



# LINE-1 retrotransposon methylation in chorionic villi of first trimester miscarriages with aneuploidy

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## Abstract

**Purpose** High frequency of aneuploidy in meiosis and cleavage stage coincides with waves of epigenetic genome reprogramming that may indicate a possible association between epigenetic mechanisms and aneuploidy occurrence. This study aimed to assess the methylation level of the long interspersed repeat element 1 (LINE-1) retrotransposon in chorionic villi of first trimester miscarriages with a normal karyotype and aneuploidy.

**Methods** The methylation level was assessed at 19 LINE-1 promoter CpG sites in chorionic villi of 141 miscarriages with trisomy of chromosomes 2, 6, 8–10, 13–15, 16, 18, 20–22, and monosomy X using massive parallel sequencing.

**Results** The LINE-1 methylation level was elevated statistically significant in chorionic villi of miscarriages with both trisomy ( $45.2 \pm 4.3\%$ ) and monosomy X ( $46.9 \pm 4.2\%$ ) compared with that in induced abortions ( $40.0 \pm 2.4\%$ ) ( $p < 0.00001$ ). The LINE-1 methylation levels were specific for miscarriages with different aneuploidies and significantly increased in miscarriages with trisomies 8, 14, and 18 and monosomy X ( $p < 0.05$ ). The LINE-1 methylation level increased with gestational age both for group of miscarriages regardless of karyotype ( $R = 0.21, p = 0.012$ ) and specifically for miscarriages with trisomy 16 ( $R = 0.48, p = 0.007$ ). LINE-1 methylation decreased with maternal age in miscarriages with a normal karyotype ( $R = -0.31, p = 0.029$ ) and with trisomy 21 ( $R = -0.64, p = 0.024$ ) and increased with paternal age for miscarriages with trisomy 16 ( $R = 0.38, p = 0.048$ ) and monosomy X ( $R = 0.73, p = 0.003$ ).

**Conclusion** Our results indicate that the pathogenic effects of aneuploidy in human embryogenesis can be supplemented with significant epigenetic changes in the repetitive sequences.

**Keywords** LINE-1 retrotransposon · DNA methylation · Aneuploidy · Miscarriage · Parental age

## Introduction

Humans are characterized by frequent reproductive loss during the early stages of pregnancy compared to other periods of ontogenesis. Demographic studies have shown that the chances of producing viable offspring in a woman of middle reproductive

age within a single menstrual cycle do not exceed 25% [1]. Chromosome abnormalities are the main factor that makes a significant contribution to the etiology of early embryonic death. According to various estimates, from 70 to over 90% of spontaneous abortions with abnormal karyotypes have aneuploidy [2–4]. Moreover, the incidence of aneuploidy is high at the pre-implantation stage of development both after assisted reproduction techniques [5–10] and in naturally conceived pregnancies [11]. Embryonic aneuploidy is the result of either errors in chromosome segregation in meiosis of parents or in the first mitotic divisions of the fertilized zygote [12]. Both of these mechanisms coincide with waves of epigenetic reprogramming, characterized by a sharp decrease in the level of DNA methylation of the entire genome [13]. This epigenetic reprogramming affects not only genes but also other regions of the genome, including various repetitive sequences [14], which make up approximately 50–70% of the human genome [15, 16]. The largest class of DNA

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repeats is long interspersed nuclear element 1 (LINE-1) retrotransposons (approximately 17% of the human genome) [17]. LINE-1 is the most evolutionarily young class of retrotransposons in humans, which still retains the ability to transpose; therefore, its transcriptional activity is suppressed by DNA methylation during most periods of ontogenesis.

The LINE-1 promoter is hypermethylated in mature spermatozoa, whereas in oocytes, it is hypomethylated at the diplotene stage, and at the ovulation stage, oocytes have an average level of LINE-1 promoter methylation [18]. In mouse embryos, LINE-1 is hypomethylated [18] and actively expressed at the cleavage stage, making up 13% of the total cDNA pool in the cell [19–21]. Then, *de novo* methylation occurs, and in most epiblast derivatives, including the cells of the embryo itself, LINE-1 retrotransposons are hypermethylated, while in cytotrophoblasts and in some epiblast derivatives that later belong to placental tissues, they remain less methylated [22, 23].

LINE-1 retrotransposons are assumed to play several roles in human embryo development. In particular, retrotransposons can be used as “alternative” strong promoters that ensure stable expression of embryonic genes at the cleavage stage on the background of total epigenetic reprogramming events [20, 24]. This assumption is confirmed by the fact that the suppression of the activity of one of the LINE-1 families led to disturbances in the first divisions of the embryo at the cleavage stage [25, 26]. In addition, it is possible that LINE-1 retrotransposons may provide an open chromatin conformation in the early stages of embryo development for epigenetic reprogramming and activation of the embryonic genome [27].

The periods of aneuploidy induction in meiosis and cleavage stage overlap with periods of hypomethylation and increased transcriptional activity of LINE-1 (reviewed in [28]). This may indicate a possible association between LINE-1 activity and the occurrence of aneuploidy. Indeed, overexpression of LINE-1 proteins in mouse oocytes leads to the arrest of meiosis I and is accompanied by abnormalities in chromosome alignment and defects in the organization of the division spindle [29, 30]. This results in the predominant elimination of oocytes with increased LINE-1 expression before birth [29].

Thus, there may be functional relationship between aneuploidy and impairment of LINE-1 regulation by DNA methylation. Therefore, the aim of this study was to assess the level of LINE-1 methylation in the chorionic villi of first-trimester miscarriages with a normal karyotype and aneuploidy of various chromosomes.

## Materials and methods

### Materials and karyotyping

Chorionic villus samples from 141 miscarriages and 31 induced abortions were collected in the Tomsk region, Russia,

from 1995 to 2020. All the women signed informed consent forms. Approval was granted by the local Research Ethics Committee of the Research Institute of Medical Genetics (22.04.2010/No 2). Before the start of the study, the samples stored at  $-80\text{ }^{\circ}\text{C}$  without unfreezing. For all miscarriages, conventional cytogenetic analysis was performed either on trophoblast cells or cultured extraembryonic fibroblasts as described previously [31]. When conventional cytogenetic analysis was non-informative, karyotypes were assessed by chromosomal comparative genome hybridization (cCGH,  $n = 16$ ) or array comparative genome hybridization (aCGH,  $n = 9$ ). Twenty-four of 31 induced abortions (77%) had been karyotyped by conventional cytogenetic analysis and had normal karyotype.

In cCGH, for fluorescence labeling, we used 1000 ng of the test DNA (miscarriage DNA) and reference DNA (normal karyotype male DNA, Agilent Technologies, USA). Labeling of the genomic DNA libraries was carried out by nick translation in a 50- $\mu\text{l}$  reaction containing 5  $\mu\text{l}$  of  $10\times$  DNA Polymerase I Reaction Buffer, 5  $\mu\text{l}$  of mixtures of deoxyribonucleotides (0.5 mM dGTP, dATP, and dCTP and 0.1 mM dTTP), 5  $\mu\text{l}$  of bovine serum albumin (0.5 mg/ml), 1  $\mu\text{l}$  of 0.2 mM fluorescein-12-dUTP or TAMRA-5-dUTP, 5  $\mu\text{l}$  of DNase I diluted in water in the ratio 1:49, 1  $\mu\text{l}$  of *Escherichia coli* DNA Polymerase I (Pol I) (10 U/ $\mu\text{l}$ ), and Milli-Q water. Incubations were performed as follows: 16  $^{\circ}\text{C}$  for 1.5 h in a thermostat «CH-100» (BioSan, Latvia). The reaction was stopped by adding 1  $\mu\text{l}$  of 0.5 M EDTA (pH 8.0). Labeled DNA samples were coprecipitated with 50-fold excess Cot-1 DNA and dissolved in 15  $\mu\text{l}$  of hybridization mixture (2 parts formamide, 1 part dextran sulfate and  $20\times$  SSC). These samples dissolved in the hybridization mixture were denatured at 98  $^{\circ}\text{C}$  for 3 min and then cooled to 37  $^{\circ}\text{C}$  for 30 min before being applied to denatured normal chromosome spreads as described below.

Metaphase spreads from cultured blood lymphocytes were prepared according to standard protocols. The slides were incubated in 10 mg/ml RNase A/ $2\times$  SSC for 20 min. Then, slides were washed three times with  $2\times$  SSC, each wash lasting 5 min, and then immersed in pepsin solution (70  $\mu\text{l}$  of 10% pepsin, 70  $\mu\text{l}$  of 37% HCl, and 70 ml of  $\text{H}_2\text{O}$ ) at 37  $^{\circ}\text{C}$  for 5 min. The slides were fixed with paraformaldehyde (1% paraformaldehyde; 1%  $\text{MgCl}_2$  in PBS) for 10 min at room temperature and then washed in  $1\times$  PBS for 5 min. All slides were dehydrated through an alcohol series (70%, 90%, and 100% ethanol for 5 min each) and air dried. Denaturation of the slides was achieved by applying 70% deionized formamide/ $2\times$  SSC under a coverslip and heating the slides in an oven at 80  $^{\circ}\text{C}$  for 2 min. Immediately after denaturation, the coverslips were removed, and the slides were washed in 70% ethanol chilled to  $-20\text{ }^{\circ}\text{C}$ . Slides were then put through an alcohol series and dried before the denatured probe was finally added. A coverslip was placed on top

and sealed with rubber glue. Hybridization of the probe proceeded in a ThermoBrite hybridization chamber (Abbott Molecular, USA) over 72 h, during which time the slides were kept in a humidified chamber at 37 °C.

After hybridization, the slides were washed three times in 0.4× SSC/0.3% NP-40 Wash Solution for 2 min at 73 °C and then in 2× SSC/0.1% NP-40 Wash Solution for 5 min at room temperature. Metaphase chromosomes were stained with DAPI. Slides were photographed using an Axio Imager Z2 fluorescence microscope (Carl Zeiss, Germany) with a set of corresponding filters. Processing of the cCGH results was carried out with Isis CGH software (Metasystems, Germany).

Array CGH analysis of miscarriage DNA was performed using the GenetiSure Pre-Screen Microarray Kit, 8 × 60 K, according to the manufacturer’s protocol (Agilent Technologies, USA). Labeling of samples was performed by a GenetiSure Pre-Screen Amp and Labeling Kit (Agilent Technologies, USA). Data analysis was performed using Cytogenomics software (v. 2.0.6.0) (Agilent Technologies, USA).

Based on karyotype analysis, all miscarriages were divided into the following subgroups: miscarriages with trisomy (*n* = 74), monosomy X (*n* = 16) (Table 1), and normal karyotype (*n* = 51). Subgroups were checked for parental and gestational age matching. The numbers of miscarriages with trisomy or monosomy of specific chromosomes are given in Table 2.

**FISH**

In 49 miscarriages with trisomy, the karyotyping results were validated by fluorescent in situ hybridization (FISH). At the same time, the degree of mosaicism of trisomy or monosomy of a single chromosome was assessed with lower and upper thresholds of 10% and 90%, respectively. Centromeric (2, 6, 8, 9, 10, 15, 18, 20, X, and Y) and subtelomeric (13q, 14q, 16q, 21q, and 22q) DNA probes were used for analysis. All DNA probes were kindly provided by Prof. Mariano Rocchi at the University of Bari. FISH was performed as previously described [32].

**Table 2** Number of miscarriages with aneuploidy of different chromosomes

Aneuploidy	Trisomy													45,X
	2	6	8	9	10	13	14	15	16	18	20	21	22	
<i>n</i>	6	1	3	3	2	1	2	5	33	2	1	13	4	16

**Targeted bisulfite massive parallel sequencing**

Chorionic villous trophoblast cells were enriched by maceration of chorionic villi in 70% acetic acid by a modified protocol [33]. The isolated cells were washed in phosphate buffer. Genomic DNA was isolated from chorionic villi using the phenol-chloroform method. Sodium bisulfite conversion was performed using an EZ DNA Methylation-Direct Kit (Zymo Research, Irvine, CA, USA). Based on the bisulfite converted consensus sequence of the LINE-1 promoter (GenBank accession: X58075.1), we carefully designed primers to cover the 19 most important CpG sites: forward 5’-TATTAGGGAGTGTAGATAGTGGG-3’ and reverse 5’-CCTCTAAACCAAATATAAAAATATAATCTC-3’. Primers with attached Illumina adapters were synthesized, and the DNA product was amplified by PCR using the modified Illumina 16S rRNA protocol with BioMaster HS-Taq PCR mix (Biolabmix, Russia). A Nextera XT index kit (Illumina, USA) was used for indexing libraries with standard Illumina i7 and i5 barcodes by a second PCR using Phusion high-fidelity DNA polymerase (NEB, USA). The quality and quantity of amplified libraries were assessed by a Qubit fluorometer (Thermo, USA) and pooled together in equimolar quantities. The size of the pooled library was assessed using a Bioanalyzer 2100 instrument (Agilent Technologies, USA) with High Sensitivity DNA Kit (Agilent Technologies, USA) and was sequenced by a MiSeq (Illumina, USA) using a Reagent Micro Kit v2 (300 cycles) in paired-end mode, with 30% PhiX added.

**Table 1** Characteristics of groups of miscarriages with a normal karyotype, trisomy, and monosomy and induced abortions

Group	<i>n</i>	Gestational age (weeks)	Maternal age (years)	Paternal age (years)	Couples with recurrent miscarriages	Anembryonic pregnancies
Miscarriages						
Trisomy	74	9.5 ± 1.6 (6–12)	30.8 ± 6.3 (16–43)	32.5 ± 6.2 (20–48)	22	17
Monosomy X	16	10.5 ± 1.7 (6–12)	27.1 ± 6.0 (18–38)	30.1 ± 4.3 (22–37)	3	2
Normal karyotype	51	9.6 ± 2.0 (5–13)	29.9 ± 5.5 (20–41)	32.4 ± 6.7 (21–46)	15	9
Induced abortions	31	9.0 ± 1.7 (7–12)	29.1 ± 6.6 (18–44)	30.8 ± 6.4 (23–37)	–	–
Total	172				40	28

## NGS data processing

Sequenced reads were demultiplexed by standard Illumina i7 and i5 barcodes using the Genome Analysis Toolkit (GATK, v4.1.3.0) [34]. Read quality was assessed using FastQC, and residual adapter sequences as well as low-quality reads were removed with Trimmomatic [35]. Read alignment was performed using bwa-meth. To avoid contamination in the library preparation step, we first mapped reads to the human genome assembly (February 2009 release, hg19/GRCh37) with masked repeat sequences, and then, unmapped reads were aligned to the reference CpG-rich region of the LINE-1 element (GenBank accession: X58075.1) [36]. Human genome repeats were masked using BEDTools [37] and RepeatMasker sequences [38], as previously described [39]. DNA methylation data in the CpG context were extracted from obtained BAM files using MethylDackel (<https://github.com/dpryan79/MethylDackel>). The results are presented as the methylation index, the ratio of the number of cytosines to the sum of cytosine and thymine in a given site. Further statistical analysis was carried out in Statistica 8.0 (StatSoft). Normality was assessed using Kolmogorov-Smirnov. Mann-Whitney rank test was used for group comparison. The correlation was analyzed by Spearman test.

## Results

The mean level of LINE-1 methylation was significantly higher in both groups of miscarriages with aneuploidy, with trisomy ( $45.2 \pm 4.3\%$ ) and monosomy X ( $46.9 \pm 4.2\%$ ) but not in miscarriages with a normal karyotype ( $42.6 \pm 5.6\%$ ) compared with that in induced abortions ( $40.0 \pm 2.4\%$ ) (Fig. 1a). The methylation level was analyzed separately for 19 sites in the LINE-1 promoter (Fig. 1b). Methylation levels for individual CpG sites in the LINE-1 promoter varied quite significantly relative to the mean LINE-1 methylation level. However, an increase in the level of methylation in groups of spontaneous abortions occurred proportionally for all analyzed CpG sites in the LINE-1 promoter, which allowed us to operate further with mean values (Fig. 1c).

The level of LINE-1 methylation was significantly increased in subgroups of miscarriages with aneuploidy of specific chromosomes, namely chromosomes 2 ( $p = 0.01$ ), 8 ( $p = 0.008$ ), 14 ( $p = 0.02$ ), 16 ( $p = 0.000003$ ), 18 ( $p = 0.04$ ), 21 ( $p = 0.001$ ), and 22 ( $p = 0.001$ ), but not of chromosomes 9 ( $p = 0.76$ ) and 15 ( $p = 0.25$ ) (Fig. 1d). The highest level of LINE-1 methylation was observed for miscarriages with trisomies 8, 14, and 18 and with monosomy X. These chromosomes contain genes that play important roles in DNA methylation (Fig. 1g).

In 49 miscarriages with trisomy, the level of mosaicism was assessed by FISH in chorionic villi. Nineteen miscarriages had mosaic karyotypes, with trisomic clone levels

ranging from 10 to 90%. The other 30 miscarriages with a higher fraction of trisomic clones were classified as having pure aneuploidy. If the increased level of LINE-1 methylation is the result of an elevated level of gene expression from the supernumerary chromosome, the level of LINE-1 methylation in mosaics should be lower than that in pure trisomies. Indeed, the level of LINE-1 methylation was slightly lower in mosaic miscarriages and pure trisomy (Fig. 1e). There was no difference in the level of LINE-1 methylation between embryos with male and female karyotypes (Fig. 1f). Moreover, the presence or absence of an embryo in fetal sac (blighted ovum or anembryonic pregnancy) and recurrent miscarriage in the maternal anamnesis had no statistically significant effect on the level of LINE-1 methylation (data not shown).

In the group of miscarriages with normal karyotypes, the level of LINE-1 methylation negatively correlated with maternal ( $R = -0.31$ ,  $p = 0.029$ ) but not paternal ( $R = -0.10$ ,  $p = 0.46$ ) age (Fig. 2a). Among miscarriages with aneuploidy, a similar negative correlation was observed only for miscarriages with trisomy 21 ( $R = -0.64$ ,  $p = 0.024$ ) (Fig. 2a). In contrast, the level of LINE-1 methylation depended on paternal but not maternal age in the group of miscarriages with trisomy 16 ( $R = 0.38$ ,  $p = 0.048$ ) and monosomy X ( $R = 0.73$ ,  $p = 0.003$ ) (Fig. 2a). There were no differences in parental age between miscarriages with aneuploidy of different chromosomes (Fig. 2b). Thus, the level of LINE-1 methylation in extraembryonic tissues of miscarriages decreased with maternal age but, in some cases (trisomy 16, monosomy X), increased with paternal age.

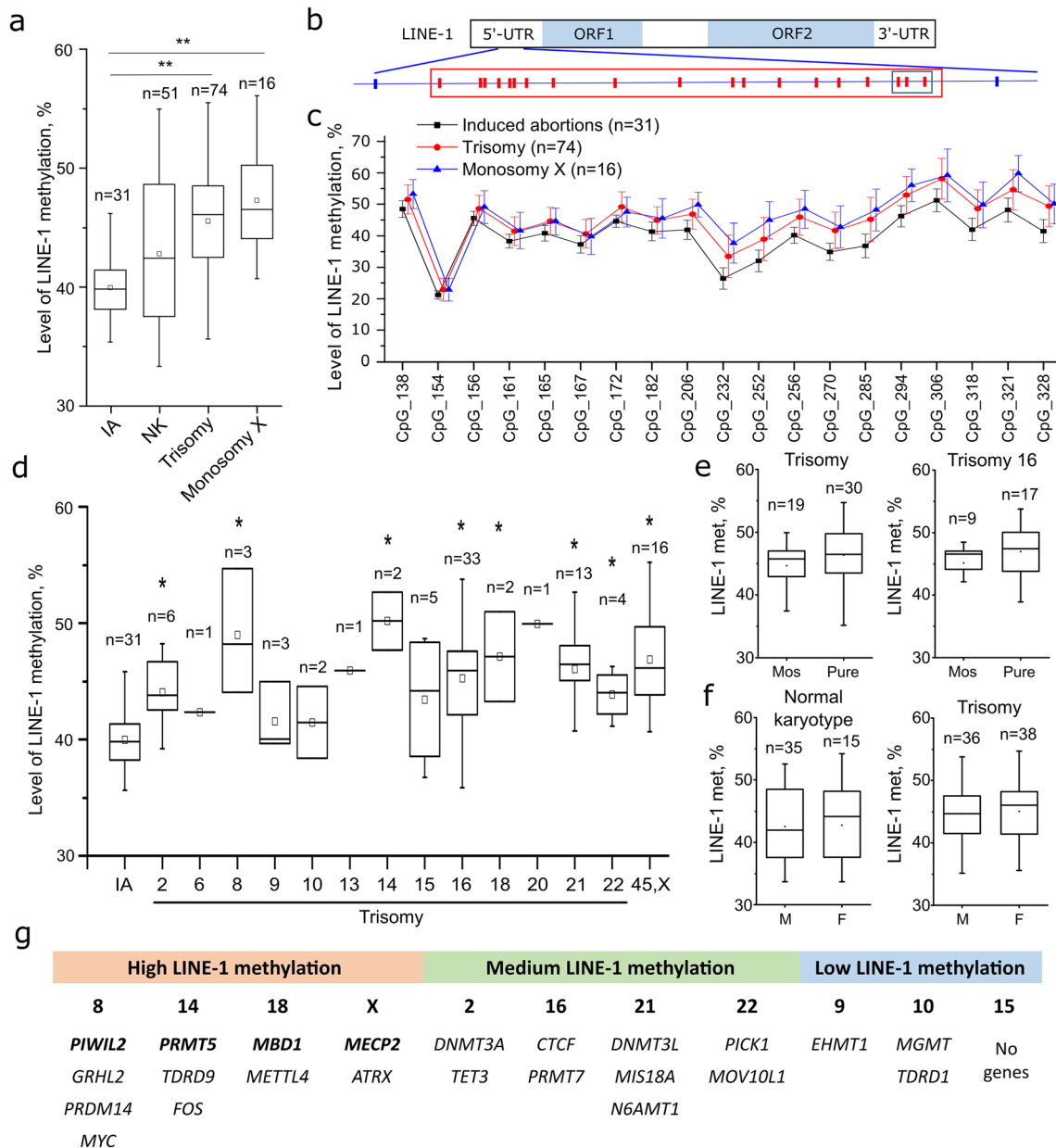
The level of LINE-1 methylation in all miscarriages regardless of karyotype weakly positively correlated with gestational age ( $R = 0.21$ ,  $p = 0.012$ ). This correlation was stronger in the group of miscarriages with trisomy 16 ( $R = 0.48$ ,  $p = 0.007$ ) (Fig. 3a). Miscarriages with trisomy 21 and monosomy X (Fig. 3b) had significantly later gestational age than miscarriages with normal karyotypes. This phenomenon is suggested to be a result of better survival of embryos with trisomy 21 and monosomy X than of embryos with aneuploidy on other chromosomes.

In the group of induced abortions, no correlations were observed between the level of LINE-1 methylation and either the age of the embryo or the age of the parents.

## Discussion

Reproductive loss in humans is an extremely frequent event, in which an important role in the etiology is played by numerical chromosomal aberrations and disturbance of the epigenetic program of development. However, the relationship among these factors of human embryonic death remains poorly understood. Several studies have focused on the profile of gene methylation in extraembryonic cells with trisomy using microarray





**Fig. 1** LINE-1 methylation in chorionic villi in groups of miscarriages with various karyotypes and induced abortions. **a** Differences between the groups of embryos. **b** Schematic representation of 19 analyzed CpG sites in the LINE-1 promoter. The red square indicates the analyzed CpG sites in this study, and the blue square indicates the most frequently analyzed CpG sites by a Qiagen pyrosequencing LINE-1 kit. **c** Methylation profile across the promoter of LINE-1 retrotransposons in miscarriages with trisomy or monosomy and in the group of induced

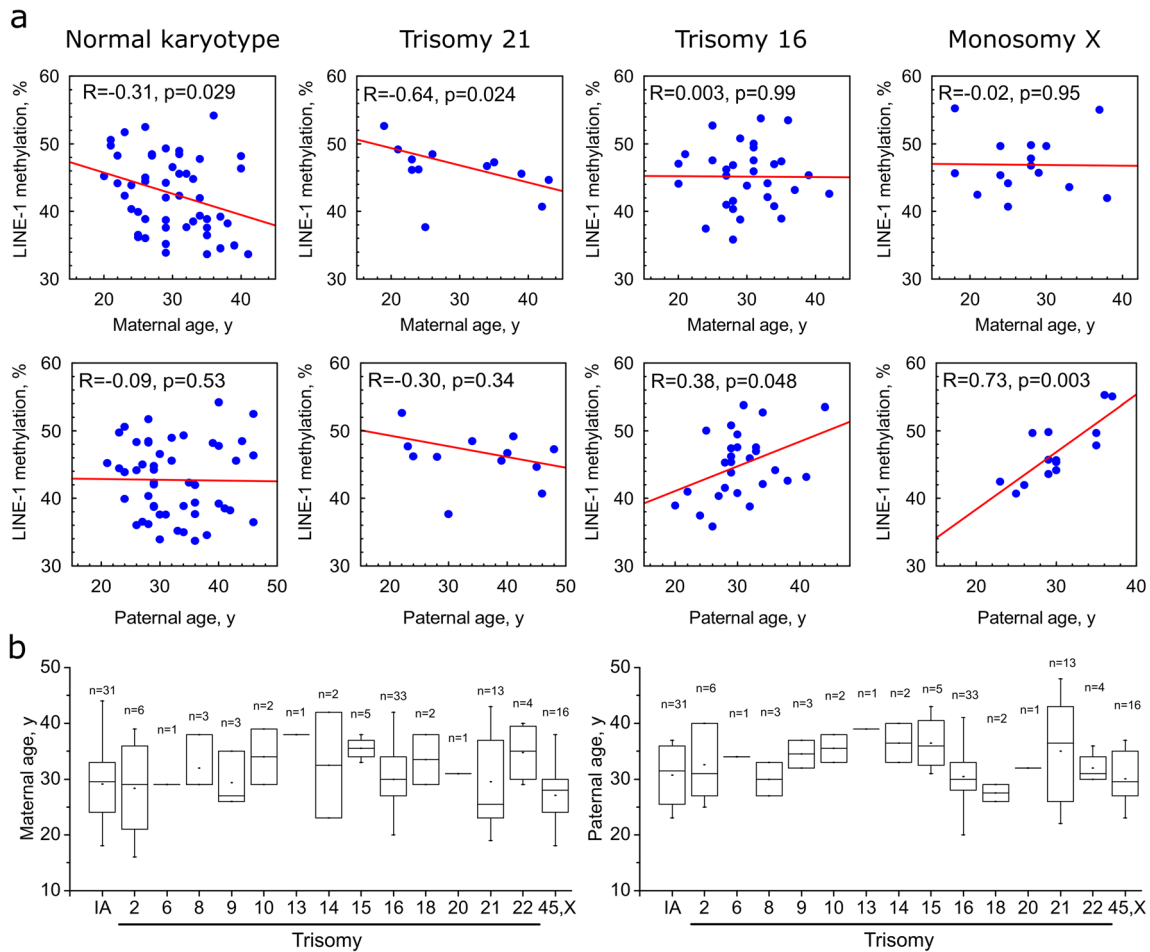
abortions (numbering of CpG sites from the beginning of the reference sequence X58075.1). **d** Level of LINE-1 methylation in miscarriages with aneuploidy of different chromosomes. **e** Level of LINE-1 methylation in miscarriages with mosaic and pure trisomy of all chromosomes or trisomy 16. **f** Level of LINE-1 methylation in miscarriages with XX and XY karyotypes. **g** Genes playing a role in DNA methylation (GO: 0006306 DNA methylation) and located on specific chromosomes. \* $p < 0.05$ ; \*\* $p < 0.00001$

techniques [40–43]. However, to our knowledge, this study is the first specifically aimed at studying the effect of aneuploidy on the level of methylation of repeated DNA sequences in embryogenesis.

In this study, we observed elevated levels of LINE-1 methylation in chorionic villi of spontaneous abortions with trisomy and monosomy of various chromosomes. This effect may reflect the effect of aneuploidy on the level of genome-wide

hypermethylation, which was shown for trisomies 16 [40, 43] and 21 [41]. However, to date, only several genes have been shown to be differentially methylated in human cells with trisomies 13 and 18 [42] and aneuploidy of the X chromosome [44], in which we also found an increased level of LINE-1 methylation.

Moreover, we have shown that the levels of LINE-1 methylation are different in miscarriages with trisomies of different



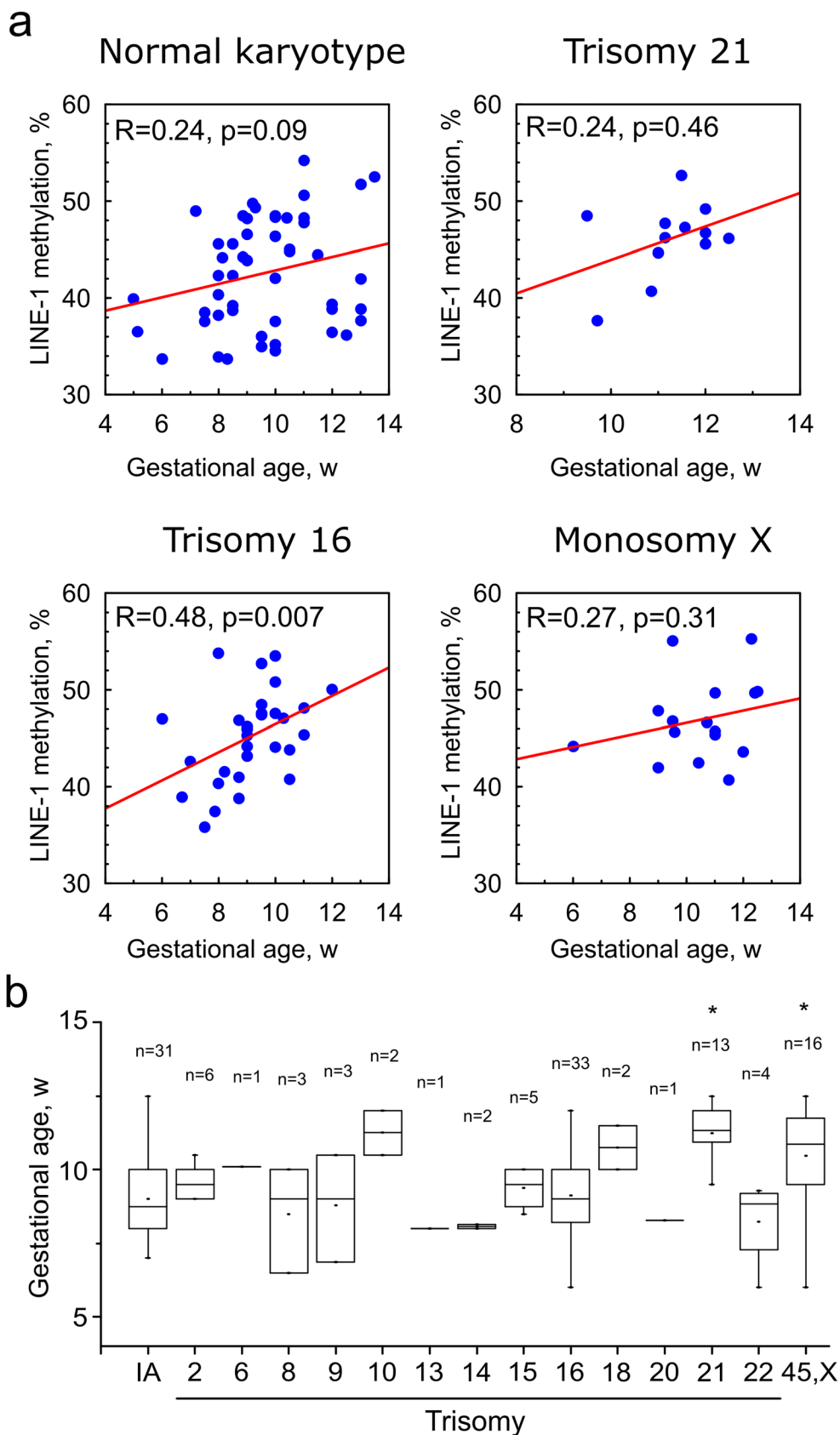
**Fig. 2** LINE-1 methylation in chorionic villi of miscarriages and parental age. **a** Correlation between the level of LINE-1 methylation and maternal and paternal ages in the groups of miscarriages with a normal karyotype,

trisomy 21, trisomy 16, and monosomy X. **b** Parental age of miscarriages with aneuploidy of different chromosomes

chromosomes, with the highest levels in trisomies 8, 14, and 18 and monosomy X. These chromosomes contain the key genes that ensure the function of the mechanism of LINE-1 control by pi-RNA and DNA methylation: *PIWIL2*, *PRMT5*, *MBD1*, and *MECP2*. *PIWIL2* is required for establishing de novo DNA methylation at retrotransposon sequences in male germ cells [45, 46], posttranscriptional suppression of retrotransposons in oocytes, and suppression of LINE-1 mobility in human induced pluripotent cells [29, 47–49]. *PRMT5*-dependent symmetric dimethylation of the histones H2AR3 and H4R3 may contribute directly to the repression of retrotransposons in hypomethylated mouse primordial germ cells and preimplantation embryos [50]. The mechanism of repression of L1 expression by DNMT3L DNA-methyltransferase involves members of the methyl-CpG-binding domain (MBD) proteins, specifically MeCP2, MBD1 [51, 52], and MBD2 [53], with genes localized on chromosomes X and 18. Other members of this family, MBD3 and MBD4, are localized on chromosomes 19 and 3, respectively, trisomy of which was not analyzed in this

study. Therefore, the increased level of DNA methylation in miscarriages with trisomy of these genes may be a result of upregulation of genes playing a role in genome defense against the LINE-1 retrotransposon. In contrast, chromosomes 9 and 10 contain genes with only minor roles in DNA methylation, and chromosome 15 has no genes annotated for DNA methylation in the Gene Ontology database. This may explain why the level of LINE-1 methylation did not increase in miscarriages with trisomies 9, 10, and 15. Chromosomes with variable levels of LINE-1 methylation in trisomic miscarriages contain several genes that are essential for DNA methylation but not particularly for defense against retrotransposons. Therefore, it is suggested that some additional factor(s) may affect the level of LINE-1 methylation in miscarriages with trisomy of these chromosomes.

The level of LINE-1 methylation increased with gestational age for both spontaneous abortions with normal karyotypes and aneuploidy of specific chromosomes. There are contradictory data regarding the stability of genome methylation levels in placental tissues during intrauterine development



**Fig. 3** LINE-1 methylation in chorionic villi of miscarriages and gestational age. **a** Correlation between the level of LINE-1 methylation and gestational age in the groups of miscarriages with a normal

karyotype, trisomy 21, trisomy 16, and monosomy X. **b** Gestational age for miscarriages with aneuploidy of different chromosomes. \* $p < 0.05$

[54–56]. A more detailed analysis showed that throughout gestation, the level of methylation in chorionic villi increased mainly in weak CpG islands and outside of CpG islands, while the level of methylation in the LINE-1 promoter remained relatively constant [23]. The increased level of LINE-1 methylation observed in our study may be the result of the accumulation of trisomy effects during the differentiation of chorionic villi.

We showed that the level of LINE-1 methylation depends on parental age. LINE-1 methylation decreases with maternal age in miscarriages with normal karyotypes and with trisomy 21. This effect is suggested to be a result of inheritance of parental gamete methylation marks in extraembryonic tissues, which recently has been shown for oocyte-derived methylation [57]. A maternal age-dependent decrease in the level of methylation was found for several genes in the cord blood of newborns [58] and even in one epigenome-wide association study at CpG sites near the *KLHL35* gene in the blood of newborns and adults [59]. However, to our knowledge, such maternal age-dependent hypomethylation was not found earlier for LINE-1 retrotransposon sequences, and there are no data on the age-dependent hypomethylation of LINE-1 retrotransposons in oocytes.

In contrast, in miscarriages with trisomy 16 and monosomy X, the level of LINE-1 methylation does not depend on maternal age but increases with paternal age. In accordance with the present results, previous studies have demonstrated the dependence of several epigenetic markers with paternal age. It is known, that LINE-1 is hypermethylated in the sperm of older individuals [60], and offspring of older fathers were shown to have hypermethylation of specific genes in the brain [61] and cord blood [62]. In mice, hypermethylation of IAP retrotransposons can be inherited by offspring through both the maternal and paternal lineages [63]. In contrast, the hypomethylation of gene promoters was found to be inherited from older father mice to offspring [64]. Moreover, paternal age impacts imprinting in placenta of mouse embryos [65]. Thus, our findings indicate that paternal age may affect the various epigenetic regulatory mechanisms in placenta including the regulation of LINE-1 retrotransposons.

May an increase in LINE-1 methylation affect the normal development of the placenta and embryo? One of the possible ways of such influence may be a disturbance of the programs of coordinated gene expression during embryogenesis. Evolutionary young LINE-1 retrotransposons can act as an alternative promoter for adjacent genes upregulated after global genome demethylation [66–68]. Among them, genes expressed in the brain, testes, placenta, embryonic tissues, and lungs are enriched [66]. Several such genes (*c-MET*, *JAK1*, *STK3/MST2*, etc.) play important roles in trophoblast differentiation and placental function [69–71]. Moreover, SINE, L1, and low-complexity repeats may barcode genes for their orchestrated regulation during development [72].

Thus, it is possible that an increased LINE-1 methylation index in chorionic villi of embryos with aneuploidy may lead to impaired gene expression regulation and subsequent death of the embryo.

The study was performed on embryos after natural conception, which makes it possible to ignore the potential effects of assisted reproductive technologies. However, one of the possible limitations is that the level of LINE-1 methylation was analyzed only in the chorionic villi and this level in other parts of the placenta and in the embryo remains unknown. In addition, we did not evaluate the effect of meiotic or mitotic origin of aneuploidy on the level of LINE-1 methylation. Finally, we cannot say exactly at what stage of embryonic development does the elevation of LINE-1 methylation occurred. However, it is most likely that this occurs during one of the epigenetic genome reprogramming waves, in gametogenesis, or during fertilization and at the cleavage stage.

Periods of epigenetic genome reprogramming and aneuploidy induction overlap with the time window at which assisted reproduction techniques are used. Therefore, the observed epigenetic changes associated with aneuploidy may be potentially enhanced by various manipulations of the embryo. This possibility requires attention to the study of epigenetic events associated with the occurrence of aneuploidy at the preimplantation stage of development.

## Conclusions

We have shown that the level of LINE-1 methylation is increased in the chorionic villi of miscarriages with trisomy and monosomy X. The different levels of LINE-1 methylation in miscarriages with trisomy of specific chromosomes indicate that the increase in LINE-1 methylation, at least in part, is the result of aneuploidy. In contrast, the effect of parental age indicates that the level of LINE-1 methylation may be inherited, suggesting an aneuploidy-independent mechanism of LINE-1 hypermethylation. Thus, our results indicate that the traditional view on the pathogenic effects of aneuploidy related to global gene expression deregulation can be supplemented with significant epigenetic changes in the largest compartment of the human genome.

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**Data availability** The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

## Compliance with ethical standards

**Conflicts of interest** The authors declare that they have no conflict of interest.

**Ethics approval** This study was performed in line with the principles of the Declaration of Helsinki. Approval was granted by the local Research Ethics Committee of the Research Institute of Medical Genetics, Tomsk NRMС.

**Consent to participate** Written informed consent was obtained from the parents of the patients for their participation.

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**Code availability** Not applicable.

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