN SALIVA, INDIVIDUAZIONE DI MARCATORI PREDITTIVI ATTRAVERSO BIOSENSORI” Fondazione IRCCS Cà Granda Ospedale Maggiore Policlinico, Milano, U.O.C. Chirurgia Maxillo-Facciale e Odontostomatologia (727_2021). Written informed consent to participate in this study was provided by the participants’ legal guardian/next of kin.

Author contributions

ZK, MM, AS, and GTo participated in the conceptualization and writing-original draft preparation. MM and GTo participated in the methodology and software. AA participated in the data curation. MC and TB participated in the methodology and software. AA and writing-original draft preparation. MM and GTo participated in the conceptualization and writing-review and editing. All authors in the formal analysis. AC and FI participated in the participation in this study was provided by the participants’ legal guardian/next of kin.

References

Armand-Labit, V., Meyer, N., Casanova, A., Bonnabau, H., Platzer, V., Tournier, E., et al. (2016). Identification of a circulating MicroRNA profile as a biomarker of metastatic cutaneous melanoma. Acta Derm. Venereol. 96, 29–34. doi: 10.2340/00015555-2156

Ballini, A., Dipalma, G., Isacco, C. G., Boccellino, M., Di Domenico, M., Santacroce, L., et al. (2020). Oral microbiota and immune system crosstalk: a translational research. Biology 9:131. doi: 10.3390/biology9060131

Boccellino, M., Vanacore, D., Zappavigna, S., Cavaleri, C., Rossetti, S., D’Aniello, C., et al. (2017). Testicular cancer from diagnosis to epigenetic factors. Oncotarget 8, 104654–104663. doi: 10.18632/oncotarget.20992

Chen, J., Yao, D., Li, Y., Shen, H., He, C., Ding, N., et al. (2013). Serum microRNA expression levels can predict lymph node metastasis in patients with early-stage cervical squamous cell carcinoma. Int. J. Mol. Med. 32, 557–567. doi: 10.3892/ijmm.2013.1424

Chen, J., Yao, D., Zhao, S., He, C., Ding, N., et al. (2014). MiR-1246 promotes SiHa cervical cancer cell proliferation, invasion, and migration through suppression of its target gene thrombospondin 2. Arch. Gynecol. Obstet. 290, 725–732. doi: 10.1007/s00404-013-3260-2

Chen, X., Lu, P., Wang, D. D., Yang, S. J., Wu, Y., Shen, H. Y., et al. (2016). The role of miRNAs in drug resistance and prognosis of breast cancer formalin-fixed paraffin-embedded tissues. Gene. 31, 221–226. doi: 10.1016/j.gene.2016.10.015

Chen, Y., and Wang, X. (2020). MiRDB: an online database for prediction of functional MicroRNA targets. Nucleic Acids Res. 48, D127–D131. doi: 10.1093/nar/gkza757

Coley, A. A., and Gao, W.-J. (2018). PSD95: a synaptic protein implicated in schizophrenia or autism? Prog. Neuropsycho. Pharmacol. Biol. Psychiatry 82, 187–194. doi: 10.1016/j.pnpbp.2017.11.012

Corradi, A., Fadda, M., Pitton, A., Patry, L., Marte, A., Rossi, P., et al. (2014). SYN2 is an autism predisposing gene: loss-of-function mutations alter synaptic vesicle cycling and axon outgrowth. Hum. Mol. Genet. 23, 90–103. doi: 10.1093/hmg/ddt401

Cossu, A. M., Mosca, L., Zappavigna, S., Misso, G., Boccetti, M., De Micco, F., et al. (2019). Long non-coding RNAs as important biomarkers in laryngeal cancer and other head and neck tumours. Int. J. Mol. Sci. 20:3444. doi: 10.3390/ijms20143444

Dana, H., Tahtasakal, R., and Sener, E. F. (2020). Animal models of autism: a perspective from autophagy mechanisms. J. Transl. Genet. Genom. 4, 251–262. doi: 10.20517/jtg.2020.25

Du, P., Lai, Y. H., Yao, D. S., Lu, Y., Chen, Y. J., and Ding, N. (2018). Lentivirus media miR-1246 knockdown inhibits tumor growth and promotes apoptosis of SiHa cells. Zhonghua Fu Chan Ke Za Zhi 25, 481–486. doi: 10.3760/cma.j.issn.0529-567x.2018.07.007

Feng, D. Q., Huang, B., Li, J., Liu, J., Chen, X. M., Xu, Y. M., et al. (2013). Selective miRNA expression profile in chronic myeloid leukemia K562 cell-derived exosomes. Asian Pac. J. Cancer Prev. 14, 7501–7508. doi: 10.7314/apjcp.2013.14.12.7501

Giaccone, A., Montella, M., Boccellino, M., Crispo, A., D’Arena, G., Bimonte, S., et al. (2017). Epigenetic changes induced by green tea catechins are associated with prostate cancer. Curr. Mol. Med. 17, 405–420. doi: 10.2174/1566524018666171219101937

Guo, S., Zhang, J., Wang, B., Zhang, B., Wang, X., Huang, L., et al. (2018). A 5-serum miRNA panel for the early detection of colorectal cancer. Onco Targets Ther. 8, 2603–2614. doi: 10.2147/OTT.S153535

Ha, S., Sohn, I.-J., Kim, N., Sim, H. J., and Cheon, K.-A. (2015). Characteristics of brains in autism spectrum disorder: structure, function and connectivity across the lifespan. Exp. Neurobiol. 24, 273–284. doi: 10.5607/en.2015.24.4.273

Hallmayer, J., Cleveland, S., Torres, A., Phillips, J., Cohen, B., Torrigoe, T., et al. (2011). Genetic heritability and shared environmental factors among twin pairs with Autism. Arch. Gen. Psychiatry 68, 1095–1102. doi: 10.1001/archgenpsychiatry.2011.76

Hasegawa, S., Eguchi, H., Nagano, H., Konno, M., Tomimaru, Y., Wada, H., et al. (2014). MicroRNA-1246 expression associated with CCNG2-mediated
chemosensitivity and resistance in pancreatic cancer. Br. J. Cancer 14, 1572–1580.
doi: 10.1038/bjc.2014.454

He, Y., Ren, S., Wang, Y., Li, X., Zhou, C., and Hirsch, F. R. (2018). Serum microRNA improving the diagnostic accuracy in lung cancer presenting with pulmonary nodules. J. Thorac. Dis. 10, 5080–5085. doi: 10.21037/jtd.2018.07.138

Hicks, S. D., Carpenter, R. L., Wagner, K. E., Pauley, R., Barros, M., Tierney-Aves, C., et al. (2020). Saliva MicroRNA differentiates children with autism from peers with typical and atypical development. J. Am. Acad. Child Adolesc. Psychiatry 59, 296–308. doi: 10.1016/j.jaac.2019.03.017

Hicks, S. D., Rajan, A. T., Wagner, K. E., Barns, S., Carpenter, R. L., and Middleton, F. A. (2018a). Validation of a salivary RNA test for childhood autism spectrum disorder. Front. Genet. 9:334. doi: 10.3389/fgene.2018.00334

Hicks, S. D., Johnson, J., Carney, M. C., Bramley, H., Olympia, R. P., Leoffert, A. C., et al. (2018b). Overlapping Mi-croRNA expression in saliva and cerebrospinal fluid accurately identifies pediatric traumatic brain injury. J. Neurotrauma 35, 64–72. doi: 10.1089/neuro.2017.0111

Hornack, N. L., Hsuan, J., Deron, B., Golovchina, N. A., Lipadis, J., Chang, B. H., et al. (2015). Serum exosomes are a multiply-invasive early biomarker of AML. Sci. Rep. 12:11295. doi: 10.1038/srep11295

Huang, F., Long, Z., Chen, Z., Li, J., Hu, Z., Qiu, R., et al. (2015). Investigation of gene regulatory networks associated with autism spectrum disorder based on MiRNA expression in China. PLoS One 10:0129502. doi: 10.1371/journal.pone.0129502

Isacco, G. C., Ballini, A., De Vito, D., Nguyen, K. C. D., Cantore, S., Bottalico, L., et al. (2021). Rebalancing the oral microbiota as an efficient tool in endocrine, metabolic and immune disorders. Endocr. Metab. Immune Disord. Drug Targets 21, 777–784. doi: 10.2174/1871530320666200729142504

Isibey, H., Kusao, M., Murayama, G., Nemoto, T., Kon, T., Ogasawara, M., et al. (2018). Changes in the expression of circulating microRNAs in systemic lupus erythematosus patient blood plasma after passing through a plasma adsorption membrane. Ther. Apher. Dial. 22, 278–289. doi: 10.1001/1774-9887.12695

Ivanov, Y. D., Malsagova, K. A., Goldaeva, K. V., Pleshakova, T. O., Shumov, I. D., Galulin, R. A., et al. (2022). “Silicon-on-insulator”-based sensoron for the revelation of MicroRNA markers of autism. Genes 22:199. doi: 10.3390/genes1202199

Jiang, L., and Sun, Q. (2018). The expression profile of human peripheral blood mononuclear cell miRNA is altered by antibody-dependent enhancement of infection with dengue virus serotype 3. Viruses 20:1250. doi: 10.3390/v11070108-0963-1

Jones, K., Nourse, J. P., Keane, C., Bhattacharj, A., and Gandhi, M. K. (2014). Plasmablastic disease patients with HIV have increased microRNA expression. PLoS One 9:e105343. doi: 10.1371/journal.pone.0105343

Kichukova, T., Petrov, V., Popov, N., Minchev, D., Naimov, S., Minkov, I., et al. (2018). Identification of serum Mi-croRNA signatures associated with autism spectrum disorder as promising candidate biomarkers. Heliyon 7:e07462. doi: 10.1016/j.heliyon.2021.e07462

Kim, G., An, H. J., Lee, M. J., Song, I. Y., Jeong, J. Y., Lee, J. H., et al. (2016). Hsa-miR-1246 and hsa-miR-1290 are associated with stemness and invasiveness of non-small cell lung cancer. Lung Cancer 91, 15–22. doi: 10.1016/j.lungcan.2015.11.013

Kreukels, G., Polychroni, C., Kaplan, J. L., Oikonomopoulou, A., Ziring, D., Hommes, D. W., et al. (2015). A microRNA signature in pediatric ulcerative colitis: deregulation of the miR-4284/CXCL5 pathway in the intestinal epithelium. Inflamm. Bowel Dis. 21, 996–1015. doi: 10.1097/MIB.0000000000000339

Li, J. J., Huang, M. J., Li, Z. L., Li, W., Wang, F., Wang, L., et al. (2018). Identification of potential whole blood MicroRNA biomarkers for the blood stage of adult imported falciparum malaria through integrated miRNA and miRNA expression profiling. Biochem. Biophys. Res. Commun. 390, 471–477. doi: 10.1016/j.bbrc.2018.10.072

Machida, T., Tomofuji, T., Maruyama, T., Yoneda, T., Ekuni, D., Azuma, T., et al. (2016). miR-1246 and miR-4644 in salivary exosome as potential biomarkers for pancreaticobiliary tract cancer. Oncol. Rep. 36, 2375–2381. doi: 10.3892/or.2016.5021

Madhavan, B., Yue, S., Galli, U., Rana, S., Gross, W., Muller, M., et al. (2015). Combined evaluation of a panel of protein and miRNA serum-exosome biomarkers for pancreatic cancer diagnosis increases sensitivity and specificity. Int. J. Cancer 1, 2616–2627. doi: 10.1002/ijc.29324

Miao, X., Li, Z., Zhang, Y., and Wang, T. (2021). MicroRNA-4284 inhibits colon cancer epithelial-mesenchymal transition by down-regulating Perilipin 5. STEMedicine 2:e85.
Tordjman, S., Davlantis, K. S., Georgieff, N., Geoffray, M.-M., Speranza, M., Anderson, G. M., et al. (2015). Autism as a disorder of biological and behavioral rhythms: toward new therapeutic perspectives. *Pediatr. Endocrinol. Rev.* 13, 1–15.

Urdinguio, R. G., Fernandez, A. F., Lopez-Nieva, P., Rossi, S., Huertas, D., Kulis, M., et al. (2010). Disrupted MicroRNA expression caused by MeCP2 loss in a mouse model of Rett syndrome. *Epigenetics* 5, 656–663. doi: 10.4161/epi.5.7.13055

Vallés, A. S., and Barrantes, F. J. (2021). Dysregulation of neuronal nicotinic acetylcholine receptor-cholesterol crosstalk in autism spectrum disorder. *Front. Mol. Neurosci.* 11:744597. doi: 10.3389/fnmol.2021.744597

van’t Hof, M., Tisseur, C., van Berckelear-Onnes, I., van Nieuwenhuyzen, A., Daniels, A. M., Deen, M., et al. (2021). Age at autism spectrum disorder diagnosis: a systematic review and meta-analysis from 2012 to 2019. *Autism* 25, 862–873. doi: 10.1177/1362361320971107

Vanacore, D., Boccellino, M., Rossetti, S., Cavaliere, C., D’Aniello, C., Di Franco, R., et al. (2017). MiR-1246 is upregulated in breast cancer: a potential biomarker for breast cancer progression. *Oncotarget* 8, 50240–50251. doi: 10.18632/oncotarget.16933

Vlachos, I. S., Zagganas, K., Paraskevopoulou, M. D., Georgakilas, G., Karagkouni, D., Vergoulis, T., et al. (2015). DIANA-MiRPath v3.0: deciphering MicroRNA function with experimental support. *Nucleic Acids Res.* 43, W460–W466. doi: 10.1093/nar/gkv403

Wei, C., Li, Y., Huang, K., Li, G., and He, M. (2018). Exosomal miR-1246 in body fluids as a potential biomarker for gastrointestinal cancer. *Biomark. Med.* 12, 1185–1196. doi: 10.2217/bmm-2017-0440

Wiąsniewska-Kowalnik, B., and Nowakowska, B. A. (2019). Genetics and epigenetics of autism spectrum disorder: current evidence in the field. *J. Appl. Genet.* 60, 37–47. doi: 10.1007/s13353-018-00480-w

Wong, S., Napoli, E., Krakowiak, P., Tassone, F., Hertz-Picciotto, I., and Giulivi, C. (2016). Role of PS3, mitochondrial DNA deletions, and paternal age in autism: a case-control study. *Pediatrics* 137:e20151888. doi: 10.1542/peds.2015-1888

Wu, X., Li, W., and Zheng, Y. (2020). Recent progress on relevant microRNAs in autism spectrum disorders. *Int. J. Mol. Sci.* 21:5904. doi: 10.3390/ijms21165904

Xu, Y. F., Hanafon, B. N., Zhao, Y. D., Postier, R. G., and Ding, W. Q. (2017). Plasma exosome miR-196a and miR-1246 are potential indicators of localized pancreatic cancer. *Oncotarget* 18, 77028–77040. doi: 10.18632/oncotarget.20332

Yamakawa, K. (2016). “Mutations of voltage-gated sodium channel genes SCN1A and SCN2A in epilepsy, intellectual disability, and autism,” in *Neuronal and Synaptic Dysfunction in Autism Spectrum Disorder and Intellectual Disability*, eds C. Sala and C. Verpelli (San Diego, CA: Academic Press), 233–251.

Yan, H., Wang, S., Yu, H., Zhu, J., and Chen, C. (2013). Molecular pathways and functional analysis of miRNA expression associated with paclitaxel-induced apoptosis in hepatocellular carcinoma cells. *Pharmacology* 92, 167–174. doi: 10.1159/000354585

Yang, F., Nam, S., Brown, C. E., Zhao, R., Starr, R., Ma, Y., et al. (2014). A novel berbamine derivative inhibits cell viability and induces apoptosis in cancer stem-like cells of human glioblastoma, via up-regulation of miRNA-4284 and JNK/AP-1 signaling. *PLoS One* 14:e94443. doi: 10.1371/journal.pone.0094443

Zakareia, F. A., Al-Ayadh, L. Y., and Al-Drees, A. A. (2012). Study of dual angiogenic/neurogenic growth factors among Saudi autistic children and their correlation with the severity of this disorder. *Neuroscienc*e 17, 213–218.

Zhai, L. Y., Li, M. X., Pan, W. L., Chen, Y., Li, M. M., Pang, J. X., et al. (2018). In situ detection of plasma exosomal MicroRNA-1246 for breast cancer diagnosis by a Au nanoflare probe. *ACS Appl. Mater. Interfaces* 10, 39478–39486. doi: 10.1021/acsami.8b12725

Zhang, L., Lu, Q., and Chang, C. (2020). Epigenetics in health and disease. *Adv. Exp. Med. Biol.* 1253, 3–55. doi: 10.1007/978-981-15-3449-2_1