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Specific Regional and Age-Related Small Noncoding RNA Expression Patterns Within Superior Temporal Gyrus of Typical Human Brains Are Less Distinct in Autism Brains

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Abstract

Small noncoding RNAs play a critical role in regulating messenger RNA throughout brain development and when altered could have profound effects leading to disorders such as autism spectrum disorders (ASD). We assessed small noncoding RNAs, including microRNA and small nucleolar RNA, in superior temporal sulcus association cortex and primary auditory cortex in typical and ASD brains from early childhood to adulthood. Typical small noncoding RNA expression profiles were less distinct in ASD, both between regions and changes with age. Typical micro-RNA coexpression associations were absent in ASD brains. miR-132, miR-103, and miR-320 micro-RNAs were dysregulated in ASD and have previously been associated with autism spectrum disorders. These diminished region- and age-related micro-RNA expression profiles are in line with previously reported findings of attenuated messenger RNA and long noncoding RNA in ASD brain. This study demonstrates alterations in superior temporal sulcus in ASD, a region implicated in social impairment, and is the first to demonstrate molecular alterations in the primary auditory cortex.

Keywords

auditory cortex, micro-RNA, small nucleolar RNA, superior temporal sulcus, autism

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Whole transcriptome studies are providing new insights into the molecular bases for normal regional heterogeneity in the human brain.[1-3] Messenger RNA signatures vary enormously between anatomical regions and cell types, although they are highly conserved between individuals. The human neocortex displays a relatively homogeneous transcriptional pattern, and the closer the 2 cortical regions, the more similar their transcriptomes. However, transcriptomes associated with primary sensorimotor cortices are quite distinct from adjacent association cortex.[1,2] The molecular mechanisms responsible for normal human brain region-specific and cell-specific gene expression patterns are not known, but they could relate at least in part to regulatory control of small noncoding RNA.

Small noncoding RNAs include micro-RNA, small nucleolar RNA, and other small RNAs. Micro-RNA are ~22-nucleotide noncoding regulatory RNA that function by base-pairing with complementary sequences in target messenger RNA. There are more than a thousand known human micro-RNA, each targeting dozens to several hundred target messenger RNA to predominantly promote target messenger RNA degradation or repress messenger RNA translation. Small noncoding RNAs have critical regulatory functions in normal brain, including regulating gene expression to coordinate brain development, neurogenesis, neuronal maturation, cell fate specification, brain patterning, and higher-level motor, sensory, and cognitive processes, including learning and memory.[4]

Though many small noncoding RNA studies have been performed in animals, few studies have assessed small noncoding RNAs in the human brain.[5-9] Similarly, though small noncoding

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RNAs are implicated in many brain diseases, including schizophrenia, Fragile X syndrome, Rett syndrome, and autism spectrum disorders (ASD),[10] few studies have directly investigated the human brain. Small noncoding RNAs could play a significant role in ASD, as suggested by recent studies demonstrating that the normal messenger RNA expression patterns typically found between brain regions are attenuated, or less distinct, in ASD brain.[11-13] Because micro-RNA / small noncoding RNA regulate messenger RNA, and regional differences of messenger RNA expression are attenuated in ASD brain, we postulated that regional differences of micro-RNA / small noncoding RNA would also be attenuated in ASD brain. Additionally, from our magnetic resonance imaging (MRI) and cellular postmortem brain studies, we know that the ASD brain undergoes an aberrant developmental trajectory across the life span,[14] and therefore, we hypothesized that age-related small noncoding RNA expression patterns would be dysregulated in ASD brain as well.

To pursue this, we examined 2 regions in the human superior temporal gyrus (STG): the superior temporal sulcus (STS) and the primary auditory cortex (PAC). Though these regions are adjacent to one another, they have very different brain functions. The STS is association cortex, involved in social perception, joint attention, and interpreting facial gaze and speech inputs.[15-17] Abnormalities in such functions are common in disorders with social impairment, including ASD.[18-20] PAC is a primary sensory cortex that modulates auditory processing, a function not usually associated with ASD impairments. Structurally, these regions exhibit distinct patterns of cellular and neurochemical organization.[15-17,21-26] Thus, from a functional anatomic perspective, one might expect to see associated differences in gene expression across regions in typically developing individuals.

Based on previous messenger RNA studies showing region-specific messenger RNA expression patterns in human brains,[1] we postulated that there are region-specific and age-specific small noncoding RNA expression patterns in typically developing brains that would be attenuated, or less distinct, in ASD brain. Thus, the objectives of this study were to (1) demonstrate regional and age-related small noncoding RNA expression profiles within typical human STS and PAC that can serve as a basis for comparison to neuropsychiatric disorders and, as an example, (2) show how these regional and age-related profiles may differ with aberrant development in children and adults with ASD.

Materials and Methods

Postmortem Brain Tissue

Regional and age-related small noncoding RNA expression patterns were assessed within 2 regions of the human superior temporal gyrus: STS and adjacent PAC. A total of 28 human brain samples were obtained from the Autism Tissue Program collection at the Harvard Brain Tissue Resource Center. These included 14 fresh-frozen postmortem human brains; 6 typically developing brains (TYP) (6 STS and 6 PAC samples) and 8 age-matched ASD brains (8 STS and 8 PAC samples) (Table 1). The STS included polymodal association cortex of Brodmann area 22 and PAC included Brodmann areas 41 and 42. Samples were taken from the same coronal section in the posterior portion of the temporal lobe in each brain. PAC was taken from the crown of Heschl gyrus, where it is identified on both cytoarchitectonic and functional maps,[27] and the STS was taken from the dorsal, upper wall of the STS immediately opposite the Heschl gyrus[21,28] (see Figures 1 and 2).

Table 1. Demographics for Each Subject.

Case no.	Primary diagnosis	Sex	Age, years
1	ASD	F	5
2	ASD	F	11
3	ASD	M	15
4	ASD	F	29
5	ASD	M	30
6	ASD	M	30
7	ASD	F	49
8	ASD	M	50
9	TYP	F	4
10	TYP	M	17
11	TYP	M	24
12	TYP	M	36
13	TYP	F	39
14	TYP	M	40

Abbreviations: ASD, autism spectrum disorders subjects; F, female; M, male; TYP, typically developing subjects.

Small Noncoding RNA Microarray Processing

Total RNA was isolated from 10 to 75 mg of brain tissue using the RecoverAll Total Nucleic Acid Isolation Kit (Ambion, Austin, TX). The heat de-paraffinization step was omitted since fresh-frozen tissue was used. Yields of RNA averaged 200 ng/mg of brain tissue. Quality of RNA was assessed using Agilent 2100 Bioanalyzer with the RNA 6000 Nano kit (Agilent) and RNA quantity was measured using NanoDrop ND-1000. RIN numbers ranged between 2.2 and 2.6, which is considered of low quality for messenger RNA studies. Therefore, the current study only evaluated small noncoding RNA, which may be less affected by the lower tissue quality. Total RNA was processed on Affymetrix micro-RNA 3.0 microarrays according to the manufacturer's protocol (Affymetrix, Santa Clara, CA). All within-chip quality control hybridization metrics were within normal ranges, indicating successful sample processing. Cel data files were imported into Partek Genomics Suite 6.6 (Partek Inc, St Louis, MO). Data were normalized using RMA (Robust Multichip Averaging).

Analysis of Differential Small Noncoding RNA Expression Between STS and PAC

Mixed effects linear regression models assessed regional small noncoding RNA expression in typically developing (TYP) and in autism spectrum disorders (ASD) subjects in the STS compared to the PAC (Partek Genomics Suite 6.6). The statistical models included the following variables: Subject (random effect), Diagnosis (typically developing, ASD), Region (STS, PAC), Sex (male, female), Age (continuous variable), and an interaction term of Diagnosis*Region. The interaction term provided the data described below and in Table 2. Random effects included within-subject correlation among

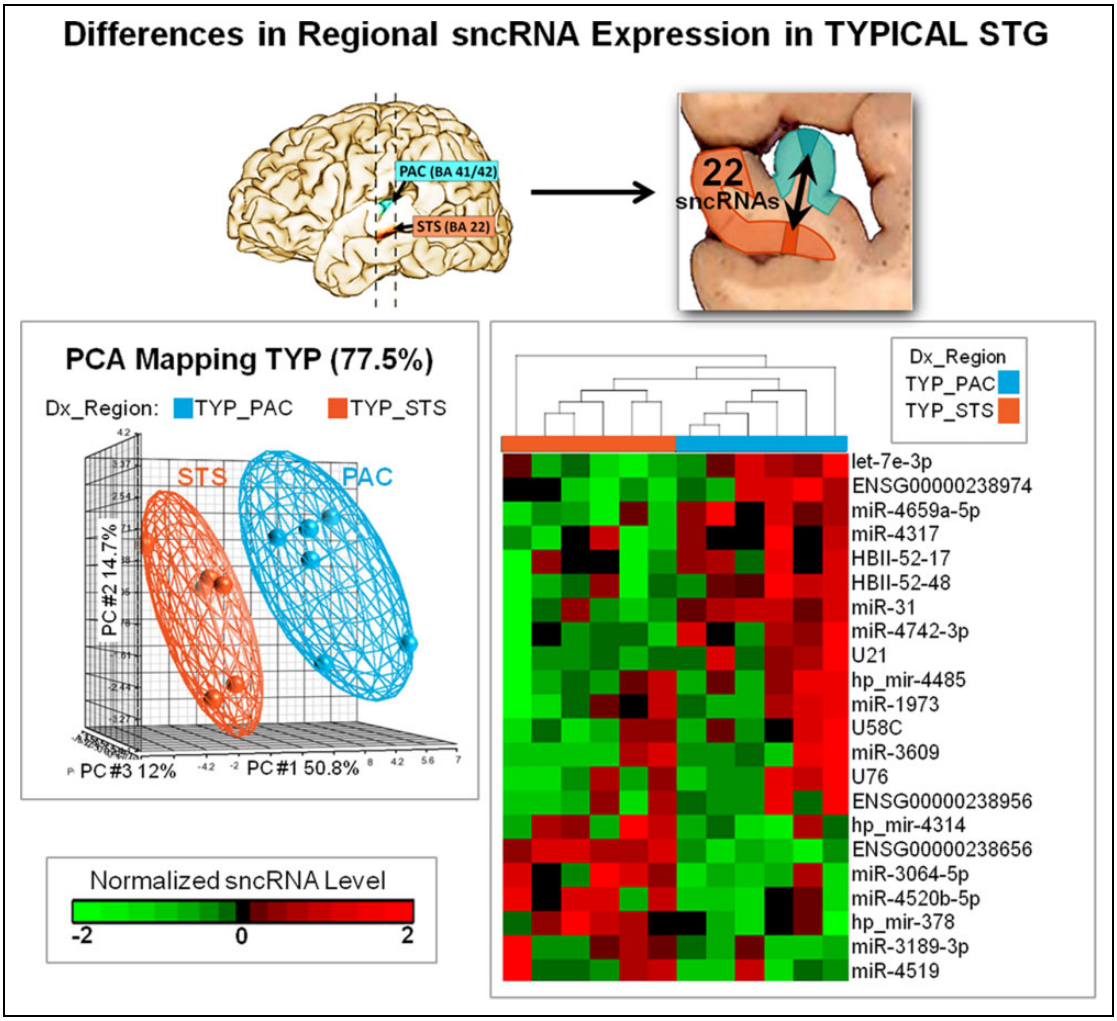

Figure 1. Differences in regional small noncoding RNA expression in the superior temporal gyrus (STG) of typically developing (TYP) subjects. A total of 22 small noncoding RNA differed in expression between the superior temporal sulcus (STS) and the primary auditory cortex (PAC) of the STG in typical subjects (Table 2). These 22 small noncoding RNA were subjected to a principal components analysis (PCA) and hierarchical clustering analysis. PAC is identified by light blue and STS by an orange color. The centroids for the PCA plots indicate 2 standard deviations around the group's centroid, and individual subjects are indicated by the small spheres. For the hierarchical clustering, subjects are on the X-axis, small noncoding RNA are on the Y-axis, 2-fold increase of expression is indicated by bright red color, and a 2-fold decrease of expression is indicated by bright green color (see normalized small noncoding RNA level color bar).

brain regions. We used the REML method of variance estimate for unbalanced designs[29] and the Fisher's least significant difference contrast method[30] to estimate regional differential small noncoding RNA expression in each of the diagnosis groups: TYP_STS versus TYP_PAC, and ASD_STS versus ASD_PAC. The small noncoding RNAs with $P < .005$ and a |fold-change| >1.2 were considered significant.

Analysis of Changes of Expression of Small Noncoding RNA in the STS and PAC With Age

We performed an analysis of age-related small noncoding RNA in STS for typically developing brain and ASD brain (Table 3), and age-related small noncoding RNA in PAC for typically developing brain and ASD brain (Table 4). From the lists of small noncoding RNAs in Tables 3 and 4, we focused subsequent analyses and figures on the mature micro-RNAs (Table 5) with expression levels >2 (n = 1123 mature micro-RNAs) in all samples. We focused on mature micro-RNA to decrease the chance for false positive associations, and because a number of mature micro-RNA are characterized experimentally and/or have high-confidence, computationally predicted, sequence-specific messenger RNA targets in the brain.

We used Spearman rank correlation coefficient (r_S) as a measure of association between micro-RNA expression levels and age in the STS and PAC regions for both typically developing and ASD groups. r_S is a nonparametric measure of statistical dependence between 2 variables. A perfect Spearman correlation of +1 or −1 occurs when each variable is a perfect linear function of the other. Associations between small noncoding RNA and micro-RNA levels and age were considered significant at $P < .005$.

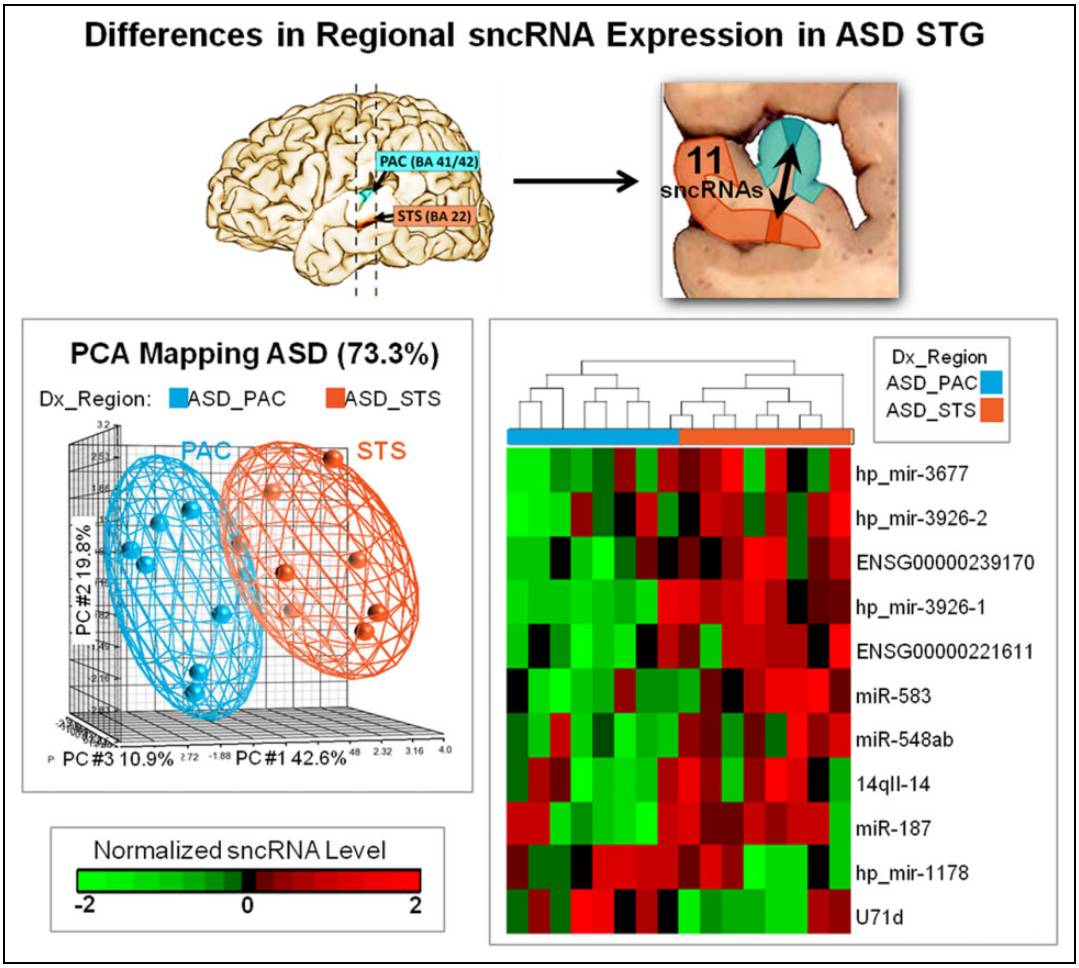

Figure 2. Differences in regional small noncoding RNA expression in the superior temporal gyrus (STG) of autism spectrum disorders (ASD) subjects. A total of 11 small noncoding RNAs differed in expression between the STS (STS) and the primary auditory cortex (PAC) of the STG in ASD subjects (Table 2). These 11 small noncoding RNAs were subjected to a principal components analysis (PCA) and hierarchical clustering analysis. PAC is identified by light blue and STS by an orange color. The centroids for the PCA plots indicate 2 standard deviations around the group's centroid, and individual subjects are indicated by the small spheres. For the hierarchical clustering, subjects are on the X-axis, small noncoding RNA are on the Y-axis, 2-fold increase of expression is indicated by bright red, and a 2-fold decrease of expression is indicated by bright green color (see normalized small noncoding RNA level color bar). Though there are fewer small noncoding RNAs in ASD STG compared to TYP STG, they separate PAC and STS on both PCA and cluster plots.

Unsupervised Hierarchical Clustering and Principal Components Analyses

To visualize regional differences in small noncoding RNA expression, hierarchical clustering and principal components analysis were performed as described previously.[31]

Coexpression Analyses of Age-Related Micro-RNA Expression in STS and PAC

Coexpression matrices were generated using the Spearman rank correlation similarity method for regulated micro-RNAs for each age-related analysis in STS and PAC in typically developing brains (Partek Genomics Suite). Only significant correlations were retained in the matrix ($P < .005$). Nonsignificant correlations ($P \geq .005$) were set to zero. This coexpression matrix was imported into *Genesis*[32] to perform hierarchical clustering, which allows for identification of coexpressed micro-RNA modules in TYP_STS and TYP_PAC. To identify deviations from this coordinate expression, coexpression matrices were derived for ASD_STS and ASD_PAC for the age-related significant micro-RNA in the corresponding regions of typically developing brains. To evaluate how these coexpression modules identified in typical subjects behaved in ASD, we arranged the ASD coexpression matrices to match the hierarchical clustering order in typically developing brains. This allowed us to visually assess deviations in ASD from the patterns of typical brain development for both STS and PAC (Figure 5).

Analysis of Micro-RNA Targets in Typically Developing and ASD Brains

Mature micro-RNAs with region and age-related regulation in typically developing and ASD brain were used to query databases

Table 2. Regionally Regulated sncRNAs Between STS and PAC in Brains of TYP and ASD Subjects.[a]

Regional analysis	Up in STS vs PAC	Down in STS vs PAC	Total	Transcript
TYP: STS vs PAC				
Mature miRNA	4	7	11	↑ miR-3064-5p, miR-3189-3p, miR-4519, miR-4520b-5p ↓ let-7e-3p, miR-31, miR-1973, miR-3609, miR-4317, miR-4659a-5p, miR-4742-3p
Stem-Loop miRNA	2	1	3	↑ mir-378, mir-4314 ↓ mir-4485
snoRNA	1	7	8	↑ ENSG00000238656, ↓ U21, U58C, U76, HBII-52-17, HBII-52-48, ENSG00000238956, ENSG00000238974
Total	7	15	22	
ASD: STS vs PAC				
Mature miRNA	3	0	3	↑ miR-187, miR-548ab, miR-583
Stem-Loop miRNA	3	1	4	↑ mir-3677, mir-3926 -1, mir-3926-2 ↓ mir-1178
snoRNA	3	1	4	↑ 14qII-14, ENSG00000221611, ENSG00000239170 ↓ U71d
Total	9	2	11	

Abbreviations: ASD, autism spectrum disorders; miRNA, micro-RNA; PAC, primary auditory cortex; sncRNAs, small noncoding RNAs; snoRNA, small nucleolar RNA; STS, superior temporal sulcus; TYP, typically developing.
[a]↑ denotes upregulated small noncoding RNA, and ↓ denotes downregulated small noncoding RNA in STS compared to PAC. Numbers in the table indicate the numbers of significantly regulated sncRNAs ($P < .005$, and $|\text{fold-change}| > 1.2$).

Table 3. Age-Regulated sncRNAs in STS in Brains of TYP and ASD Subjects.[a]

Age-related analysis	Increase	Decrease	Total	Transcript
TYP-STS				
Mature miRNA	10	8	18	See Table 5
Stem-Loop miRNA	2	6	8	↑ mir-30d, mir-1537 ↓ mir-222, mir-548n, mir-620, mir-1248, mir-3179-3, mir-4477b
snoRNA	2	20	22	↑ U38A, ENSG00000202374 ↓ ACA5, ACA13, ACA24, ACA40, ACA46, HBII-296B, U15A, U23, U31, U46, U90, ENSG00000252543, ENSG00000200042, ENSG00000202216, ENSG00000202498, ENSG00000208308, ENSG00000212342, ENSG00000212397, ENSG00000212551, ENSG00000239046
Total	14	34	48	
ASD-STS				
Mature miRNA	1	3	4	See Table 5
Stem-Loop miRNA	3	1	4	↑ mir-146b, mir-640, mir-4477b ↓ let-7a-1
snoRNA	4	1	5	↑ U17a, ENSG00000239055, ENSG00000206603, ENSG00000212445 ↓ U89
Total	8	5	13	

Abbreviations: ASD, autism spectrum disorders; miRNA, micro-RNA; PAC, primary auditory cortex; sncRNAs, small noncoding RNAs; snoRNA, small nucleolar RNA; STS, superior temporal sulcus; TYP, typically developing.
[a]↑ indicates expression of the small noncoding RNA increases with age, and ↓ indicates expression decreases with age. Numbers indicate the numbers of significantly regulated sncRNA (Spearman rank correlation $P < .005$).

of known target genes (TargetScan) as we previously described.[33] Only putative targets with experimental validation or high confidence of prediction, and expressed in the nervous system, were considered for further analysis. Minor micro-RNA isoforms did not have predicted targets in TargetScan and were not considered in the analyses where micro-RNA targets were examined. The target

Table 4. Age-Regulated sncRNAs in PAC in Brains of TYP and ASD Subjects.[a]

Age-related analysis	Increase	Decrease	Total	Transcript
TYP_PAC				
Mature miRNA	16	11	27	See Table 5
Stem-Loop miRNA	11	7	18	↑ let-7i, mir-151, mir-200c, mir-219 -1, mir-550a-1, mir-1260, mir-1285-2, mir-3138, mir-3194, mir-4454, mir-4487 ↓ mir-323b, mir-409, mir-485, mir-3690, mir-4476, mir-4531, mir-4797
snoRNA	8	17	25	↑ U71a, ENSG00000199392, ENSG00000201407, ENSG00000207217, ENSG00000238437, ENSG00000239171, ENSG00000252119, ENSG00000252190 ↓ 14qII-17, 14qII-20, 14qII-21, 14qII-22, ACA13, ACA5, HBI-61, HBII-210, HBII-420, HBII-99, U45C, ENSG00000201009, ENSG00000212149, ENSG00000212528, ENSG00000221060, ENSG00000238342, ENSG00000238390
scaRNA	0	1	1	↓ ACA66
Total	35	36	71	
ASD_PAC				
Mature miRNA	0	2	2	↓ miR-93-3p, miR-3607-5p
Stem-Loop miRNA	0	2	2	↓ mir-7-2, mir-92a-1
snoRNA	0	1	1	↓ ENSG00000238816
Total	0	5	5	

Abbreviations: ASD, autism spectrum disorders; miRNA, micro-RNA; PAC, primary auditory cortex; scaRNA, small Cajal body–specific RNA; sncRNAs, small noncoding RNAs; snoRNA, small nucleolar RNA; STS, superior temporal sulcus; TYP, typically developing.
[a]↑ indicates expression of small noncoding RNA increase with age, and ↓ indicates expression of small noncoding RNA decrease with age. Numbers in the table indicate the numbers of significantly regulated sncRNA (Spearman rank correlation $P < .005$).

Table 5. Age-Regulated Mature miRNAs in STS and PAC in Brains of TYP and ASD Subjects.[a]

Age-related analysis	Increase	Decrease	Total	Transcript
TYP_STS	10	8	18	↑ miR-99b-3p, miR-223, miR-320a, miR-320b, miR-320c, miR-345, miR-1234, miR-1306, miR-2110, miR-4786-3p ↓ miR-103a-2-5p, mir-136-3p, miR-147b, miR-378b, miR-504, miR-1244, miR-3152-3p, miR-4299
ASD_STS	1	3	4	↑ miR-1260b ↓ miR-424-3p, miR-484, miR-3916
TYP_PAC	16	11	27	↑ miR-29b, miR-29c, miR-193b-5p, miR-320a, miR-612, miR-1249, miR-2053, miR-4310, miR-4421, miR-4440, miR-4487, miR-4506, miR-4717-3p, miR-4725-3p, miR-4740-5p, miR-4799-3p ↓ miR-132, miR-132-5p, miR-370, miR-431-3p, miR-432, miR-433, miR-654-3p, miR-676-5p, miR-2115-3p, miR-4291, miR-4804-3p
ASD_PAC	0	2	2	↓ miR-93-3p, miR-3607-5p

Abbreviations: ASD, autism spectrum disorders; miRNA, micro-RNA; PAC, primary auditory cortex; STS, superior temporal sulcus; TYP, typically developing.
[a]↑ indicates increasing small noncoding RNA expression with age, and ↓ indicates decreasing small noncoding RNA expression with age. Numbers in the table indicate the numbers of significantly regulated mature miRNA (Spearman rank correlation $P < .005$).

analyses revealed putative messenger RNA targets of the differentially expressed mature micro-RNAs in typically developing and ASD brain. These putative targets were subjected to IPA pathway-enrichment analysis (Ingenuity Pathway Analysis; Qiagen) as previously described.[31] A Benjamini-Hochberg multiple testing–corrected $P < .05$ was considered to be statistically significant for overrepresentation of molecules in a pathway (Figure 6). Gene Ontology (GO) enrichment analysis of putative targets was performed in DAVID Bioinformatics Resources Functional Annotation Tool (Benjamini-Hochberg $P < .05$).[34,35]

Test for Enrichment of Autism Spectrum Disorders– and Other Disease-Associated Genes

To determine if the target genes of the differentially expressed micro-RNAs relate to other diseases, we examined their enrichment in 22 disease-related gene sets. The ASD–related gene set was from AutDB (SFARI, MindSpec).[36] The other disease-related gene sets were from the Genotator database.[37] Enrichment was assessed in neurological diseases (Alzheimer's, Huntington's, Parkinson's, and epilepsy), psychiatric disorders (schizophrenia, major depressive

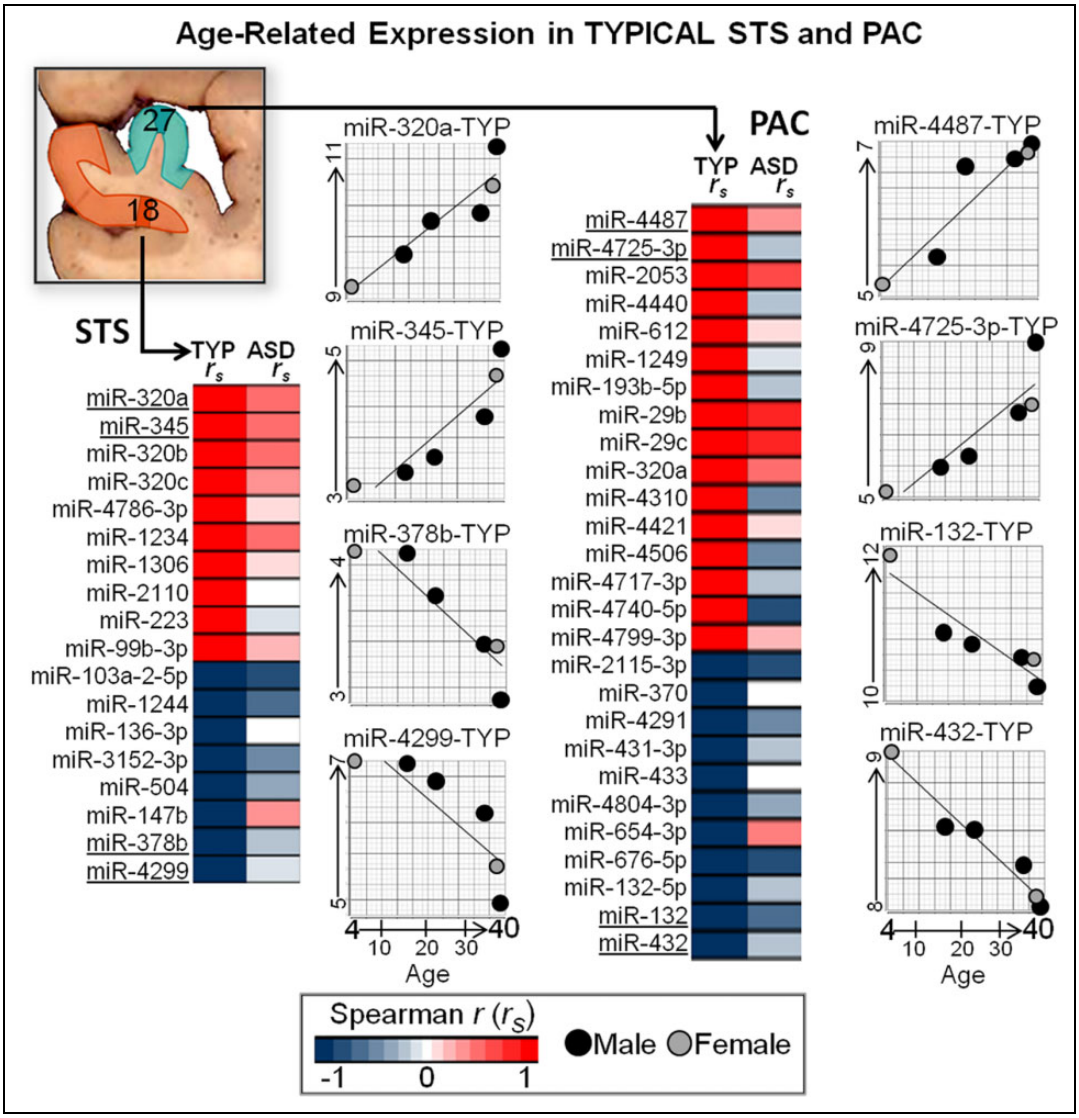

Figure 3. Mature micro-RNAs that change expression with age in STS (STS) and primary auditory cortex (PAC) of typical subjects. Typical STS (TYP_STS) is shown on the left and typical PAC (TYP_PAC) on the right. The heat map on the left is for STS and on the right is for PAC. The heat maps represent the strength of the age-related association with the expression levels of the regulated micro-RNAs in TYP_STS or TYP_PAC (Spearman rank correlation, r_S values) compared to their corresponding associations in ASD_STS and ASD_PAC, respectively (dark red, $r_S = +1$; dark blue, $r_S = -1$). Scatter plots for the top 2 positive age-correlating mature micro-RNAs (miR-320a, miR-345) and the top 2 negative age-correlating micro-RNAs (miR-378b, miR-4299) for STS are shown on the left. Scatter plots for the top 2 positive age-correlating micro-RNAs (miR-4487, miR-4725-3p) and the top 2 negative age-correlating micro-RNAs (miR-132, miR-432) for PAC are shown on the right. Micro-RNAs displayed in scatter plots are underlined in heat maps. For the scatter plots, micro-RNA expression is on the Y-axis, age is on the X-axis (4-40 years of age), males are indicated by black dots, and females by gray dots.

disorders, bipolar disorders, obsessive-compulsive disorders, panic disorders, dissociative disorders) and neurodevelopmental disorders (autism spectrum disorders, Angelman syndrome, Asperger's syndrome, developmental delay, Fragile X syndrome, Prader-Willi syndrome, Rett syndrome, Tourette syndrome, tuberous sclerosis, microcephaly, macrocephaly, Timothy syndrome). Enrichment was tested using the hypergeometric probability function *phyper* in R, with the population size set to 20 687 (all protein-coding genes in the human genome).[38] A Bonferroni correction for multiple comparisons was considered significant (Bonferroni $P < .05$, $-\log_{10}$[Bonferroni-corrected P] >1.3) (Figure 7).

Nervous System Functions Common to Micro-RNA Targets and SFARI Database

The 11 mature micro-RNA that were differentially expressed between STS and PAC in typical subjects (see results below) had 844 putative messenger RNA targets (TargetScan). Of these 844 messenger RNA targets, a total of 40 genes overlapped with the 573 autism-implicated genes from the SFARI AutDB database ($P < .05$). These 40 genes were used as an input into the Ingenuity Pathway Analysis Knowledge Base and the top 10 enriched nervous system functions were identified with a $P < .05$ (Figure 8).

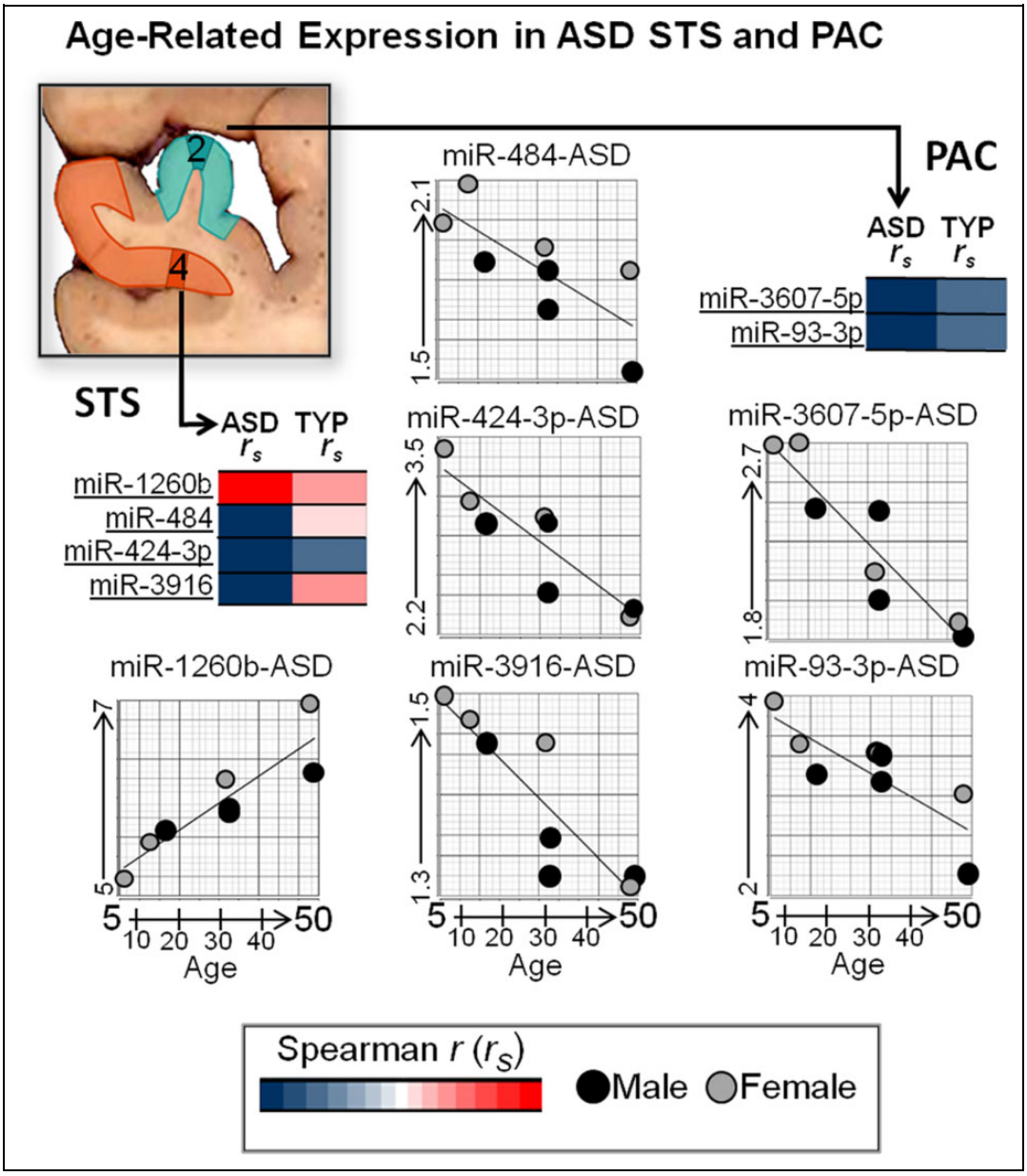

Figure 4. Mature micro-RNAs that change expression with age in superior temporal sulcus (STS) and primary auditory cortex (PAC) of autism spectrum disorders (ASD) subjects. The STS (ASD_STS) is shown in the left 2 panels and PAC (ASD_PAC) on the right. The heat maps represent the strength of the age-related association with the expression levels of the regulated micro-RNAs in ASD_STS or ASD_PAC (Spearman rank correlation, r_S values) and their corresponding associations in TYP_STS and TYP_PAC, respectively (dark red, $r_S = +1$; dark blue, $r_S = -1$). Scatter plots on the left are shown for the one positive age-correlating and 3 negative age-correlating mature micro-RNA in ASD STS, and the scatter plots on the right are shown for the 2 negatively correlating mature micro-RNA in ASD PAC. For the scatter plots, micro-RNA expression is on the Y-axis, age is on the X-axis (5-50 years of age), males are indicated by black dots, and females by gray dots. TYP. typically developing brains.

Results

Subject Characteristics

Demographic and clinical characteristics of the subjects are listed in Table 1. There were 12 typically developing brain samples (6 STS and 6 PAC) from subjects 26.7 $\pm$ 14.4 years of age (range 4-40 years), of whom 66.6% were male. There were 16 ASD samples (8 STS and 8 PAC) from subjects 27.4 $\pm$ 16.6 years of age (range 5-50 years) of whom 50% were male. There were no significant differences ($P > .05$) in age, postmortem interval or sex between the typically developing control and ASD groups. Additional information on subject medication/drug use, comorbidities, cause of death, and agonal state were limited and therefore not included in analyses.

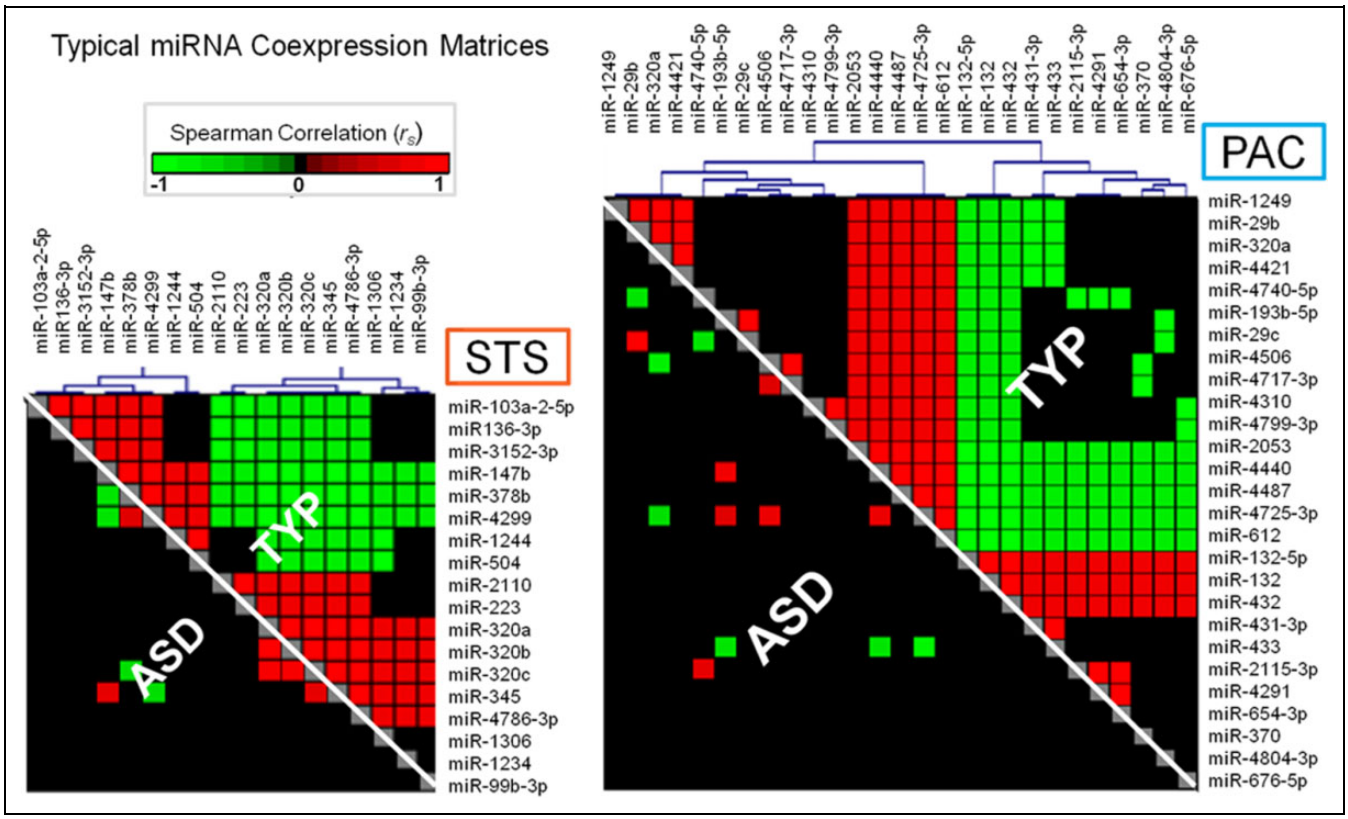

Figure 5. Coexpression relationships for micro-RNAs that changed expression as a function of age in the superior temporal sulcus (STS) (left panel) and in the primary auditory cortex (PAC) (right panel). The coexpression relationships for mature micro-RNA in typical subjects are above the white diagonal line for STS and PAC. The coexpression relationships for the corresponding mature micro-RNA in autism spectrum disorders (ASD) subjects are below the white diagonal line for STS and PAC. Bright red = Spearman rank correlation coefficient of +1; bright green = Spearman rank correlation coefficient of –1. Nonsignificant correlations ($P \geq .005$ were set to 0, black). Most of the coexpression relationships observed in typically developing brains are absent in ASD for both STS and PAC.

Regional Differences of Small Noncoding RNA Expression Between STS and PAC

Typical subjects. In typically developing control brains, there were 22 small noncoding RNAs differentially expressed between STS and PAC ($P < .005$, |fold change| >1.2) (Table 2) in the superior temporal gyrus. Principal components analysis and unsupervised hierarchical clustering of the 22 small noncoding RNAs demonstrated good separation of STS and PAC in typically developing brains (Figure 1). Eleven of the 22 small noncoding RNAs that differed in typical STS compared to PAC were mature micro-RNA, 3 were stem-loop precursor micro-RNA, and 8 were small nucleolar RNA (Table 2).

Autism spectrum disorders subjects. In ASD brains, there were 11 small noncoding RNAs differentially expressed between the STS and PAC ($P < .005$, |fold change| >1.2) (Table 2). Principal components analysis and unsupervised hierarchical clustering of the 11 small noncoding RNAs demonstrated good separation of STS and PAC in ASD brains (Figure 2). Three of the 11 small noncoding RNA that differed in ASD STS compared to the PAC were mature micro-RNA, 4 were stem-loop precursor micro-RNA, and 4 were small nucleolar RNA (Table 2). None of the small noncoding RNA differentially expressed between the STS and PAC in ASD brains were the same as those differentially expressed in typically developing control brains (Table 2). There were only upregulated mature micro-RNAs in ASD (expressed higher in STS than in PAC), whereas there were upregulated and downregulated micro-RNAs (expressed lower in STS compared to PAC) in typically developing brains.

Age-Related Changes of Small Noncoding RNA Expression in STS

Typical subjects' STS. In the STS of typically developing brains, a total of 48 small noncoding RNA changed expression as a function of age ($P < .005$) (Table 3). Eighteen of the 48 were mature micro-RNA, of which 10 increased expression with age and 8 decreased expression with age in STS (Tables 3 and 5). Figure 3 (left panels) shows the strength of the association of the expression of these 18 mature micro-RNAs with age in typically developing brains compared to that in ASD subjects (heat map shows Spearman rank correlation coefficients, r_S: dark red, +1, dark blue, –1). Note that most positively correlated

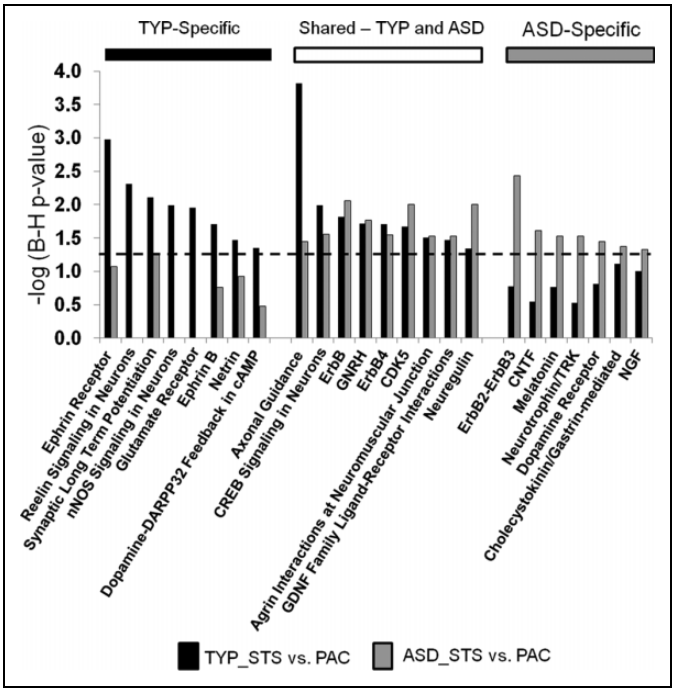

Figure 6. Enrichment of nervous system pathways that are targets of regulated micro-RNA in typically developing (TYP) and autism spectrum disorders (ASD) brains. The messenger RNA targets for the 11 mature micro-RNA (Table 2) differentially expressed in typical STS compared to primary auditory cortex (PAC) were determined using TargetScan. Similarly, the messenger RNA targets for the 3 mature micro-RNA (Table 2) differentially expressed in ASD STS compared to PAC were determined using TargetScan. Both sets of messenger RNA targets were inputed into Ingenuity Pathway Analysis and significantly regulated pathways identified. For the figure, significant pathways are above the thin black line (Benjamini-Hochberg [B-H]–corrected $P < .05$, corresponding to $-\log_{10}$[B-H P value] >1.3). Black bars indicate typical STS versus PAC pathways; and grey bars indicate ASD STS versus PAC pathways. Note that there are pathways common to TYP and ASD, as well as pathways specific for TYP subjects and specific for ASD subjects.

micro-RNA in typically developing brains were also positively correlated in ASD except that their r_S were not significant; and most negatively correlated micro-RNA in typically developing brains were also negatively correlated in ASD except that their r_S were not significant. Figure 3 also shows scatter plots (left panels) of the top 2 positive associations with age (miR-320a, miR-345) and the top 2 negative associations with age (miR-378b, miR-4299) in typically developing brain STS for males (black dots) and females (gray dots).

Autism spectrum disorders subjects' STS. In the STS of ASD brains, a total of 13 small noncoding RNA changed expression as a function of age ($P < .005$) (Table 3), with 4 being mature micro-RNA, of which 1 increased expression with age and 3 decreased expression with age (Tables 3 and 5). Figure 4 (left panels) shows the strength of the association of the expression of these micro-RNAs with age in ASD versus typically developing brains (heat map shows Spearman rank correlation coefficients: dark red, +1, dark blue, –1). Two of the negatively correlated micro-RNA in ASD were positively correlated in typically developing brains (though their r_S were not significant). Figure 4 also shows the scatter plots (left 2 panels) of the positive association with age for miR-1260B and negative associations with age for miR-484, miR-424-3p, and miR-3916 in ASD STS for males (black dots) and females (gray dots).

Age-Related Differential Small Noncoding RNA Expression in PAC

Typical subjects PAC. In the PAC of typical brains, a total of 71 small noncoding RNA changed expression as a function of age ($P < .005$) (Table 4), with 27 being mature micro-RNA, of which 16 increased expression with age and 11 decreased expression with age in typically developing brain PAC (Tables 4 and 5). Figure 3 (right panels) shows the strength of the association of the expression of the 27 micro-RNAs with age in typically developing brains compared to ASD (heat map shows Spearman rank correlation coefficients: dark red, +1; dark blue, –1). Several of the micro-RNA positively correlated with age in typically developing brains were negatively correlated in ASD (though their r_S were not significant). One of the negatively correlated micro-RNA in typically developing brains was positively correlated with age in ASD (though its r_S was not significant). Figure 3 also shows scatter plots (far right panels) of the top 2 positive associations with age (miR-4487, miR-4725-3p) and the top 2 negative associations with age (miR-132, miR-432) in typical PAC for males (black dots) and females (gray dots).

Autism spectrum disorders subjects PAC. In the PAC of ASD brains, a total of 5 small noncoding RNA changed expression as a function of age ($P < .005$) (Table 4), with 2 of the 5 being

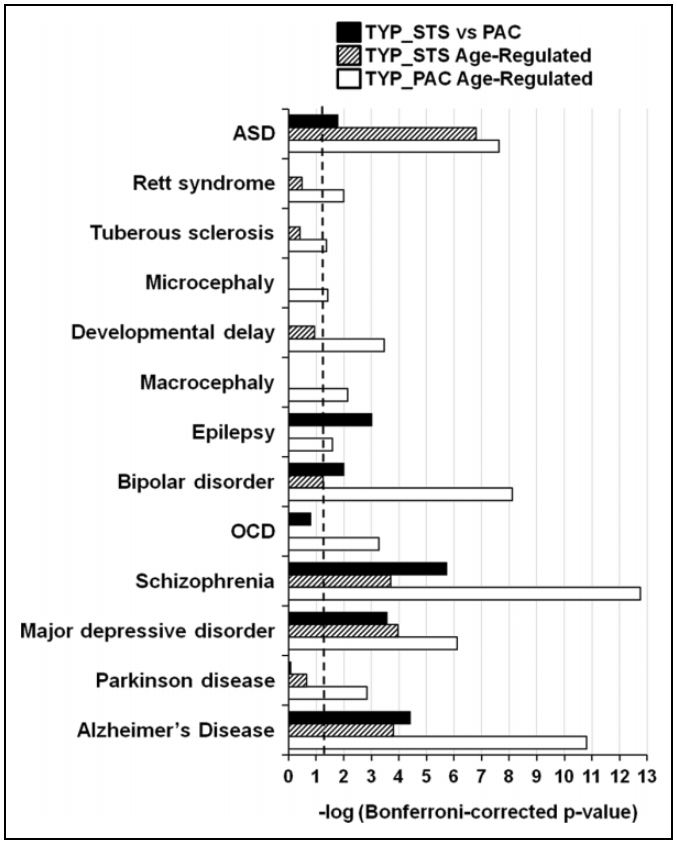

Figure 7. Enrichment in putative messenger RNA targets of the regionally and age-regulated mature micro-RNAs in typical brains that differ from autism spectrum disorders (ASD) brains and are associated with specific diseases. For this analysis, we determined whether the putative messenger RNA targets for the 11 micro-RNA in the TYP_STS versus PAC analysis (black bars), the 18 micro-RNA in the TYP_STS age-related analysis (cross-hatched bars), and the 27 micro-RNA for the TYP_PAC age-related analysis (white bars) were significantly enriched in messenger RNA gene sets from 22 different diseases. The figure shows only 11 diseases for which the Bonferroni-corrected *P* was <.05 ($\log_{10}$[Bonferroni-corrected *P*] >1.3; dotted line) for at least one of the regional- or age-related analyses.

mature micro-RNA, both of which decreased expression with age in ASD_PAC (Tables 4 and 5). Figure 4 (far right panels) shows the strength of the association of the level of these 2 micro-RNAs with age in ASD compared to typically developing brains, as well as the scatter plots of these negative associations for miR-3607-5p and miR-93-3p in ASD_PAC for males (black dots) and females (gray dots).

Coexpression Analyses of Micro-RNA that Changed With Age in STS and PAC

STS. The coexpression analysis of the age-associated mature micro-RNA in the STS of typical subjects revealed modules of negative (green) and positive (red) associations in typically developing subjects (Figure 5, left panel, triangle above diagonal). In contrast, the coexpression analysis yielded quite different results in the STS of ASD subjects with apparent loss of most of the significant negative and positive associations in ASD subjects (Figure 5, left panel, triangle below diagonal).

PAC. Similarly, the coexpression analysis of the age-associated micro-RNA in the PAC revealed modules of negative (green) and positive (red) coexpression relationships in the age-associated mature micro-RNAs in the PAC of typical controls (Figure 5, right panel, triangle above diagonal). In contrast, the coexpression pattern is different in the PAC of ASD subjects with apparent loss of most of the significant negative and positive associations in ASD subjects (Figure 5; right panel, triangle below diagonal).

Overlap of Micro-RNA in This Study With Previous Autism Spectrum Disorders Micro-RNA Studies

The overlap of our findings with dysregulated micro-RNAs reported in previous ASD studies is shown in Table 6.[39-42] We found 58 mature micro-RNAs differentially expressed by region or age in the STS or PAC either in typically developing brains or in ASD. Of these, 11 micro-RNAs or their gene family members have been reported to be dysregulated in ASD cerebellar cortex[39] and/or ASD lymphoblastoid cell lines[40-42] (Table 6). Seven of the 11 micro-RNAs (miR-93, miR-132, miR-193, miR-320, miR-431, miR-432, miR-484) have been reported to be significantly differentially expressed in subsets of ASD brain samples of cerebellar cortex compared to the non-ASD control set (out of their 28 significant micro-RNAs, with 26 unique family IDs, *P* of overlap = .0002) (Table 1, from Abu-Elneel et al[39]). Five micro-RNAs were differentially expressed in the lymphoblastoid cell lines of affected versus unaffected monozygotic twins discordant for ASD diagnosis or ASD severity (out of their 43 significant micro-RNAs, with 39 unique micro-RNA family IDs, *P* of overlap = .046) (Table 1, from Sarachana et al[40]). Two micro-RNAs (miR-132, miR-320) were differentially expressed in lymphoblastoid cell lines between ASD and controls (out of their 9, with 7 unique micro-RNA family IDs, *P* of overlap = .047) (Table 1, from Talebizadeh et al[42]). One micro-RNA (miR-548) was differentially expressed in the lymphoblastoid cell lines between ASD–affected siblings versus unaffected siblings (out of their 12 significant micro-RNAs, with 10 unique micro-RNA family IDs, *P* of overlap = .413, nonsignificant) (Table 2, from Ghahramani Seno et al[41]).

Messenger RNA Target Pathways of Differentially Expressed Micro-RNAs for the Superior Temporal Sulcus Versus the PAC

There were 11 micro-RNAs in typical brains that were differentially expressed between the STS and PAC (Table 2), which had a total of 844 predicted messenger RNA targets (TargetScan). In contrast, there were only 3 differentially expressed micro-RNAs in the ASD brain between the STS and the PAC (Table 2), which had a total of 51 predicted messenger RNA targets. Based on these messenger RNA targets, biological

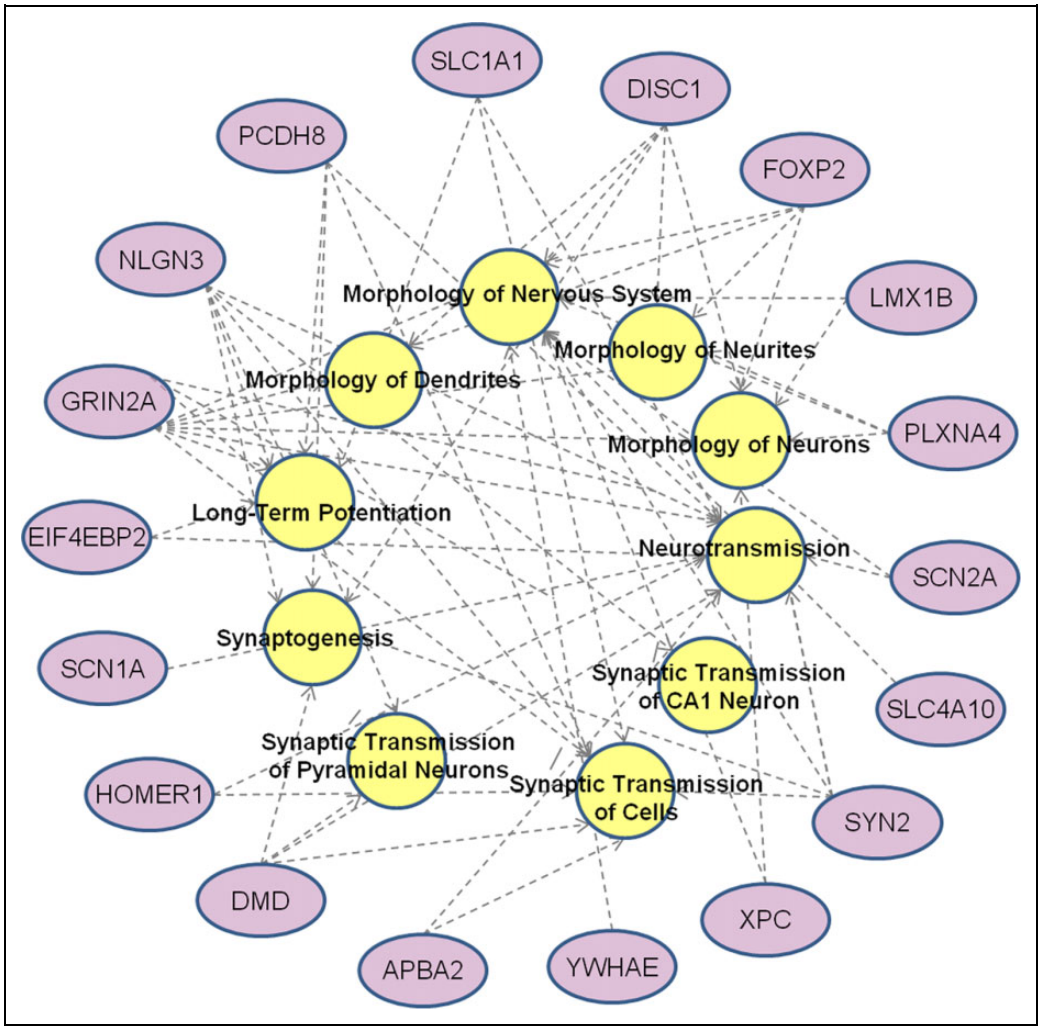

Figure 8. Top 10 enriched nervous system–related biological functions among the genes overlapping between the micro-RNA targets in typically developing regional analysis and autism-implicated genes. Of the 844 messenger RNA targets of the 11 mature micro-RNA (Table 2) in the typical superior temporal sulcus (STS) versus primary auditory cortex (PAC) analysis, there were 40 genes that overlapped with the 573 autism-implicated genes from the SFARI AutDB database ($P < .05$) (data provided on request). These 40 genes were inputed into an Ingenuity Pathway Analysis to yield the top 10 nervous system–related functions shown in light yellow ($P < .05$). Of the 40 genes, 18 genes (shown in light purple) were most associated with these top 10 functions.

processes for neuron function, synaptic transmission, and transcription were enriched in typical brains, but not in ASD brains (unpublished data, available on request).

To provide one example of the effect of the dysregulated micro-RNA on functional pathways, we compared the messenger RNA targets for micro-RNA differentially expressed in typically developing brain STS versus PAC and the messenger RNA targets for micro-RNA differentially expressed for ASD STS versus PAC for nervous system functional pathways (Figure 6). The canonical pathways derived from those messenger RNA targets showed some pathways specific for typical brain, some specific for ASD brain, and some pathways shared by both (Figure 6). Note that the dysregulated micro-RNA in ASD brain would result in dysregulation of pathways such as ephrin receptor, ephrin B, netrin, and reelin signaling in the ASD brain (Figure 6, typically developing brain–specific), and aberrant regulation of pathways, such as NGF, neurotrophin, CNTF, and melatonin signaling (Figure 6, ASD-Specific). In spite of these many abnormalities there are a number of pathways shared between typically developing and ASD subjects (Figure 6)—even though the micro-RNA implicated in the shared pathways were different for typically developing compared to ASD subjects (Tables 4 and 5).

Putative Messenger RNA Targets of Differentially Expressed Micro-RNA Implicated in Disease

We next showed that target genes of the micro-RNA that varied as a function of region and age in typically developing brains but not in ASD (and were therefore dysregulated in ASD) have been implicated in a variety of diseases (Figure 7). Both region- and age-related micro-RNA target

Table 6. Overlap of the Micro-RNA Regulated in This Study Compared With Other Studies of ASD Micro-RNA Transcriptomes.

STS, PAC ASD, TYP this study	Cerebellar cortex ASD vs control[39]	LCL ASD vs control[40]	LCL ASD vs control[42]	LCL ASD vs control[41]
miR-93-3p	miR-93	miR-93		
miR-132	miR-132	miR-132	miR-132	
miR-132-5p				
miR-193b-5p	miR-193b			
miR-320a	miR-320a		miR-320	
miR-320b				
miR-320c				
miR-431-3p	miR-431			
miR-432	miR-432			
miR-484	miR-484			
miR-29b		miR-29b		
miR-29c				
miR-103a-2-5p		miR-103		
miR-136-3p		miR-136		
miR-548ab				miR-548o

Abbreviations: ASD, autism spectrum disorders; LCL, lymphoblastoid cell lines; PAC, primary auditory cortex; STS, superior temporal sulcus; TYP, typically developing controls.

genes in superior temporal gyrus of typically developing brains have been implicated in ASD (Figure 7). However, targets of the ASD–dysregulated micro-RNAs are not specific for ASD–associated genes, because they are associated with many other disorders (Figure 7). Significant associations for typically developing brain STS versus PAC, TYP_STS age-related, and TYP_PAC age-related are obtained for ASD–implicated genes, as well as schizophrenia, manic depressive disorders, and Alzheimer's disease (Figure 7).

Messenger RNA Targets of Dysregulated Micro-RNA Overlap With Autism-Implicated Genes

There were micro-RNAs differentially regulated in STS compared to the PAC of typical subjects that had messenger RNA target genes that are implicated in ASD. These micro-RNAs are important for ASD because they were regulated in typical but not ASD brains. Of the 844 messenger RNA targets of the 11 micro-RNAs (Table 2) in the typical STS versus PAC analysis, there were 40 genes that overlapped with the 573 autism-implicated genes from the SFARI AutDB database ($P < 0.05$) (data provided on request). These 40 genes were inputted into an Ingenuity Pathway Analysis to yield the top 10 nervous system functions ($P < .05$) (Figure 8). The 18 genes most associated with these top 10 functions are also shown (Figure 8). Among the 18 genes were those involved in neurotransmission (hypergeometric probability of overlap $P = 2.4 \times 10^{-9}$), including neuroligin 3, Homer homolog 1, ionotropic glutamate receptor, synapsin 2, voltage-gated sodium channels, protocadherin 8, and eukaryotic translation initiation factor 4E–binding protein 2 (Figure 8). Glutamate receptor signaling was also specifically enriched ($P = .01$).

Similarly, there were also age-regulated micro-RNAs in STS and PAC of typical subjects which had messenger RNA targets implicated in ASD. These micro-RNAs are important for ASD because they were regulated in typical but not ASD brains. For the 18 micro-RNAs regulated as a function of age in typical STS (Table 5), these had 1591 messenger RNA targets (TargetScan), of which 83 were shared with the 573 autism-implicated genes from the SFARI AutDB database ($P < .05$). For the 27 micro-RNA regulated as a function of age in typical PAC but not ASD PAC (Table 5), these had 2867 messenger RNA targets (TargetScan), of which 169 were shared with the 573 autism-implicated genes from the SFARI AutDB database ($P < .05$) (data for all of the above analyses provided on request).

Discussion

As we postulated, specific expression patterns of micro-RNA and other small noncoding RNA differ significantly between STS and PAC within normal human superior temporal gyrus and change significantly with age. In contrast, there were fewer differences of micro-RNA / small noncoding RNA between STS and PAC in ASD brain, and there was loss of the typical age-related changes. Given that micro-RNAs/ small noncoding RNAs regulate many messenger RNA targets, this likely contributes to the attenuated messenger RNA expression patterns recently reported between regions in ASD brain compared to typical brains.[11,13] Moreover, a number of micro-RNA gene targets regulated in the STS and PAC of typical but not ASD subjects are ASD candidate genes.[36] The lack of differentiation in micro-RNA-mediated transcriptional changes in ASD brain would affect axon guidance, dendritic growth, synaptic plasticity, neurotransmission, and other processes that likely contribute to ASD pathophysiology.[43,44]

Role of Micro-RNA/Small Noncoding RNA in Typically Developing Superior Temporal Gyrus

Recent whole genome studies of human brain show tremendous brainwide variation in differential gene expression and coexpression relationships.[1] Though the neocortex displays a relatively homogeneous transcriptional pattern, there are distinct transcriptome signatures associated with primary sensorimotor cortices including the PAC.[1] Given the many micro-RNA and other small noncoding RNA differentially expressed in the PAC compared to STS in this study as a function of brain region and age, and the fact that micro-RNA regulate dozens to a few hundred messenger RNA targets each, it is likely that the micro-RNA/ small noncoding RNA differences in the PAC and STS observed in this study explain many of the transcriptional messenger RNA differences observed in typically developing superior temporal gyrus reported in other studies.[1] Region-specific

micro-RNA/ small noncoding RNA likely regulate cascades of genes that mediate either specific cognitive functions in STS or auditory processing in the PAC and may also have region-specific roles in learning and memory.[4,45-47]

Attenuated Regional and Age-Related Micro-RNA / Small Noncoding RNA in ASD Superior Temporal Sulcus and Primary Auditory Cortex

The small noncoding RNA and micro-RNA expression patterns in the STS and PAC discussed above for typical human brains were markedly attenuated in ASD brains. If one considers the predicted brain messenger RNA targets of the mature micro-RNAs in ASD compared to typical brains, this translates to 16 times fewer putative targets in the STS and PAC of ASD (51 targets) compared to typical (844 targets) brains. Notably, fewer messenger RNA and long noncoding RNA differences have been reported among ASD brain regions. Regional messenger RNA differences between the frontal and temporal cortex are attenuated in ASD brain,[11] and there are fewer messenger RNA and long noncoding RNA differences between the prefrontal cortex and cerebellum of ASD brain compared to controls.[11,13] It is possible that decreased numbers of regulated messenger RNA in the ASD cortex may be related to decreased numbers of regulated micro-RNA. Alternatively, these results may be due to general dysregulation of transcription.

In addition, fewer small noncoding RNAs / micro-RNAs changed expression with age in ASD compared to typical brains. Previous studies show that many micro-RNAs are regulated in an age-dependent manner in normal human brains.[8,48] We found that several more small noncoding RNAs / micro-RNAs changed with age in typical STS compared to ASD STS. Given STS is a primary component of the ''social brain''[17] and there are structural changes in the temporal lobe of ASD subjects,[49] the aberrant attenuated micro-RNA expression in ASD STS suggests aberrant molecular functions that likely contribute to core social abnormalities in ASD.

Surprisingly, dysregulation of small noncoding RNAs in ASD was not limited to STS association cortex. The PAC in ASD also showed decreased numbers of age-regulated micro-RNA and drastically altered coexpression modules compared to typical development. Though ASD is not usually thought to be associated with sensory abnormalities, ASD subjects often have auditory, visual, and tactile hyperreactivity or hyporeactivity.[50,51] Moreover, enhanced auditory perception and acoustic startle response to weak stimuli are thought to contribute to atypical auditory processing in ASD[52] and is reported to interrupt behavioral adaptation.[53] Though it is unclear whether molecular changes in the PAC contribute to core ASD symptoms, the PAC is interconnected with STS and other social brain regions and thus could contribute to circuit dysfunction in ASD. Moreover, age-related attenuation of small noncoding RNA responses in ASD PAC suggests lifelong abnormalities in superior temporal gyrus circuitry.

Role of Specific Micro-RNA in Superior Temporal Gyrus of Typical and ASD Brains

There were 2 classes of ASD–related, dysregulated small noncoding RNA/micro-RNA identified in this study: (1) those that were regionally or age regulated in typical human brain but not in ASD brain and (2) those that were regionally or age regulated in ASD but not in typical brain. The first class would result in failure to regulate typical messenger RNA targets in ASD brain, and the second class would result in aberrant regulation of messenger RNA targets in ASD brain. Both would contribute to abnormal molecular pathology in ASD, though it is possible that the second class contributes to compensatory functions.

Though all of the dysregulated small noncoding RNA, including micro-RNA, reported in this study would bind a number of target messenger RNA/genes and regulate their function, the specific functions of only a few have been identified in the brain. Indeed, all of the micro-RNA reported here will require further study in cellular and animal models to assess their role in normal brain. To date, only miR-132, miR-103, miR-320, and miR-378—the family members of which are dysregulated in this study—have been studied in the human brain. Each is discussed briefly below.

miR-132 decreases with age in the PAC of typical brains and has an established role in brain development and disease.[44,54,55] It is regulated by neuronal activity and neurotrophins, it increases dendritic spine density and size, and promotes mature spine morphology.[56-59] miR-132 regulates dendritic spine morphology in an experience-dependent manner to maintain plasticity.[60-62] Thus, miR-132 could relate to the alterations in dendritic spine density reported in ASD brain.[63,64]

Though miR-132 decreases with age in the PAC in the typical human brain, it does not in ASD brain. Altered miR-132 expression is reported in the cerebellum and lymphoblastoid cell lines in ASD and in the prefrontal cortex in schizophrenia.[39,40,42] Moreover, miR-132 regulates the *MeCP2* gene, which is mutated in Rett syndrome, an X-linked disorders with shared features of, and often comorbid with, ASD.[65] These findings suggest that miR-132 is required for brain plasticity, and its dysregulation could contribute to aberrant cortical maturation seen in the ASD brain.[49,66]

miR-103a-2-5p is downregulated with age in typical STS in this study, and miR-103 is downregulated with age in typical human dorsolateral prefrontal cortex.[48] miR-103, along with other micro-RNA, is involved in fine-tuning of neuronal migration.[67] The normal downregulation of miR-103a-2-5p with age in the STS does not occur in ASD brain. miR-103 is differentially expressed in lymphoblastoid cell lines of monozygotic twins discordant for ASD.[40] miR-103 targets are enriched in the genomic regions of long noncoding RNAs that are dysregulated in ASD brain.[13] This may contribute to aberrant neuronal connectivity in ASD circuits,[68] and, along with its role in neuronal migration, suggests miR-103 plays a potential role in ASD neuropathology.

miR-320 family members are found to increase with age in typical human STS and PAC in this study, whereas

micro-RNA-320 decreases with age in the prefrontal cortex.[8] miR-320 induces neurite outgrowth, increases neurite length, decreases proliferation of Neuro-2A (N2A) cells,[69] responds to oxidative stress, and regulates glycolysis.[70] Because miR-320 does not increase with age in ASD as it does in typical superior temporal gyrus, this might relate to ASD developmental abnormalities and perhaps to mitochondrial dysfunction reported in ASD.[71]

miR-378b expression in typical human brains decreases with age in STS, and hp_mir-378 (stem-loop precursor) is overexpressed in the STS versus PAC. miR-378 together with other micro-RNAs modulates learning, memory, and exploration behavior.[72] Because age-related changes of miR-378b are not observed in ASD STS, miR-378b could contribute to ASD pathophysiology as well.[73]

Role of Small Nucleolar RNAs in Superior Temporal Gyrus of Typical and ASD Brains

Other classes of small noncoding RNA displayed regional and age-related regulation in typical brain including small nucleolar RNAs, such as HBII-48 and HBII-52. Their rodent homologs, MBII-48 and MBII-52, affect learning and memory in mice.[74,75] HBII-52 regulates 5-HT2C receptor subunit messenger RNA and loss of function of MBII-52 increased serotonin 2C receptor pre-RNA editing and altered 5HTR-2C-mediated behavior.[76] These small nucleolar RNAs were dysregulated in ASD brains in this study, and could relate to studies that have reported 5HT abnormalities in ASD.[77]

HBII-48 and HBII-52 (SNORDs115) are both located on chromosome 15q11.2, which is notable since maternally inherited duplications involving chromosomal region 15q11–15q13 are among the most common chromosomal abnormalities in ASD.[78] In addition, small nucleolar RNA MB-II-52 regulates alternative splicing,[79,80] which is of interest because abnormalities of alternative splicing have been reported in the brain[11] as well as blood[31] of patients with ASD.

Conclusions

Here we found that in 2 structurally and functionally distinct regions of the superior temporal gyrus, STS, and PAC, there are differential expression patterns of small noncoding RNAs. These specific expression patterns, in addition to appropriate coexpression of micro-RNAs, for different brain regions as well as changes with age are required to shape a typical human brain and produce neurotypical function throughout the life span. These patterns were not observed in ASD brains, likely contributing to dysregulation of messenger RNA and consequently alterations in brain structure and function. If fact, the micro-RNA families found to be dysregulated in the current study have previously been associated with neurodevelopmental disorders. Aberrant small noncoding RNA expression patterns were not limited to the region commonly associated with ASD social impairments, the STS, but also present in a primary sensory region, the PAC. The lack of differential regional and age-related small noncoding RNA expression patterns in ASD brain support findings of attenuated messenger RNA expression profiles, which consequently would profoundly alter typical human brain development and function. Future studies will need to confirm these findings in a larger sample size with high-quality brain tissue, as well as assessment of small noncoding RNA and messenger RNA in the same human brain samples.

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Declaration of Conflicting Interests

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References

1. Hawrylycz MJ, Lein ES, Guillozet-Bongaarts AL, et al. An anatomically comprehensive atlas of the adult human brain transcriptome. *Nature*. 2012;489:391-399.
2. Miller JA, Ding SL, Sunkin SM, et al. Transcriptional landscape of the prenatal human brain. *Nature*. 2014;508:199-206.
3. Geschwind DH, Konopka G. Neuroscience in the era of functional genomics and systems biology. *Nature*. 2009;461:908-915.
4. Saab BJ, Mansuy IM. Neuroepigenetics of memory formation and impairment: the role of microRNAs. *Neuropharmacology*. 2014; 80:61-69.
5. Ziats MN, Rennert OM. Identification of differentially expressed microRNAs across the developing human brain. *Mol Psychiatry*. 2014;19:848-852.
6. Shao NY, Hu HY, Yan Z, et al. Comprehensive survey of human brain microRNA by deep sequencing. *BMC Genomics*. 2010;11: 409.
7. Hu HY, Guo S, Xi J, et al. MicroRNA expression and regulation in human, chimpanzee, and macaque brains. *PLoS Genet*. 2011;7: e1002327.
8. Somel M, Guo S, Fu N, et al. MicroRNA, mRNA, and protein expression link development and aging in human and macaque brain. *Genome Res*. 2010;20:1207-1218.
9. Somel M, Liu X, Tang L, et al. MicroRNA-driven developmental remodeling in the brain distinguishes humans from other primates. *PLoS Biol*. 2011;9:e1001214.
10. Im HI, Kenny PJ. MicroRNAs in neuronal function and dysfunction. *Trends Neurosci*. 2012;35:325-334.

11. Voineagu I, Wang X, Johnston P, et al. Transcriptomic analysis of autistic brain reveals convergent molecular pathology. *Nature*. 2011;474:380-384.
12. Chow ML, Pramparo T, Winn ME, et al. Age-dependent brain gene expression and copy number anomalies in autism suggest distinct pathological processes at young versus mature ages. *PLoS Genet*. 2012;8:e1002592.
13. Ziats MN, Rennert OM. Aberrant expression of long noncoding RNAs in autistic brain. *J Mol Neurosci*. 2013;49:589-593.
14. Schumann CM, Nordahl CW. Bridging the gap between MRI and postmortem research in autism. *Brain Res*. 2011;1380:175-186.
15. Allison T, Puce A, McCarthy G. Social perception from visual cues: role of the STS region. *Trends Cogn Sci*. 2000;4:267-278.
16. Redcay E. The superior temporal sulcus performs a common function for social and speech perception: implications for the emergence of autism. *Neurosci Biobehav Rev*. 2008;32:123-142.
17. Kennedy DP, Adolphs R. The social brain in psychiatric and neurological disorders. *Trends Cogn Sci*. 2012;16:559-572.
18. Achiron A, Chapman J, Tal S, Bercovich E, Gil H, Achiron A. Superior temporal gyrus thickness correlates with cognitive performance in multiple sclerosis. *Brain Struct Funct*. 2013;218: 943-950
19. Baron-Cohen S, Ring HA, Wheelwright S, et al. Social intelligence in the normal and autistic brain: an fMRI study. *Eur J Neurosci*. 1999;11:1891-1898.
20. Aeby A, De Tiege X, Creuzil M, et al. Language development at 2 years is correlated to brain microstructure in the left superior temporal gyrus at term equivalent age: a diffusion tensor imaging study. *NeuroImage*. 2013;78:145-151.
21. Barger N, Sheley MF, Schumann CM. Stereological study of pyramidal neurons in the human superior temporal gyrus from childhood to adulthood. *J Comp Neurol*. 2014;523:1054-1072.
22. Economo Cv. *Cytoarchitectonics of the Human Cerebral Cortex*. London: Oxford University Press; 1929.
23. Galaburda A, Sanides F. Cytoarchitectonic organization of the human auditory cortex. *J Comp Neurol*. 1980;190:597-610.
24. Morosan P, Rademacher J, Palomero-Gallagher N, Zilles K. Anatomical organization of the human auditory cortex: cytoarchitecture and transmitter receptors. In: Heil P, König E, Budinger E, eds. *Auditory Cortex: Towards a Synthesis of Human and Animal Research*. Mahwah, NJ: Lawrence Erlbaum; 2004:27-50.
25. Morosan P, Schleicher A, Amunts K, Zilles K. Multimodal architectonic mapping of human superior temporal gyrus. *Anat Embryol*. 2005;210:401-406.
26. Sweet RA, Dorph-Petersen KA, Lewis DA. Mapping auditory core, lateral belt, and parabelt cortices in the human superior temporal gyrus. *J Comp Neurol*. 2005;491:270-289.
27. Da Costa S, van der Zwaag W, Marques JP, Frackowiak RS, Clarke S, Saenz M. Human primary auditory cortex follows the shape of Heschl's gyrus. *J Neurosci*. 2011;31:14067-14075.
28. Fullerton BC, Pandya DN. Architectonic analysis of the auditory-related areas of the superior temporal region in human brain. *J Comp Neurol*. 2007;504:470-498.
29. Thompson WA Jr. The problem of negative estimates of variance components. *Ann Math Stat*. 1962;33:273-289.
30. Tamhane AC, Dunlop DD. *Statistics and Data Analysis: From Elementary to Intermediate*. Upper Saddle River, NJ: Prentice Hall; 2000:473-474.
31. Stamova BS, Tian Y, Nordahl CW, et al. Evidence for differential alternative splicing in blood of young boys with autism spectrum disorders. *Mol Autism*. 2013;4:30.
32. Sturn A, Quackenbush J, Trajanoski Z. Genesis: cluster analysis of microarray data. *Bioinformatics*. 2002;18:207-208.
33. Liu DZ, Ander BP, Tian Y, et al. Integrated analysis of mRNA and microRNA expression in mature neurons, neural progenitor cells and neuroblastoma cells. *Gene*. 2012;495:120-127.
34. Huang da W, Sherman BT, Lempicki RA. Systematic and integrative analysis of large gene lists using David bioinformatics resources. *Nat Protoc*. 2009;4:44-57.
35. Huang da W, Sherman BT, Lempicki RA. Bioinformatics enrichment tools: paths toward the comprehensive functional analysis of large gene lists. *Nucleic Acids Res*. 2009;37:1-13.
36. Basu SN, Kollu R, Banerjee-Basu S. Autdb: a gene reference resource for autism research. *Nucleic Acids Res*. 2009;37: D832-D836.
37. Wall DP, Pivovarov R, Tong M, et al. Genotator: a disease-agnostic tool for genetic annotation of disease. *BMC Med Genom*. 2010;3:50.
38. Encode PC. An integrated encyclopedia of DNA elements in the human genome. *Nature*. 2012;489:57-74.
39. Abu-Elneel K, Liu T, Gazzaniga FS, et al. Heterogeneous dysregulation of microRNAs across the autism spectrum. *Neurogenetics*. 2008;9:153-161.
40. Sarachana T, Zhou R, Chen G, Manji HK, Hu VW. Investigation of post-transcriptional gene regulatory networks associated with autism spectrum disorders by microRNA expression profiling of lymphoblastoid cell lines. *Genome Med*. 2010;2:23.
41. Ghahramani Seno MM, Hu P, Gwadry FG, et al. Gene and miRNA expression profiles in autism spectrum disorders. *Brain Res*. 2011;1380:85-97.
42. Talebizadeh Z, Butler MG, Theodoro MF. Feasibility and relevance of examining lymphoblastoid cell lines to study role of microRNAs in autism. *Autism Res*. 2008;1:240-250.
43. Qureshi IA, Mehler MF. Emerging roles of non-coding RNAs in brain evolution, development, plasticity and disease. *Nat Rev Neurosci*. 2012;13:528-541.
44. Mellios N, Sur M. The emerging role of microRNAs in schizophrenia and autism spectrum disorders. *Front Psychiatry*. 2012; 3:39.
45. Rudenko A, Tsai LH. Epigenetic modifications in the nervous system and their impact upon cognitive impairments. *Neuropharmacology*. 2014;80:70-82.
46. Olde Loohuis NF, Kos A, Martens GJ, Van Bokhoven H, Nadif Kasri N, Aschrafi A. MicroRNA networks direct neuronal development and plasticity. *Cell Mol Life Sci*. 2012;69: 89-102.
47. Follert P, Cremer H, Beclin C. MicroRNAs in brain development and function: a matter of flexibility and stability. *Front Mol Neurosci*. 2014;7:5.
48. Beveridge NJ, Santarelli DM, Wang X, et al. Maturation of the human dorsolateral prefrontal cortex coincides with a dynamic

shift in microRNA expression. *Schizophr Bull*. 2014;40: 399-409.
49. Schumann CM, Bloss CS, Barnes CC, et al. Longitudinal magnetic resonance imaging study of cortical development through early childhood in autism. *J Neurosci*. 2010;30: 4419-4427.
50. Gomes E, Pedroso FS, Wagner MB. Auditory hypersensitivity in the autistic spectrum disorders. *Pro Fono*. 2008;20:279-284.
51. Marco EJ, Hinkley LB, Hill SS, Nagarajan SS. Sensory processing in autism: a review of neurophysiologic findings. *Pediatr Res*. 2011;69:48R-54R.
52. Takahashi H, Nakahachi T, Komatsu S, Ogino K, Iida Y, Kamio Y. Hyperreactivity to weak acoustic stimuli and prolonged acoustic startle latency in children with autism spectrum disorders. *Mol Autism*. 2014;5:23.
53. Lane AE, Young RL, Baker AE, Angley MT. Sensory processing subtypes in autism: association with adaptive behavior. *J Autism Dev Disord*. 2010;40:112-122.
54. Coolen M, Bally-Cuif L. MicroRNAs in brain development and physiology. *Curr Opin Neurobiol*. 2009;19:461-470.
55. Schratt G. Fine-tuning neural gene expression with microRNAs. *Curr Opin Neurobiol*. 2009;19:213-219.
56. Vo N, Klein ME, Varlamova O, et al. A camp-response element binding protein-induced microRNA regulates neuronal morphogenesis. *Proc Natl Acad Sci U S A*. 2005;102: 16426-16431.
57. Wayman GA, Davare M, Ando H, et al. An activity-regulated microRNA controls dendritic plasticity by down-regulating p250gap. *Proc Natl Acad Sci U S A*. 2008;105:9093-9098.
58. Impey S, Davare M, Lesiak A, et al. An activity-induced microRNA controls dendritic spine formation by regulating rac1-pak signaling. *Mol Cell Neurosci*. 2010;43:146-156.
59. Impey S, McCorkle SR, Cha-Molstad H, et al. Defining the CREB regulon: a genome-wide analysis of transcription factor regulatory regions. *Cell*. 2004;119:1041-1054.
60. Mellios N, Sugihara H, Castro J, et al. Mir-132, an experience-dependent microRNA, is essential for visual cortex plasticity. *Nat Neurosci*. 2011;14:1240-1242.
61. Tognini P, Putignano E, Coatti A, Pizzorusso T. Experience-dependent expression of mir-132 regulates ocular dominance plasticity. *Nat Neurosci*. 2011;14:1237-1239.
62. Nudelman AS, DiRocco DP, Lambert TJ, et al. Neuronal activity rapidly induces transcription of the CREB-regulated microRNA-132, in vivo. *Hippocampus*. 2010;20:492-498.
63. Hutsler JJ, Zhang H. Increased dendritic spine densities on cortical projection neurons in autism spectrum disorders. *Brain Res*. 2010;1309:83-94.
64. Penzes P, Cahill ME, Jones KA, VanLeeuwen JE, Woolfrey KM. Dendritic spine pathology in neuropsychiatric disorders. *Nat Neurosci*. 2011;14:285-293.
65. Klein ME, Lioy DT, Ma L, Impey S, Mandel G, Goodman RH. Homeostatic regulation of MeCP2 expression by a CREB-induced microRNA. *Nat Neurosci*. 2007;10:1513-1514.
66. Amaral DG, Schumann CM, Nordahl CW. Neuroanatomy of autism. *Trends Neurosci*. 2008;31:137-145.
67. Moncini S, Salvi A, Zuccotti P, et al. The role of mir-103 and mir-107 in regulation of cdk5r1 expression and in cellular migration. *PLoS One*. 2011;6:e20038.
68. Geschwind DH, Levitt P. Autism spectrum disorders: developmental disconnection syndromes. *Curr Opin Neurobiol*. 2007; 17:103-111.
69. White RE, Giffard RG. MicroRNA-320 induces neurite outgrowth by targeting ARPP-19. *Neuroreport*. 2012;23:590-595.
70. Tang H, Lee M, Sharpe O, et al. Oxidative stress-responsive microRNA-320 regulates glycolysis in diverse biological systems. *FASEB J*. 2012;26:4710-4721.
71. Rossignol DA, Frye RE. Mitochondrial dysfunction in autism spectrum disorders: a systematic review and meta-analysis. *Mol Psychiatry*. 2012;17:290-314.
72. Parsons MJ, Grimm CH, Paya-Cano JL, et al. Using hippocampal microRNA expression differences between mouse inbred strains to characterise miRNA function. *Mamm Genome*. 2008;19:552-560.
73. Levy SE, Mandell DS, Schultz RT. Autism. *Lancet*. 2009;374: 1627-1638.
74. Rogelj B, Giese KP. Expression and function of brain specific small RNAs. *Rev Neurosci*. 2004;15:185-198.
75. Rogelj B. Brain-specific small nucleolar RNAs. *J Mol Neurosci*. 2006;28:103-109.
76. Doe CM, Relkovic D, Garfield AS, et al. Loss of the imprinted snoRNA mbii-52 leads to increased 5htr2c pre-RNA editing and altered 5HT2CR-mediated behaviour. *Hum Mol Genet*. 2009;18: 2140-2148.
77. Gabriele S, Sacco R, Persico AM. Blood serotonin levels in autism spectrum disorders: a systematic review and meta-analysis. *Eur Neuropsychopharmacol*. 2014;24:919-929.
78. Cook EH Jr, Lindgren V, Leventhal BL, et al. Autism or atypical autism in maternally but not paternally derived proximal 15q duplication. *Am J Hum Genet*. 1997;60:928-934.
79. Kishore S, Khanna A, Zhang Z, et al. The snoRNA mbii-52 (snoRD 115) is processed into smaller RNAs and regulates alternative splicing. *Hum Mol Genet*. 2010;19:1153-1164.
80. Soeno Y, Taya Y, Stasyk T, Huber LA, Aoba T, Huttenhofer A. Identification of novel ribonucleo-protein complexes from the brain-specific snoRNA mbii-52. *RNA*. 2010;16:1293-1300.