LINE-1 and Alu methylation signatures in autism spectrum disorder and their associations with the expression of autism-related genes

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Long interspersed nucleotide element-1 (LINE-1) and Alu elements are retrotransposons whose abilities cause abnormal gene expression and genomic instability. Several studies have focused on DNA methylation profiling of gene regions, but the locus-specific methylation of LINE-1 and Alu elements has not been identified in autism spectrum disorder (ASD). Here we interrogated locus- and family-specific methylation profiles of LINE-1 and Alu elements in ASD whole blood using publicly-available Illumina Infinium 450 K methylation datasets from heterogeneous ASD and ASD variants (Chromodomain Helicase DNA-binding 8 (CHD8) and 16p11.2del). Total DNA methylation of repetitive elements were notably hypomethylated exclusively in ASD with CHD8 variants. Methylation alteration in a family-specific manner including L1P, L1H, HAL, AluJ, and AluS families were observed in the heterogeneous ASD and ASD with CHD8 variants. Moreover, LINE-1 and Alu methylation within target genes is inversely related to the expression level in each ASD variant. The DNA methylation signatures of the LINE-1 and Alu elements in ASD whole blood, as well as their associations with the expression of ASD-related genes, have been identified. If confirmed in future larger studies, these findings may contribute to the identification of epigenomic biomarkers of ASD.

Abbreviations

16p11.2del  16P11.2 deletion
3'UTR   3' Untranslated region
5'UTR   5' Untranslated region
ASD   Autism spectrum disorder
CASP1    Caspase 1
CHD8    Chromodomain helicase DNA-binding protein 8
CNV    Copy number variation
DEGs    Differentially expressed genes
DMGs    Differentially methylated genes
DMPs    Differentially methylated positions
DMRs    Differentially methylated regions
DNAm    DNA methylation

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Autism spectrum disorder (ASD) is a complex neurodevelopmental disorder characterized by two behavioral impairments: (i) deficits in social interactions and communication, and (ii) restricted interests and repetitive behaviors, according to the Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition criteria. According to the Centers for Disease Control and Prevention (CDC), the prevalence of ASD has risen dramatically over the last decade due to better screening methods. In 2018, ASD affected approximately one out of every 44 children in the United States. ASD is currently understood as a multifactorial disorder, with the precise causes remaining unknown. Over the last two decades, research has attempted to elucidate the genetic origin of the disorder. However, genetic aberration is only found in 10–20% of ASD cases. In total, more than 60% of people with ASD are idiopathic. Several studies have shown that ASD clinical phenotypic heterogeneity is influenced by a combination of genetic and environmental factors. This evidence has highlighted non-genetic factors such as epigenetics (DNA methylation (DNAm)) and environmental interactions as key players in ASD progression. Additionally, some genetic factors that increase the risk of ASD, but only a few loci have a high impact on ASD. The 16p11.2 deletion (16p11.2 del) and Chromodomain helicase DNA-binding domain 8 (CHD8) variants are high genetic risk factors for ASD. People with 16p11.2.del are usually characterized by developmental delay, intellectual disability, or ASD. CHD8 is strongly associated with ASD and other neurodevelopmental disorders including schizophrenia and intellectual disability.

Epigenetics is a family of inheritable mechanisms that elicit control of gene expression without modification to DNA sequences. Examples of epigenetic mechanisms are DNAm, RNA modification, and histone modifications. DNAm, the most frequently studied epigenetic modification, involves the addition of methyl groups to DNA. Depending on its genomic location, the addition of a methyl group to the 5th carbon atom of cytosine can have repressive or inductive effects on the gene expression. When DNAm is not properly maintained or established, methylation abnormalities can manifest in disease development. DNAm patterns are well known to show tissue-specific differentially methylated regions (DMRs). However, most loci present similar DNAm levels across a wide variety of tissue types. Interestingly, recent work has demonstrated the utility of blood as a surrogate for human brain tissue CpG methylation. Therefore, blood-based epigenetic biomarkers have the potential to serve as non-invasive biomarkers for otherwise inaccessible tissues. For instance, some epigenetic markers in blood have been identified as biomarkers in early stages of Alzheimer’s disease.

Long interspersed nucleotide element-1 (LINE-1) and Alu elements are known as non-long terminal repeat retrotransposons that can replicate and insert themselves into different locations within the host genome. LINE-1 and Alu elements make up more than 25% of the human genome and have a copy number of over one million elements. These repetitive elements (REs) can affect the expression of host or neighboring protein-coding genes through introducing alternative promoters or enhancers, novel splicing sites, and epigenetic alteration through DNAm. Subfamilies of LINE-1 and Alu elements can be subcategorized by identifying variants in their sequences that have accumulated in the evolutionary heritage. LINE-1 has been classified into three main subfamilies during early primate evolution including L1M (mammalian-specific, oldest), L1P (primate-specific, intermediate), and L1H (human-specific, youngest) subfamilies. Alu elements have been classified into three main subfamilies including AluJ (oldest), AluS (intermediate), and AluY (youngest). The ability to transposition has been lost in the oldest subfamilies of both LINE-1 and Alu, whereas the intermediate and young subfamilies (L1PA, L1H, AluS, and AluY) are active and capable of jumping.

Current evidence suggests that aberrant DNAm of LINE-1 and Alu elements links to several diseases: ASD, pre-symptomatic dementia in type 2 diabetes, and chronic lymphocytic leukemia. Whole-genome sequencing investigation of the brains of individuals with ASD revealed that LINE-1 and Alu elements have a larger number of insertions than in normal brain tissues. The binding of methyl-CpG binding protein 2 (MeCp2), transcriptional repressor, to the LINE-1 promoter was dramatically reduced, and this was related to LINE-1 overexpression in ASD brains. The functional impact of LINE-1 and Alu elements in the ASD is currently unknown.
One possibility is that LINE-1 and Alu elements act as enhancers or alternative promoters for host genes. Our recent study discovered associations between LINE-1/Alu elements and gene expression in blood transcriptome, implying that LINE-1 and Alu may influence the expression of host genes in ASD. Additionally, we also found changes in global methylation of LINE-1 and Alu elements in the lymphoblastoid cell line of the ASD subgroup based on clinical phenotype. According to a recent study using blood samples from ASD, one of CpG sites within the LINE-1 sequence showed a slight decrease of methylation levels in ASD compared to unaffacted controls but its methylation level was highly significant in ASD with mental regression. However, locus- and family-specific methylation patterns of LINE-1 and Alu elements in ASD whole blood have not been reported.

Here, we intended to investigate the DNA methylation profiles of LINE-1 and Alu elements, as well as their associations with the expression of genes located nearby these elements. Using Illumina Infinium 450 K annotation, CpG sites mapping to LINE-1 and Alu families were identified from DNA methylation data (GSE80017, GSE113967, and GSE131706) obtained from the NCBI Gene Expression Omnibus (GEO) database. Differential methylation of LINE-1 and Alu elements was examined in a total, locus- and subfamily-specific manner for each ASD variant, including heterogeneous ASD (n = 52), ASD with 16p11.2 del (n = 7), and ASD with CHD8 variants (n = 15).

Biological functions and interactome networks of genes located nearby LINE-1 and Alu elements were predicted by Ingenuity Pathway Analysis (IPA). We subsequently identified these genes that were reproducibly differentially expressed in transcriptome data obtained from multiple ASD cohorts.

Results

DNA methylation profile of LINE-1 and Alu elements in the heterogeneous ASD. A total of 22,352 probes mapping to LINE-1 and Alu elements were identified on the Infinium 450 K platform for differential DNA methylation analysis. The analyses were performed for heterogeneous ASD (n = 52) versus non-ASD (n = 48). Firstly, we measured the total methylation by combining all positions mapping to LINE-1 and Alu elements as the total of CpGs. In the comparison of heterogeneous ASD against non-ASD (Fig. 1a), there was no significant difference in total methylation of REs between these cohorts (Δβ = 0.003, p = 0.098). However, when we performed the methylation profile of REs by which LINE-1 and Alu elements were analyzed separately, we found that 2802 (LINE-1) and 4363 (Alu) differentially methylated positions (DMPs) were significantly differentially methylated (P<0.05) in the heterogeneous ASD compared to non-ASD (Figs. 1b and 2a–c). All these loci included 2471 hypomethylated loci (LINE-1: 1437 loci, Alu: 1304 loci; P<0.05) and 4424 hypermethylated loci (LINE-1: 1365 loci, Alu: 3059 loci; P<0.05). Due to the different activity of subfamilies of RE, LINE-1 and Alu elements were clustered by evolution age into three categories including old age (L1M, AluI), intermediate age (L1P, L1PB, AluS), young age (L1HS, L1PA, AluY), and related (HAL1, FAM, FLAM, FRAM). The methylation of LINE-1 and Alu elements were changed in a subfamily-specific manner. We discovered that LINE-1 was considerably hypermethylated in young and intermediate age families, including L1H (Δβ = 0.013, p = 0.00001) and L1P (Δβ = 0.005, p = 0.027), but HAL1 was hypomethylated (Δβ = −0.003, p = 0.03) (Fig. 1d). Methylation of Alu elements was significantly hypermethylated in the old and intermediate age families: AluI (Δβ = 0.006, p = 0.004) and AluS (Δβ = 0.005, p = 0.016), respectively (Fig. 1e). These findings indicated that methylation of LINE-1 and Alu elements in the heterogeneous ASD was altered in family- and locus-specific manner rather than globally.

LINE-1 and Alu methylation signatures in the homogeneous ASD (16p11.2del and CHD8 variants). Due to the heterogeneity in the ASD population, we also intended to investigate the methylation profile of LINE-1 and Alu elements in genetically homogeneous ASD, as identified in the original article of GSE113967, including ASD individuals with 16p11.2del (n = 7) and CHD8 variant (n = 15). As for the results of ASD with 16p11.2del compared with non-ASD, we found no significant changes in the total methylation of REs compared with non-ASD (Δβ = −0.002, p = 0.771, Supplementary Fig. S1a). However, the analyses identified 70 significantly locus-specific DMPs of REs in ASD with 16p11.2del including 27 DMPs at LINE-1 (5 hypomethylated loci, 22 hypermethylated loci, Supplementary Fig. S1b) and 43 DMPs at Alu elements (23 hypomethylated loci, 20 hypermethylated loci, Supplementary Fig. S1c). When LINE-1 and Alu positions were categorized into families, there was no significant difference in methylation of LINE-1 and Alu elements by family (Supplementary Fig. 1d,e).

Subsequently, we analyzed data for ASD with CHD8 variants by using the same approach. We found that total methylation of REs was exclusively hypomethylated in the ASD with CHD8 variants (Δβ = −0.006, p = 0.042, Fig. 3a). Analyzing by the position, the majority of DMPs at LINE-1 and Alu elements were hypomethylated (616 loci or 88.63%, P<0.05) of the total identified 695 DMPs observed (Fig. 3b,c). Among all significant DMPs in the ASD with CHD8 variants, 528 DMPs were mapped to LINE-1, while 167 DMPs were Alu elements. Moreover, changes in LINE-1 and Alu methylation regarding to their families were observed in ASD with CHD8 variants. In contrast to the differences found in the heterogeneous ASD, young and intermediate age LINE-1 elements were significantly hypomethylated including L1H (Δβ = −0.015, p = 0.0038) and L1P (Δβ = −0.010, p = 0.0186) (Fig. 3d). Hypomethylation of Alu elements was also observed in old age, intermediate age, and related families: AluI (Δβ = −0.010, p = 0.0154), AluS (Δβ = −0.008, p = 0.0443), FAM (Δβ = −0.008, p = 0.0399), FRAM (Δβ = −0.007, p = 0.0255) respectively (Fig. 3e). These findings suggest that DNA methylation signatures were a widespread reduction in LINE-1 and Alu regions which occurred at a specific family in the ASD with CHD8 variants but not in ASD with 16p11.2del.

Genomic distribution of LINE-1 and Alu methylation in heterogeneous and homogeneous ASD. To determine the differential DNA methylation of LINE-1 and Alu elements by genomic features, we performed enrichment analysis using Fisher’s exact test. CpG positions at LINE-1 and Alu elements were categorized to 1500 and 200 within the transcriptional start site (TSS1500 and TSS200, respectively), the 5’ untranslated region...
(5’UTR), the first exon (1st exon), gene body (Body), and 3’ untranslated region (3’UTR). In the heterogeneous ASD signatures, CpG sites at LINE-1 were significantly enriched in TSS1500 ($p = 0.0005$) and Body ($p < 0.0001$) (Supplementary Fig. S2a). Whereas $\text{Alu}$ elements were significantly enriched in TSS1500 ($p < 0.0001$), 5’UTR ($p < 0.0001$), Body ($p < 0.0001$), and 3’UTR ($p = 0.0086$) (Supplementary Fig. S3a). However, DNAm across all retrotransposons by genomic location did not significantly differ between non-ASD and heterogeneous ASD (Supplementary Figs. S2b and S3b). DNAm signatures of the ASD with 16p11.2del and $\text{CHD8}$ variants were significantly enriched in Body ($p = 0.04$) and TSS1500 ($p < 0.0001$) respectively (Supplementary Fig. S4b,c). This result shows that the changes of probes mapping to TSS1500 and gene bodies are more likely to have an association with gene expression in ASD in both heterogeneous and homogenous ASD.

**Biological functions and pathways of LINE-1 and $\text{Alu}$ methylation signatures in ASD and ASD variants.** To determine the biological significance of LINE-1 and $\text{Alu}$ methylation signatures identified in...
Figure 2. Unsupervised hierarchical cluster heatmap of the significant differentially methylated loci of the repetitive elements in non-ASD and ASD. Clustering of the 7165 significant differentially methylated loci (combining of LINE-1 and Alu loci) (a). Clustering of the 2802 significant differentially methylated loci (LINE-1) (b). Clustering of the 4363 significant differentially methylated loci (Alu) (c). The color scale indicates methylation level (M value), from low (blue) to high (red). Green color represents ASD and white color represents non-ASD.

Each ASD cohort, we predicted the biological function and pathway of genes located nearby DMPs of LINE-1 and Alu elements using IPA software. We found that neurological diseases were significantly enriched among genes associated with LINE-1 and Alu methylation signatures in the heterogeneous ASD (p range: 0.00495–3.33E-26, 2274 genes) and ASD with CHD8 variants (p range: 0.0258–0.000117, 302 genes) as shown in Supplementary Tables S1 and S2. The categories ASD and intellectual disability were exclusively associated with LINE-1 and Alu methylation signatures in the heterogeneous ASD (p = 2.56E–06, 253 genes). Whereas Huntington’s disease, familial encephalopathy, and brain lesion were commonly associated with both ASD signatures. For ASD with 16p11.2del variant, LINE-1 and Alu methylation signatures in this cohort were significantly associated with developmental disorders (p range: 0.0393–0.00222, 9 genes) (Supplementary Table S3). However, only one gene was associated with the disease, possibly caused by a small number of genes associated with LINE-1 and Alu methylation of this ASD variant. Additionally, we discovered that several canonical pathways linked to ASD were associated with genes located nearby LINE-1 and Alu methylation signatures in each ASD cohort. More precisely, we found that the α-adrenergic signaling pathway was significantly associated in the heterogeneous ASD (p = 0.00269, 28 genes) and ASD with CHD8 variants (p = 0.00646, 7 genes). Axonal guidance signaling pathway involved in nervous system development was significantly associated with LINE-1 and Alu methylation signatures of ASD with 16p11.2del and CHD8 variants. These results indicate that genes associated with LINE-1 and Alu methylation signatures in ASD were involved with neurological diseases and ASD-comorbid disorders as well as canonical pathways known to be implicated in ASD. The list of all significant biological functions and pathways in each ASD variant is shown in Supplementary Tables S1, S2, and S3.

Interactome networks or gene regulatory networks revealed the interaction of genes located nearby LINE-1 and Alu methylation signatures of each ASD variant. The functions and pathways implicated in ASD were highlighted in the networks. The interactome of the heterogeneous ASD was associated with ASD and mental retardation, as well as canonical pathways implicated in ASD such as retinoic acid receptor (RAR) and AMP-activated protein kinase (AMPK) signaling (Supplementary Fig. S5). In ASD with 16p11.2del, we found that the interactome related to axonal guidance and sirtuin signaling pathway (Supplementary Fig. S6a). The interactome of ASD with CHD8 was related to familial encephalopathy and movement disorder which conditions found in ASD individuals29,30. The interactomes were also associated with neuronal function including axonal guidance and synaptogenesis signaling pathways (Supplementary Fig. S6b,c).

Identification of unique target loci located nearby LINE-1 and Alu signatures in heterogeneous ASD. To investigate the associations of locus-specific LINE-1 and Alu methylation to target gene or neighboring gene expression in the ASD, we identified DEGs from multiple ASD studies obtained from the GEO DataSets. This approach reflected the heterogeneity of the ASD population because these studies were compiled from a different ASD cohort. There were 12,419 DEGs identified from seven datasets including four studies used peripheral blood samples and three studies used post-mortem brain tissues from ASD individuals (Supplementary Table S4). We subsequently overlapped the list of DEGs with differentially methylated genes (DMGs: genes located nearby LINE-1 and Alu signatures). The overlapping revealed 1847 DMGs in the heterogeneous ASD that were differentially expressed in several ASD studies, with 155 of them being autism-related genes in the SFARI database. We identified 43 top DMGs, |Δβ| ≥ 5%, inversely related to gene expression, and differentially expressed in at least two studies (Supplementary Table S5). Interestingly, two of the top DMGs, potassium voltage-gated channel subfamily Q member 3 (KCNQ3) and ubiquitin conjugating enzyme E2 H (UBE2H) (Table 1), were genes in the SFARI database and were enriched in the gene regulatory network related to ASD and mental retardation (Supplementary Fig. S5).

The genomic regions of LINE-1 and Alu methylation signatures within the DMGs are shown in Supplementary Fig. S7. We identified DMRs by mapping all probes located nearby LINE-1 and Alu signatures using the UCSC genome browser. The findings revealed that AluSg7 (cg16926147), which is located on the gene body of the KCNQ3 gene (Supplementary Fig. S7a), was hypermethylated and KCNQ3 expression level was significantly reduced in blood and post-mortem brain tissues. Interestingly, we discovered that several probes in this region,
including those in the promoter region were not changed. This result suggests that LINE-1 and Alu methylation at DMRs may facilitate gene expression indicated by the inverse relationship between LINE-1/Alu methylation and gene expression. As well as AluY (cg08998414) within UBE2H gene (Supplementary Fig. S7b) and L1PA3 (cg24094412) within hyperpolarization activated cyclic nucleotide gated potassium channel 1 (HCN1) (Supplementary Fig. S7c), we also observed that AluY and L1PA3 methylation were inversely related to the gene expression levels in both blood and brain tissues of ASD cohort. Moreover, we found several DMGs that were not reported in the SFARI database but the expression of these DMGs in the blood and post-mortem brain tissues was inversely related to LINE-1 and Alu methylation such as N-deacetylase and N-sulfotransferase 1 (NDST1) (cg12611243: L1MC1), ubiquitin specific peptidase 6 (USP6) (cg23416909: L1M5), and formin binding protein 1 (FNBP1) (cg13916261: AluSg) (Table 1). These associations suggest that DMPs at LINE-1 and Alu elements may affect the expression of genes located nearby these DMPs in the heterogeneous ASD cohort.

**Figure 3.** Methylation of repetitive elements (LINE-1 and Alu) in non-ASD (n = 48) and ASD patients who carry CHD8 variants (n = 15). DNA methylation (a). Volcano plots of mean change in methylation (Δβ) of LINE-1 (b) and Alu (c) against −log10 FDR-adjusted p value (P_{FDR}) of ASD compared with non-ASD; the green line represents P_{FDR} = 0.05, the red line represents 10% of methylation changes, green dots represent hypomethylation loci, and orange dots represent hypermethylation loci. Changes in DNA methylation (Δβ) of ASD with CHD8 compared with non-ASD by a subfamily of LINE-1 (d) and Alu (e). Mean ± SD. *p < 0.05.
Identification of unique target loci located nearby LINE-1 and Alu signatures in ASD variants. To investigate the associations of unique LINE-1 and Alu methylation signatures to target gene or neighboring gene expression in the genetically homogeneous ASD, we obtained 39 and 101 DMPs that were found exclusively in the ASD with 16p11.2 del and CHD8 variants, respectively (Supplementary Fig. S4a, Supplementary Table S6). Within the analysis among heterogeneous ASD, ASD + 16p11.2 del and CHD8 variants, the probes cg27005715 and cg08394597 were found in the overlap (Supplementary Fig. S4a). Furthermore, two other probes (cg26962295 and cg26620682) were found in the overlapped ASD + 16p11.2 del and ASD + CHD8 variants (Supplementary Fig. S4a). We re-analyzed them for ASD variant versus the heterogeneous ASD. Next, we conducted the same strategy used for the heterogeneous ASD to select the candidate DMPs by overlapping with the transcriptome data. The overlapping of unique DMPs with transcriptome data revealed 11 sensitivity and specificity of unique LINE-1 and Alu methylation signatures to target gene or neighboring gene expression in the genetically homogeneous ASD, we obtained 39 and 101 DMPs that were found exclusively in the ASD with 16p11.2 del and CHD8 variants, respectively (Supplementary Fig. S4a, Supplementary Table S6). Within the analysis among heterogeneous ASD, ASD + 16p11.2 del and CHD8 variants, the probes cg27005715 and cg08394597 were found in the overlap (Supplementary Fig. S4a). Furthermore, two other probes (cg26962295 and cg26620682) were found in the overlapped ASD + 16p11.2 del and ASD + CHD8 variants (Supplementary Fig. S4a). We re-analyzed them for ASD variant versus the heterogeneous ASD. Next, we conducted the same strategy used for the heterogeneous ASD to select the candidate DMPs by overlapping with the transcriptome data. The overlapping of unique DMPs with transcriptome data revealed 11 and 31 unique DMPs in the ASD with 16p11.2 del and CHD8 variants, respectively (Supplementary Tables S7 and S8). Among the unique DMPs, we found several genes linked to neurodevelopmental disorder and ASD, including XK related 6 (XKR6) (Fig. 4a), zinc finger protein 107 (ZNF107) (Fig. 4b), and myeloma-overexpressed gene 2 protein (MYEOV2) (Fig. 4c) in the ASD with 16p11.2 del. The significant DMPs at AluY (cg21300361) within XKR6 was hypermethylated, while AluSa (cg01772945) within ZNF107 and L1MB3 (cg13749777) within MYEOV2 were hypomethylated. Interestingly, these genes were differentially expressed in the blood transcriptome of multiple ASD cohorts, and their expression was inversely relative to LINE-1 and Alu methylation levels (Table S2).

For ASD with CHD8 variants, we found that all LINE-1 and Alu elements located on candidate genes were markedly hypomethylated, as expected from total and family-specific methylation levels. These DMPs consist of L1MC5 (cg22706070) within Euchromatic Histone Lysine Methyltransferase 2 (EHMT2) (Fig. 5a), AluIo (cg06421197) within caspase 1 (CASP1) (Fig. 5b), and AluSx (cg18699242, cg01963623, cg02169692) within ubiquitin-specific peptidase 18 (USP18) (Fig. 5c). EHMT2 was significantly increased in the blood of ASD, while CASP1 was increased in both the blood and brain of multiple ASD cohorts (one probe was decreased). These changes were inversely relative to LINE-1 and Alu methylation levels within that gene (Table 2). We found that the expression of USP18 was not inversely relative to AluSx methylation located on the gene. Additionally, the DMRs of XKR6, ZNF107, MYEOV2, EHMT2, and CASP1 genes revealed LINE-1 and Alu probes as well as non-LINE-1/Alu probes located in the same DMRs (Figs. 4a–c and 5a,b).

### Sensitivity and specificity of unique LINE-1 and Alu signatures in ASD variants.

To predict diagnosis of the genetically homogenous ASD by using LINE-1 and Alu methylation signatures, we subsequently conducted ROC curves analysis of these loci and other probes within unique DMRs to distinguish each homogenous ASD variant from non-ASD and ASD with non-specific variants. For ASD with 16p11.2 del, AluY within XKR6 (cg21300361) exhibited high sensitivity and specificity (AUC = 0.905, 95%CI = 0.83–0.98) to distinguish ASD with 16p11.2 del from non-ASD and ASD with CHD8 variants as shown in the ROC curves (Fig. 4d). In addition, the ROC curves of AluSa within ZNF107 (cg01772945) and L1MB3 within MYEOV2 (cg13749777) also exhibited high AUC value (AluSx: AUC = 0.900, 95%CI = 0.83–0.97 and L1MB3: AUC = 0.841, 95%CI = 0.74–0.95) (Fig. 4e,f). In the ASD with CHD8 variants, LINE-1 and Alu methylation signatures within candidate DMPs showed moderate sensitivity and specificity as demonstrated by AUC values (AUC range: 0.712–0.819) compared with the specificity of unique locus in ASD with 16p11.2 del, including L1MC5 (cg22706070) within EHMT2 (Fig. 5d), AluIo (cg06421197) within CASP1 (Fig. 5e), and AluSx (cg18699242, cg01963623, cg02169692) within USP18 (Fig. 5f). Our findings suggest that these novel DMPs at the LINE-1 and

| ProbeID       | Subfamily | Delta | P_adj  | Gene ID              | log_FC | q-value |
|---------------|-----------|-------|--------|----------------------|--------|---------|
| cg13916261    | AluSa     | 1.05E-04 | 0.354 | FNBP1                | 0.013  |
| cg13916261    | AluSa     | 1.05E-04 | 0.156 | ILMN_1797342         | 0.000  |
| cg16926147    | AluSa    | 3.1E-04 | 0.099 | KCNN3                | 0.018  |
| cg16926147    | AluSa    | 3.1E-04 | 0.634 | KCNN3                | 0.000  |
| cg5899414     | AluY     | 1.85E-04 | 0.130 | UBE2H                | 0.035  |
| cg5899414     | AluY     | 1.85E-04 | 0.126 | UBE2H                | 0.026  |
| cg5899414     | AluY     | 1.85E-04 | 0.171 | UBE2H                | 0.023  |
| cg5899414     | AluY     | 1.85E-04 | 0.253 | USP6                 | < 0.001|
| cg2416909     | L1MS     | 3.44E-04 | 0.614 | USP6                 | < 0.001|
| cg2416909     | L1MS     | 3.44E-04 | 0.231 | NSD1                 | 0.050  |
| cg2416909     | L1MS     | 3.44E-04 | 0.209 | NSD1                 | 0.021  |
| cg2416909     | L1MS     | 3.44E-04 | 0.275 | NSD1                 | 0.029  |
| cg2416909     | L1MS     | 3.44E-04 | 0.428 | HCN1                 | 0.002  |
| cg2416909     | L1MS     | 3.44E-04 | 0.315 | HCN1                 | 0.041  |

Table 1. Utilized gene expression microarrays/RNA-sequencing for the differential gene expression analysis of the target genes of heterogeneous ASD.
Alu elements could be used for clinical purposes. However, an independent cohort is required for validation, as we were limited by the percentage of ASD individuals affected by these genetic variants.

Discussion
Epigenetic modification is an important mechanism linking environmental and genetic factors, especially during the developmental process. There are accumulating evidences suggest that ASD heterogeneity is influenced by a combination of genetic and environmental factors4–6,31–33. DNA methylation status of LINE-1 and Alu elements account for more than 25% of the human genome and 50% of genomic methylation17,36. Altered DNA methylation of these REs is involved with genomic instability and biologically relevant such as gene expression17,37–42. In this study, we interrogated locus- and family-specific methylation profiles of LINE-1 and Alu elements in ASD whole blood. In the heterogeneous ASD, we found no difference in total methylation of REs (LINE-1 and Alu) (Fig. 1a), which is consistent with our previous studies using lymphoblastoid cell lines that found no difference when all ASD were combined. In addition to Shpyleva’s study, total methylation of LINE-1 in the ASD brain was also not significantly altered26. The possibility is that the aberration of total methylation of REs may rely on family-specific REs or

Figure 4. Genomic location and specificity of the unique differentially methylated regions in ASD with 16p11.2 deletion. Genomic location on XK related 6 (XKR6) (a), Zinc Finger Protein 107 (ZNF107) (b) and Myeloma overexpressed 2 (MYEOV2) (c): blue line represents hypomethylation, red line represents hypermethylation. The receiver operating characteristic (ROC) analysis of the unique differentially methylated regions of ASD with 16p11.2 deletion (n = 7) was performed against non-ASD (n = 48) and ASD with CHD8 variants (n = 15). Specificity and sensitivity of the unique differentially methylated regions for XKR6 (d), ZNF107 (e) and MYEOV2 (f).
revealed that NPCs have high retrotransposition of LINE-1. They also discovered low DNAm at the LINE-1 promoter as well as a high copy number of LINE-1 in brain tissues when compared to other somatic cells.

We identified significant (p < 0.05) at LINE-1 and Alu elements in the heterogeneous ASD compared with non-ASD and the most of the DMPs were typically hypermethylated. We observed these hypermethylated loci mapped to L1P, L1H, AluI, and AluS elements, which are intermediate and youngest LINE-1, and oldest and intermediate Alu, respectively. This implies that hypermethylation suppressed the most active LINE-1 and Alu subfamilies (intermediate and young REs) in the heterogeneous ASD. However, the hypomethylation of intermediate and young LINE-1 and Alu was shown in ASD with CHD8 variants. Both hypermethylation and hypomethylation of REs may interfere with gene expression of themselves and inserted genes in ASD.

It is important to note that LINE-1 and Alu elements play important roles in human brain development and brain somatic mosaicism. LINE-1 and Alu elements can regulate nearby genes during brain development. LINE-1 and Alu retrotransposition occurred more frequently in the brain than in germline cells. Furthermore, Coufal’s study, which compared LINE-1 activity in fetal neural progenitor cells (NPCs) to other somatic cells, revealed that NPCs have high retrotransposition of LINE-1. They also discovered low DNAm at the LINE-1 promoter as well as a high copy number of LINE-1 in brain tissues when compared to other somatic cells. Thus, we also performed analysis in the validation cohort using methylation data from post-mortem brain tissues of ASD including prefrontal cortex and subventricular zone regions (GSE80017 and GSE131706, respectively). We identified significant (p < 0.05) at LINE-1 and Alu elements in the prefrontal cortex and subventricular zone of ASD, respectively. When DMPs from whole blood were compared to DMPs in subventricular zone area (Supplementary Fig. S8b,f). While as significant DMPs in whole blood of ASD with 16p11.2del intersect with both prefrontal cortex and subventricular zone reached only 0.2% (Supplementary Fig. S8c,d). This warrant additional investigation into how relevant these DMPs are to ASD, and will necessitate to analyze a larger dataset of DMPs in ASD brain tissue. A validation of the data could open the way to the potential medical application of specific DMP tracking as marker to identify high risk ASD patient from blood test.

### Table 2. Utilized gene expression microarrays/RNA-sequencing for the differential gene expression analysis of the target genes of ASD with 16p11.2 deletion and CHD8 variants.

| Probe ID      | Elements | Delta | P_FDR  | GSE   | Gene ID | Gene log2FC | q-value   |
|---------------|----------|-------|--------|-------|---------|-------------|-----------|
| cg2062537     | L1MB3    | −0.079| 0.0002 | GSE25507 | 1553515_at | MYEOV2    | 0.107     | 0.0496   |
| cg13749477    | L1MB3    | −0.038| 0.0004 | GSE25507 | 1553515_at | MYEOV2    | 0.107     | 0.0496   |
| cg0762869     | L1MB3    | −0.101| 0.0028 | GSE25507 | 1553515_at | MYEOV2    | 0.107     | 0.0496   |
| cg01772945    | AluSq    | −0.048| 0.0003 | GSE18123 | 205739_x_at | ZNF107    | 0.382     | 0.0017   |
| cg09168728    | HAL1     | 0.042 | 0.0039 | GSE18123 | 202651_at | LPGAT1    | 0.16      | 0.0038   |
| cg21300361    | AluY     | 0.024 | 0.0139 | GSE18123 | 1553640_at | XR6       | −0.949    | 0.0206   |
| cg21300361    | AluY     | 0.024 | 0.0139 | GSE25507 | 1553640_at | XR6       | 0.134     | 0.0112   |

ASD with CHD8 variant

| Probe ID      | Elements | Delta | P_FDR  | GSE   | Gene ID | Gene log2FC | q-value   |
|---------------|----------|-------|--------|-------|---------|-------------|-----------|
| cg06421197    | AluJo    | −0.034| 0.0266 | GSE18123 | 2060311_at | CASP1      | 0.14      | 0.0122   |
| cg06421197    | AluJo    | −0.034| 0.0266 | GSE24133 | ILMN_2326509 | CASP1     | −0.16     | 0.0258   |
| cg06421197    | AluJo    | −0.034| 0.0266 | GSE24133 | ILMN_2326512 | CASP1     | −0.17     | 0.0213   |
| cg06421197    | AluJo    | −0.034| 0.0266 | GSE24133 | ILMN_1727762 | CASP1     | −0.25     | <0.0001  |
| cg06421197    | AluJo    | −0.034| 0.0266 | GSE59288 | 834 | CASP1 | 0.61      | 0.0096   |
| cg09604414    | AluSx    | −0.067| 0.0336 | GSE25507 | 229079_at | EHMT2     | 0.09      | 0.0471   |
| cg22706070    | L1MC5    | −0.008| 0.0496 | GSE25507 | 229079_at | EHMT2     | 0.09      | 0.0471   |
| cg12699692    | AluSx    | −0.109| 0.0275 | GSE24133 | ILMN_3240420 | USP18     | −0.31     | 0.025    |
| cg12699692    | AluSx    | −0.109| 0.0275 | GSE24133 | ILMN_3240420 | USP18     | −0.31     | 0.025    |
| cg18699242    | AluSx    | −0.106| 0.0448 | GSE24133 | ILMN_3240420 | USP18     | −0.31     | 0.025    |
| cg18699242    | AluSx    | −0.106| 0.0448 | GSE24133 | ILMN_3240420 | USP18     | −0.31     | 0.025    |

Restrict to specific locations. Reducing the heterogeneity of ASD by classifying ASD based on clinical phenotype may be beneficial, as demonstrated by previous findings from our investigators. Utilized gene expression microarrays/RNA-sequencing for the differential gene expression analysis of the target genes of ASD with 16p11.2 deletion and CHD8 variants.
Our findings suggest that epigenetic dysregulation of LINE-1 and Alu elements in ASD may alter the function of autism-related genes regulated by these elements. To address this, we predicted the biological functions and networks of genes located nearby DMPs of LINE-1 and Alu elements. Neurological diseases and canonical pathways implicated in ASD were significantly associated with these genes (Supplementary Table S1). Moreover, interactome networks associated with ASD revealed several autism-related genes (Supplementary Fig. S5). Especially, AluSg7 (cg16926147) within KCNQ3 gene and L1PA3 (cg24094412) within HCN1 gene were hypermethylated and inversely related to aberrant gene expression in the blood and post-mortem brain tissues of several ASD cohort studies. Hypomethylated DMPs were also discovered in the most active Alu family, AluY (cg08998414), which is located on the UBE2H gene and has an inverse relationship with gene expression. KCNQ3 encodes a protein involved in neuronal excitability; people with a de novo variant of this gene experience ASD features, and some were diagnosed with ASD50. HCN1 encodes a hyperpolarization-activated cation channel that is widely expressed in the brain regions51. HCN1 mutation causes epileptic encephalopathy.

**Figure 5.** Genomic location and specificity of the unique differentially methylated regions in ASD with CHD8 variants. Genomic location on Euchromatic Histone Lysine Methyltransferase 2 (EHMT2) (a), Caspase 1 (CASP1) (b) and Ubiquitin Specific Peptidase 18 (USP18) (c): blue line represents hypomethylation, the red line represents hypermethylation. The receiver operating characteristic (ROC) analysis of the unique differentially methylated regions of ASD with CHD8 variants (n = 15) was performed against non-ASD (n = 48) and ASD with 16p11.2 deletion (n = 7). Specificity and sensitivity of the unique differentially methylated regions for EHMT2 (d), CASP1 (e) and USP18 (f).
and this mutation is associated with intellectual disability and autistic traits\(^2\). UBE2H encodes an E2 ubiquitin-conjugating enzyme family protein that is involved in the protein ubiquitination mechanism. Genetic association and screening studies have shown that this gene is present in ASD individuals\(^3,5,35,36\). Another interesting result is hypomethylation in the HAL1 family which was found exclusively in the heterogeneous ASD. HAL1 or half-L1 encodes only ORF1p which enhances the efficiency of their transposition, but the origin, biological properties, and subfamilies have not been well studied\(^3\). HAL1 subfamilies were also not well annotated in our data. However, this result warrants further research of their biological activity in the ASD.

Here, we discovered LINE-1 and Alu methylation signatures in these genetically homogeneous ASD (both 16p11.2 del and CHD8 variants). In the ASD with 16p11.2del, only locus-specific changes at LINE-1 and Alu elements were observed (Supplementary Fig. S1). We identified unique DMPs which target genes differentially expressed in the several ASD cohort studies including AluY within XKR6 (cg21300361), AluSq within ZNF107 (cg01772945), and L1MB3 within MYEOV2 (cg13749477). These genes were genetic risk variants for ASD identified in genome-wide association study (GWAS), single nucleotide polymorphisms (SNPs), and copy number variation (CNV) studies\(^36,37\). In the case of ASD with the CHD8 variants, we observed a widespread reduction of LINE-1 and Alu methylation levels in total methylation and the active LINE-1 and Alu families (L1P, L1H, and AluS). This change has far-reaching implications for even the oldest and fossil family (Alu) and FAM, as well as FRAM family. Furthermore, the unique LINE-1 and Alu methylation signatures of ASD with CHD8 variants, such as L1MC5 (cg22706070) within EHM72, AluJo (cg06421197) within CASP1, and AluSx (cg18699242, cg01963623, cg02169692) within USP18, were also hypomethylated. However, we found that these alterations are inconsistent with the heterogeneous ASD profile, in which most DMPs were hypermethylated. These results may be caused by disease-specific genetic variants of CHD8 that is a huge difference from the ASD without any genetic variants or with undefined ones. CHD8 is a chromatin remodeling/modifier factor that plays a role in the transcription process required for brain development\(^31\). LINE-1 and Alu elements have an activation and a repressive chromatin mark that is bound by several chromatin remodeling/modifier factors\(^39,42,47,58\). aberrant CHD8 function may be leading to changes in genome-wide epigenetic marks which can affect a variety of gene regulatory mechanisms. The inverse relationship between LINE-1/Alu methylation and gene expression was also observed in the ASD with CHD8 variants. EHM72, located nearby L1MC5, is a histone lysine methyltransferase involved with gene activation or repression. Gene and protein expression levels of EHM72 were significantly increased in the post-mortem brain tissues of ASD\(^59,60\). CASP1 encodes cysteine-aspartic acid protease (caspase) enzyme involving apoptosis, monocyte cell fate, and immune response\(^61\). CASP1, located nearby AluJo, was significantly elevated in the peripheral blood mononuclear cells of ASD\(^62\), as well as overexpressed in two ASD studies including blood and post-mortem brain tissues. USP18 is a protein in the ubiquitin pathway which is essential for cell cycle, cell differentiation, and proliferation\(^63\) and its CNV has been reported in ASD individuals\(^64\). In transcriptome data obtained from several ASD studies, USP18 was significantly decreased, but not inversely related to hypomethylated positions of AluSx located in the upstream region of USP18 gene. However, three probes (cg18699242, cg01963623, and cg27281093) at the same regions have been reported to be hypomethylated and they are the CDH8 signature in the previous study\(^65\). Our findings showed that DNAm of LINE-1 and Alu elements, located in the target genes, are connected with ASD-related genes. Moreover, biological functions and interactome of the genes located nearby LINE-1 and Alu methylation signatures in the ASD cohorts were associated with neurological diseases and developmental disorders, as well as canonical pathways implicated in ASD. Unlike genetic changes, epigenetic alterations are not recorded in the genome and cannot be identified by genome sequencing. DNAm signatures are identified by comparing the methylation patterns of affected individuals to those typically developing control individuals. Several DNAm signatures have been established, and their effectiveness is demonstrated as epigenetic markers for identifying variations of uncertain significance as pathogenic or benign\(^66\). Although ASD pathogenesis occurs in the brain tissue, other systems such as the immune\(^25\), metabolic\(^26\), and gastrointestinal systems\(^69\) are also affected in ASD individuals. DNAm in the blood is highly correlated to brain tissue samples and reflects environmental exposure\(^70\). The discovery of distinct LINE-1 and Alu methylation signatures in ASD blood outlines their clinical potential to be used as non-invasive biomarkers. We conducted ROC curves analysis to predict a sensitivity and specificity of diagnosis with ASD using unique DMPs at LINE-1 and Alu elements identified in the blood of ASD individuals. Our findings show that LINE-1 and Alu methylation can be used to identify ASD with specific variants from unaffected individuals and classify them. However, additional research is required to determine its sensitivity and specificity in large and independent ASD cohorts.

Because of the limitation of available post-mortem brain tissues for each ASD with genetic variants in publicly available datasets, our analyses were carried out using Illumina Infinium 450 K methylation array from ASD whole blood. Further research with a large number of post-mortem brain tissues and whole genome bisulfite sequencing or Epic850K array (an updated version with twice coverage) is required. There are confounding factors that may interfere the methylation data such as gender, age, and blood cell type composition. However, according to the original article, these factors have not been demonstrated to have no effect on the DNAm signatures of this dataset\(^65\). As our study does not offer sufficient statistical power to include these covariates within the model, we cannot exclude the possibility that the observed differences in methylation could in part be linked to these factors. However, to ensure that our analysis is minimally disrupted by such factors, we performed a Chi-square test to determine whether sex is independent or no relationship to control, heterogeneous ASD, and ASD with variants used in our model (Pearson's Chi-square p = 0.198), as well as age comparisons. We found no significant difference between the control group versus heterogeneous ASD or ASD with variants using the non-parametric Mann Whitney U-test (p = 0.691 for control vs heterogeneous ASD, p = 0.068 for control vs ASD with 16p11.2 del, and p = 0.507 for control vs ASD with CHD8 variants). In this study, we discovered links between REs methylation and expression of target genes located nearby REs. A functional assay is required to demonstrate the functional impact of these associations in ASD. Moreover, we did not perform multiple test corrections in the
analysis of the validation cohort using post-mortem brain tissues, due to the small sample size which affects the statistical analysis power. Additionally, methylome and transcriptome datasets used in our study were obtained from different cohorts. However, transcriptome data from several ASD studies may reflect the heterogeneity of ASD, and one of the ASD cohorts in our analyses is a heterogeneous group. It is important to note that changes in LINE-1 and Alu methylation may occur as a result of a genetic factor in the genetically homogeneous ASD.

Conclusions
Locus-specific DNAm of LINE-1 and Alu elements in ASD, as well as its associations with gene expression, were firstly reported in our study. Our analyses revealed LINE-1 and Alu methylation changes in a locus- and family-specific manner which were different according to each ASD cohort. By integrating methylome and transcriptome data, the target genes of LINE-1 and Alu elements were identified. These target genes were differentially expressed in multiple ASD cohorts, and their functions were related to neurological diseases and developmental disorders such as ASD. Therefore, disruption of these functions may lead to ASD features (Fig. 6). Our research also demonstrated that the LINE-1 and Alu signatures could be applied to diagnose and classify people with ASD. Finally, our finding will provide a better understanding of the impact of LINE-1 and Alu elements in ASD, at least in the blood. Our study provides evidence supporting future studies on the role of LINE-1 and Alu related to ASD neuropathology using human post-mortem brain tissues. However, further functional studies will be necessary to investigate the subsequent impact upon the target genes and fully elucidate the role of REs in ASD biology.

Methods
Data collection. Differentially methylated retrotransposon loci were identified in publicly available Illumina Infinium 450 K datasets through GEO DataSets: http://www.ncbi.nlm.nih.gov/ gds[25]: GSE113967[30]. In this dataset, ethical approval was granted by the Research Ethics Boards of the respective institutions (University of Michigan SickKids, Holland Bloorview Kids Rehabilitation Hospital, Western University, McMaster University)[36]. Data were collected from the heterogeneous ASD (n = 52), ASD with confirmed typical 600 Kbp deletion in 16p11.2 del (n = 7), ASD with confirmed de novo CHD8 sequence variants (n = 15), and age-matched controls (non-ASD) (n = 48) (Supplementary Table S9). The heterogeneous ASD group in our study consists of ASD individuals who were selected without consideration for genetic characteristics or clinical symptoms as described in the original article[25]. Validation was performed in a cohort of genome-wide DNAm profiling of post-mortem brain tissue in the subventricular zone of the lateral ventricles from 17 individuals with ASD and 17 without (GSE131706)[23] and in the frontal cortex from nine individuals with ASD and nine without (GSE80017) (Supplementary Table S9). The ethical considerations of these datasets were considered according to the original
Genes with differently methylated loci were analyzed in publicly available gene expression datasets from publicly available datasets accessed via GEO using the following inclusion criteria: (1) the study must include ASD cases and controls; (2) the study must use microarray/RNA-seq technology; and (3) the study must use blood or post-mortem brain tissues. Finally, we obtained seven ASD studies, four of which used blood and three of which used post-mortem brain tissues (Supplementary Table S4).

**Differential methylation of retrotranspon subfamilies.** Methylation datasets were normalized using the single-sample normalized (ssNoob) method in minfi package and corrected probes using Regression on Correlated Probes (RCP) probes on the Illumina Infinium 450K methylation array mapping to repetitive elements (LINE-1 and Alu) were extracted using RepeatMarker. To identify the variant-associated differential methylation of REs, probes with single nucleotide polymorphisms (SNPs) located at or within 10 base pairs of the target CpG site were included in the analysis. The CpG sites were mapped to LINE-1, Alu, half-L1 (HAL1), fossil Alu monomer (FAM), free right Alu monomer (FRAM), and free left Alu monomer (FLAM). Due to the evolution age of the REs, LINE-1 elements were clustered into oldest (L1M, mammalian-wide), intermediate (L1P, primate-specific), and youngest (L1HS, human-specific and L1PA, primate-amplified). Concomitantly, Alu elements were categorized into AluJ (oldest), AluS (intermediate) and AluY (youngest).

Mean β value across all loci of REs was calculated as total DNAm of REs in non-ASD, ASD with 16p11.2 del, and ASD with CHD8 variants. Differential methylation of LINE-1 and Alu subfamilies between 1) non-ASD vs ASD, 2) non-ASD vs ASD with 16p11.2 del, and 3) non-ASD vs ASD with CHD8 variants, were identified. DMPs to ASD were examined in the validation dataset. DMPs were identified in ASD, with 16p11.2 del, and ASD with CHD8 variants and non-ASD with 16p11.2 del, by two-tailed t-test with correction for false discovery rate (FDR) using the Benjamini–Hochberg (BH) method and significance defined as FDR ≤ 0.05. To find the unique DMPs of each data set, the significant loci from 1 to 3 comparisons were computed to create Venn diagrams (https://bioinfgp.cnb.csic.es/tools/venny/).

**Differential gene expression analysis.** The expression data of ASD studies were obtained from the GEO DataSets. The data from each study were analyzed separately using the Multiple Experiment Viewer (MeV) program (microarray software suite). Firstly, the data were filtered using a 70% cut-off filter to remove probes that were missing in > 30% of samples. The available data were then used for the identification of differentially expressed genes (DEGs) in ASD vs non-ASD cohort by using the Significance Analysis of Microarrays (SAM). The FDR and q-value less than 5% were considered as significant DEGs. RNA-sequencing (RNA-seq) data were obtained from the Sequence Read Archive database and re-analyzed using the Galaxy platform (https://usegalaxy.org/)77. The quality control of RNA-seq data was assessed by fastp tool. The cleaned reads were then mapped to the human reference genome (GRCh38/hg38) using HISAT2 and quantified using the Subread package FeatureCounts. Differential expression analysis was performed using the DESeq2 package. The read counts were normalized using the median ratio method of the DESeq2 and the remove unwanted variation (RUV) tool. The genes with a p value (p) with Benjamini–Hochberg correction of less than 0.05 were considered significant.

**Gene functions and pathway analysis.** To predict biological functions and gene regulatory networks associated with LINE-1 and Alu elements, a list of genes located nearby DMPs of LINE-1 and Alu elements for each ASD variant were submitted to the Ingenuity Pathway Analysis software (IPA: QIAGEN Inc., https://www.qiagenbioinformatics.com/products/ingenuitypathway-analysis). Gene regulatory networks were highlighted with log, fold change of DNAm level.

**Identification of target loci in ASD with each genetic variant.** The target RE loci of ASD with each genetic variant were identified by taking the unique DMPs from the Venn diagrams and re-analyzing the different methylation of RE loci in ASD vs ASD with 16p11.2 del or with CHD8 variants. We only selected loci which were significant in all three conditions (non-ASD vs ASD, ASD vs ASD with 16p11.2 del and ASD vs ASD with CHD8 variants) by two-tailed t-test with correction for multiple hypothesis testing using the BH method and significance defined as FDR ≤ 0.05. Moreover, the DMRs, located nearby the significantly distinct DMPs, were identified in ASD with 16p11.2 del or with CHD8 variants.

**Statistical analyses.** Differentially methylated loci were identified by two-tailed t-tests and multiple testing correction for array data was performed by BH procedure for false discovery rate adjustment (PFDR ≤ 0.05 was considered to be significant). Fisher’s exact test was used to identify enrichment by genomic location of REs. DEGs were identified using SAM analysis with significance defined as FDR ≤ 0.05 by the BH method. Gene function and pathway analysis were performed in IPA using Fisher’s exact test with BH correction for multiple testing (PFDR ≤ 0.05 was considered to be significant). All statistical analyses were performed in R (version 4.0.5) and RStudio (version 1.4.1103) using the ggplot2, plotROC, pheatmap, and GraphPad Prism (version 7.0b); data are presented as mean ± SD, and p ≤ 0.05 were considered to be significant.

**Data availability** The datasets used and/or analyzed during the current study are available in the Gene Expression Omnibus, GSE113967, GSE131706, GSE80017, GSE59288, GSE64018, GSE28521, GSE18123, GSE25507, GSE42133, GSE89594.
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Author contributions
T.S. performed transcriptome analysis, biological function, and interactome prediction, and drafted the manuscript under the supervision of T.S., T.T. and C.S. C.S., N.I., D.G., and T.P. performed DNAm analysis for LINE-1 and Alu elements. T.S. supervised transcriptome analysis and interpretation. T.S. and V.W.H. supervised the biological function and interactome prediction using IPA. C.S. had primary responsibility for the final content. All authors read and approved the final manuscript.

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Competing interests
The authors declare no competing interests.

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