Variation in DNA methylation in the KvDMR1 (ICR2) region in first-trimester human pregnancies

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Objective: To investigate the levels of DNA methylation in the KvDMR1 (KvLQT1 differentially methylated region 1) in embryonic and extra-embryonic tissues.
Design: Cross-sectional study.
Setting: University medical center and clinical hospital.
Patient(s): Embryonic and/or extraembryonic tissues (umbilical cord, chorionic villus, chorion, decidua, and/or amnion) collected from 27 first-trimester pregnancies (up to 12 weeks of gestation, single embryos) from elective abortions, extravillous trophoblasts (EVTs) from the top of individual chorionic villi, and chorionic villi from 10 normal full-term placentas collected after birth.
Intervention(s): None.
Main Outcome Measure(s): DNA methylation of the KvDMR1 region evaluated using quantitative analysis of DNA methylation followed by real-time polymerase chain reaction (qAMP) and bisulfite sequencing (bis-seq) analysis.
Result(s): The results showed variability in KvDMR1 DNA methylation in different tissues from the same pregnancy. The average of DNA methylation was not different between the embryo, umbilical cord, amnion, and chorionic villi, despite the relatively low level of methylation observed in the amnion (33.50% ± 14.48%). Chorionic villi from term placentas showed a normal methylation pattern at KvDMR1 (42.60% ± 6.08%). The normal methylation pattern at KvDMR1 in chorionic villi (as well as in EVTs) from first-trimester placentas was confirmed by bis-seq.
Conclusion(s): Our results highlight an existing heterogeneity in DNA methylation of the KvDMR1 region during first trimester and a consistent hypomethylation in the amnion in this period of gestation. (Fertil Steril® 2019;111:1186–93. Copyright ©2019 American Society for Reproductive Medicine, Published by Elsevier Inc.)
El resumen está disponible en Español al final del artículo.

Key Words: Bisulfite sequencing, DNA methylation, KvDMR1, pregnancy, real-time qPCR

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The disruption of epigenetics marks early in life in embryonic and fetal development can influence human biology and long-term health in adulthood (1, 2). Intrauterine adverse conditions appear to be related to developmental disorders and may lead to an increased risk of developing (metabolic) diseases during adulthood, such as type 2 diabetes mellitus and cardiovascular diseases (3–5).

Loss of imprinting has been reported for paternally or maternally imprinted genes in first-trimester placentas (6). The occurrence of loss of imprinting can vary between tissues from the affected individual, which may reflect different time points during the developmental window where it occurs (7). Epimutations in these genes have been detected in embryonic and extraembryonic tissues of individuals conceived both naturally or with the help of artificial reproductive techniques (ART) (8, 9). The disruption of imprinting has also been reported in first-trimester human placentas from uncomplicated pregnancies, reflecting a dynamic process during the development of this organ (6).

Imprinting control region 2 (ICR2), which includes the KvDMR1 (KvLQT1 differentially methylated region 1), mapped to human chromosome 11p15.5 (Fig. 1A), is responsible for the imprinting regulation of several genes involved in placental and embryonic growth and development (10). KvDMR1 is a differentially methylated region (DMR) at the maternal allele, located in an intronic CpG island at the *KCNQ1* (potassium voltage-gated channel subfamily Q member 1), also known as *KvLQT1* (potassium voltage-gated channel subfamily long QT syndrome member 1). This region overlaps the promoter region of the paternally expressed long-noncoding RNA *KCNQ1OT1* (*KCNQ1* overlap transcript 1), also known as LIT1 (long intronic transcript 1), which regulates the expression of neighboring imprinted genes such as the maternally expressed *CDKN1C* (cyclin-dependent kinase inhibitor 1C) (11).

Alterations at the KvDMR1 are related to growth and developmental syndromes (12) as well as some tumors in humans (13). The prenatal period seems to be a sensitive developmental time window for epigenome perturbations affecting long-term adult health (2, 14). Considering the importance of the KvDMR1-regulated genes associated with imprinting disorders and placental development, there are few reports about the regulatory plasticity of this region, and just a small set of genes have been investigated during the first trimester in the placenta (15). Thus, we sought to evaluate the DNA methylation levels in the KvDMR1 region in embryonic and extraembryonic tissues from several human pregnancies.

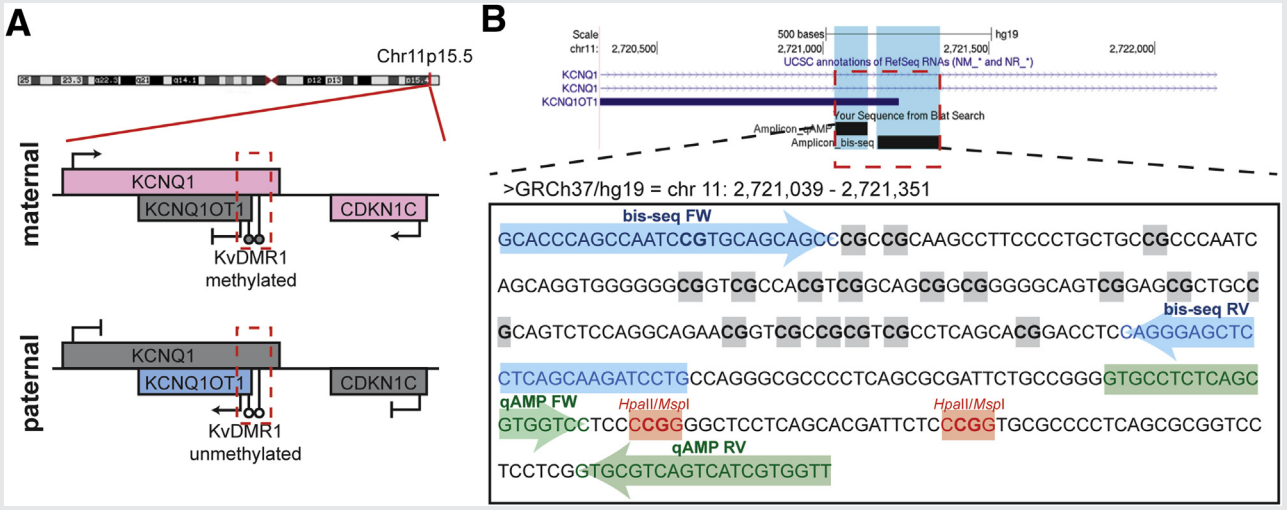
MATERIALS AND METHODS

Ethics Statement and Sample Collection

Sample collection and use of the material from the first trimester was approved by the medical ethics committee of Leiden University Medical Center in the Netherlands (P08.087), and from the third trimester was approved by the research ethics committee of the University Hospital of the Ribeirao Preto Medical School, University of São Paulo (HC-FMRP-USP) (Process CAAE #0091.0.004.000-04). All tissues analyzed were donated with informed consent.

Embryonic and extraembryonic tissues, such as umbilical cord, chorionic villus, and amnion were collected from uncomplicated, naturally conceived first-trimester pregnancies, up to 12 weeks of gestation with single embryos (Supplemental Table 1, available online), without medical indication. Samples were collected after elective abortion (with aspiration). Additionally, extravillous trophoblasts (EVTs), collected from the top of individual chorionic villus, individual chorionic villi, polled villi (bulk), and maternal decidua, as well as few samples of amnion, chorion, and umbilical cord were collected for

FIGURE 1



Structure of the KvDMR1 locus in humans. (**A**) Schematic representation of KvDMR1 imprinting domain in the Chr11p15.5. The methylation at the maternal allele (closed circles) in the intronic region of *KCNQ1* that overlaps with promoter of the *KCNQ1OT1*. The methylation at *KCNQ1OT* silences this gene, while the *CDKN1C* and *KCNQ1* are maternally expressed. In the unmethylated paternal allele (open circles) the *KCNQ1OT1* is expressed and *CDKN1C* and *KCNQ1* are paternally silenced. (**B**) Genomic position with marked the sequences of the human KvDMR1 (https://genome.ucsc.edu) analyzed by bisulfite sequencing (bis-seq) and qAMP. In bold, CpG dinucleotides; in blue, primer annealing region for bis-seq; in green, primer annealing region for qAMP; in red, restriction recognition-sites of HpaII/MspI enzymes.

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bisulfite sequencing (bis-seq) analysis. In total, 27 first-trimester pregnancies were collected.

Chorionic villi from 10 normal full-term placentas were collected after birth, single embryo, without gestational complications (see Supplemental Table 1). Individual chorionic villi were excised from each cotyledon immediately below the fetal surface. These fragments were washed in phosphate-buffered saline (PBS 1X) under a stereomicroscope (Olympus SZH10) to minimize blood contamination.

Isolation of Genomic DNA

Genomic DNA from embryonic and extraembryonic tissues as well as maternal decidua were isolated using the salting-out protocol (16) with some modifications. Briefly, samples were first washed in red-blood-lysis buffer (0.32 M sucrose, l% [v/v] Triton X-100, 5 mM $MgC1_2.6H_2O$, 12 mM Tris-HC1, pH 7.5) and centrifuged (13,000 *g*, 20 seconds). The sample pellets were then lysed (80 μL proteinase-K buffer [0.375 M NaCl, 0.12 M EDTA, pH 8.0], 8 μL proteinase K [25 mg/mL], 10 μL 20% sodium dodecyl sulfate, and 280 μL H_2O) overnight at 55°C with shaking. The samples were allowed to cool down, and 120 μL of 5 M NaCl was added. The samples were then shaken vigorously for 8 seconds and centrifuged (13,000 *g*, 5 minutes, 4°C). Next, 400 μL of the supernatant was mixed with 1 mL cold ethyl alcohol 98%, inverted a few times, and kept at −20°C overnight. The precipitated DNA was washed twice with cold ethyl alcohol 70% and centrifuged (13,000 *g*, 5 minutes, 4°C); the pellet was dried in air, resuspended in 50 μL H_2O, and stored at −20°C.

In EVTs and individual chorionic villi samples, the genomic DNA was lysed in 20 μL of 1 mg/mL proteinase K (Qiagen) in 10 mM Tris-HCl (pH 8.5) for 1 hour at 56°C and was directly used for bisulfite conversion. The DNA concentration and integrity were evaluated using a spectrophotometer (Nanodrop 2000c; Thermo Fisher Scientific).

Quantitative Analysis of DNA Methylation Using Real-Time Polymerase Chain Reaction

The quantification of KvDMR1 DNA methylation was carried out using quantitative analysis of DNA methylation using real-time PCR (polymerase chain reaction, qAMP) (17). Briefly, genomic DNA (400 ng) was digested with HpaII (New England Biolabs) or mock-digested without restriction enzyme, according to the manufacturer's instructions, in a final volume of 10 μL. The same amount of DNA was digested with the methylation-nonsensitive enzyme MspI (New England Biolabs), an *Hpa*II isoschizomer that was used as a control for digestion efficiency and to identify undesirable genetic polymorphisms.

Thereafter, primers flanking the restriction sites within CpG island at KvDMR1 (see Fig. 1B; Supplemental Table 2, available online) were used to amplify genomic DNA by quantitative PCR, as previously described elsewhere (18). The amplification was performed using 10 pmol of each primer and 2 μL of digested (*Hpa*II and *Msp*I) and mock-digested DNA, in 10 μL of final volume, using the StepOne Real-Time PCR system (Applied Biosystems) with SYBR Green PCR Master Mix (Applied Biosystems). Each reaction was performed in triplicate, and an average of the threshold cycle values was obtained. The relative quantification of DNA methylation at the KvDMR1 was calculated using the average threshold cycle of *Hpa*II digested (D) and undigested (UD) DNA using the formula $1/2^{(D\ -\ UD)}$ (18).

DNA Methylation Analysis by Bis-seq

Bisulfite conversion and PCR amplification. Genomic DNA was used for DNA bisulfite conversion using the EZ DNA Methylation-Lightning kit (Zymo Research) according to the manufacturer's instructions. The amplification of the KvDMR1 region was performed using Platinum-Taq DNA polymerase (Invitrogen) with primers containing adapters (see Fig. 1B; see Supplemental Table 2) as previously described elsewhere (19). The PCR conditions to amplify the KvDMR1 region were 95°C for 5 minutes, 50 cycles of denaturation at 94°C for 45 seconds, annealing at 59°C for 45 seconds and extension at 72°C for 45 seconds, and a final extension step at 72°C for 10 minutes.

Next-generation sequencing. Bis-seq was performed using MiSeq (Illumina). The PCR products, containing the adapters, were barcoded and converted to Illumina-compatible next-generation sequencing libraries, and the sequencing was performed using the 600bp V3 reagents kit according to the manufacturer's instructions. The FASTQ files for individual samples were generated using Illumina's pipeline (bcl2fastq2-v2-20). The adapter and indexes were removed from the sequence using Cutadapt v1.9.1 (20) software with settings: -q 30 –minimum-length 50. The paired read sequences were merged using default settings of FLASH v1.2.11 (21) and aligned to the bisulfite converted genome using Bismark v0.18.2 with settings: –ambig_bam (22), which was also used to count the reads with different methylation patterns. Visualization of the methylated CpGs in the regions of interest was performed based on Tabsat v1.0.2 (23).

Statistical Analysis

From a power calculation considering a mean difference of 28% between the groups and a standard deviation of 22%, it was necessary to include 15 samples per group to have 90% statistical power with $P<.05$. The nonparametric Mann-Whitney *U* test for independent samples was used to compare the percentage of DNA methylation at the KvDMR1 between first- and third-trimester chorionic villi. Kruskal-Wallis one-way analysis of variance was used to compare the average of DNA methylation between different tissues from pregnancies (embryo, umbilical cord, chorionic villus, and amnion). All statistical analysis was performed using the computing environment R version 3.5.1. $P<.05$ was considered statistically significant.

RESULTS

We analyzed the methylation pattern of KvDMR1 (see Fig. 1A) using the qAMP assay based on the analysis of two CpG hot spots (see Fig. 1B) in different tissues from 21 first-trimester pregnancies (Fig. 2A). The methylation pattern of KvDMR1

showed high variation among different tissues analyzed within each individual from the first-trimester pregnancies, ranging from 12% to 99% in the embryo (n = 16), 17% to 80% in the umbilical cord (n = 17), 14% to 61% in the amnion (n = 12), and 14% to 82% in chorionic villi bulk (n = 20) (see Fig. 2A). Comparing the average DNA methylation in the KvDMR1 region in the first-trimester tissues analyzed by qAMP, the amnion showed a relatively lower percentage of DNA methylation (33.50% ± 14.48%), but no statistically significant differences were observed (P=.233) when compared with the embryo (47.90% ± 20.83%), umbilical cord (46.82% ± 19.06%), or chorionic villi bulk (43.11% ± 20.99%) (see Fig. 2C).

Next, we analyzed DNA methylation in the KvDMR1 region in chorionic villi from third-trimester (full-term) placenta (n = 10) using qAMP and observed an average percentage of DNA methylation of 42.60% ± 6.08% (see Fig. 2C). When the average percentage of DNA methylation in the KvDMR1 was compared between full-term and first-trimester placenta (43.11% ± 20.99%) no statistically significant difference (P=.947) was observed (see Fig. 2C).

To compare the results obtained using qAMP (measuring DNA methylation at two CGs) with the methylation of a longer region with more CGs (19 CGs) in the imprinting control region (see Fig. 1B), we investigated the pattern of DNA methylation in the KvDMR1 region in the first-trimester placenta (N = 7 different placentas) using bis-seq. To study this in great detail, we used bulk chorionic villi (several villi pooled), individual chorionic villi (containing the inner core of extraembryonic mesoderm and trophectoderm-derived outer cells), and EVT collected from individual chorionic villi tops (containing only trophectoderm-derived outer cells and no extraembryonic mesoderm). In this way, it would be possible to distinguish imprinting defects on the trophectoderm-derived cells specifically.

The selected bis-seq region is adjacent (32 base pair distance) to the selected qAMP region (see Fig. 1B). Using bis-seq, we observed in the placentas analyzed (n = 7) consistently normal levels of DNA methylation between 35% and 65% in the chorionic villi (bulk and individual) analyzed, with few outliers per placenta (Fig. 3). This is suggestive of either biologic variation or technical bias, and it showcases the importance of having multiple samples for analysis. In addition, the individual EVTs of the placentas analyzed (n = 7) also showed normal levels of DNA methylation (see Fig. 3). The three samples of amnion included (T1–21, T1–24, T1–27) showed an average of methylated reads of 38.63% ± 3.25%. Unfortunately, we were unable to detect in the KvDMR1 region the presence of informative single-nucleotide polymorphisms, which would have allowed us to differentiate between DNA methylation in the maternal and paternal allele.

DISCUSSION

Our results showed a variation in the levels of DNA methylation in KvDMR1 region between pregnancies and also between different tissues of the same pregnancy. However, the embryos, umbilical cords, and chorionic villi (bulk, individual, or EVTs) presented average levels of DNA methylation between 35% and 65%, as expected for a bona fide imprinted region such as the KvDMR1 (24, 25). The amnion showed relatively lower levels of DNA methylation (33.50% ± 14.48% using qAMP, and 38.63% ± 3.25% using bis-seq) when compared with the other tissues analyzed but was not statistically significant. The bis-seq confirmed the normal DNA methylation levels at the KvDMR1 in placentas from the first-trimester obtained by qAMP. Using whole chorionic villi (bulk or individual) or villi tops consisting only the trophectoderm-derived ETVs showed similar results, suggestive of a normal imprinting pattern in the KvDMR1 region.

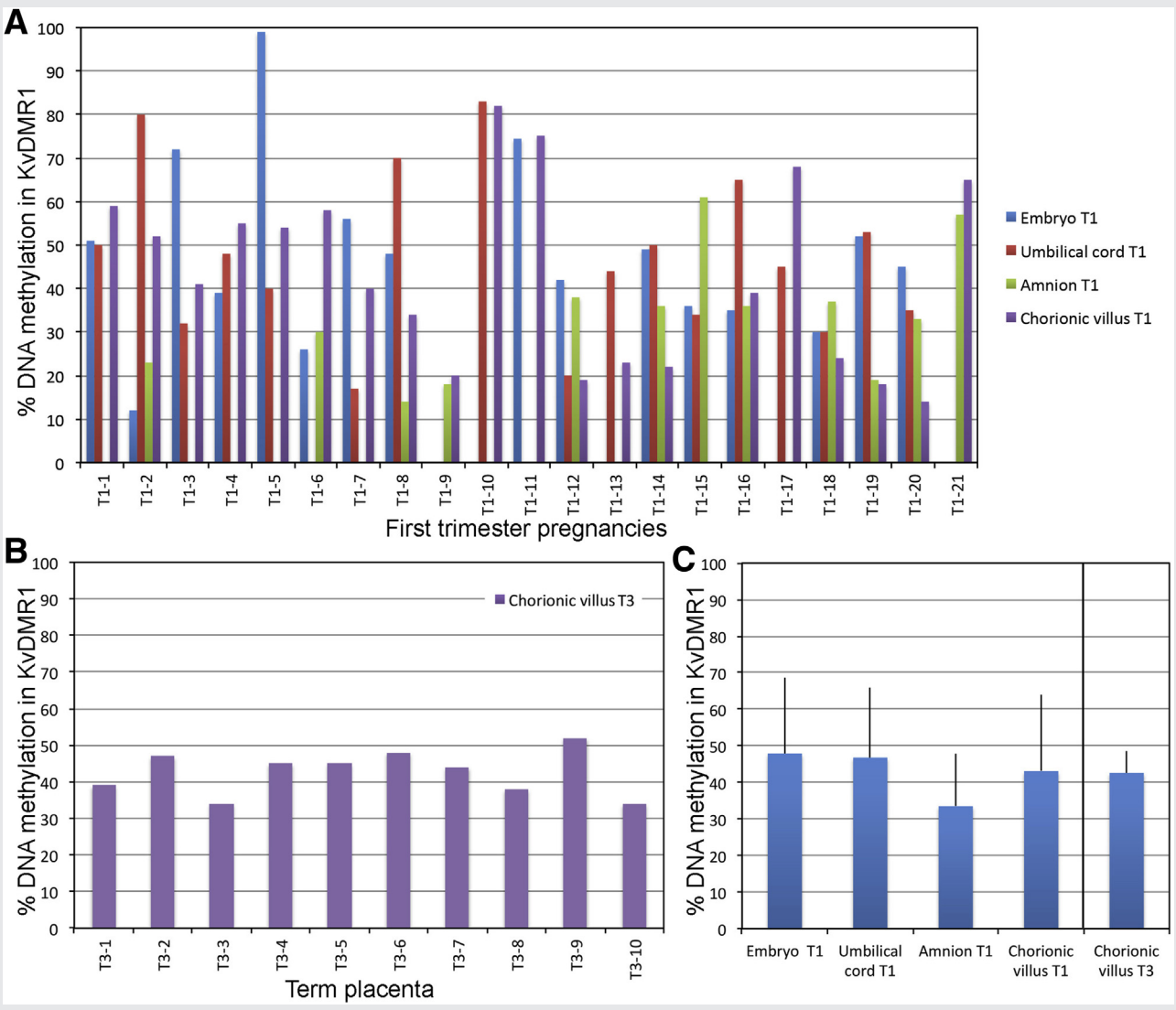
Although on average most (embryonic and extraembryonic) tissues showed a normal methylation pattern at the KvDMR1, individual samples presented hypomethylation and/or hypermethylation at this DMR. Alterations in the DNA methylation levels in the KvDMR1 region, such as hyper- or hypomethylation, could be related to gestational complications such as preeclampsia (26) or intrauterine growth restriction (27), or developmental syndromes such as Beckwith-Wiedemann syndrome (28), or could perhaps still be corrected during gestation (29). However, it is known that the level of DNA methylation in different DMRs may vary from the standard 50:50 ratio (24, 25, 30, 31). These values may be different among different tissues and even in individuals affected or unaffected by classical imprinting disorders (28, 32). The variation observed in the KvDMR1 region could reflect the physiologic fluctuations observed in normal adult tissues (25).

The methylation pattern based on parent-of-origin at ICR DMRs, which leads to parental-specific monoallelic gene expression, is generally maintained in somatic tissues. However, imprinting in the placenta is more dynamic, and some placenta-specific imprinted genes may escape epigenetic regulation (30, 31). Schroeder et al. (33) showed that 37% of the placental genome in the third trimester is covered by partially methylated domains, which are stable during gestation and between individuals. In gene bodies, disregarding the CpG islands, the average methylation was 48.2% in the partially methylated domains and 79.3% in the highly methylated domains. In promoter regions and CpGs islands, the percentage of DNA methylation was lower than in the other chromosomal domains.

The results from the qAMP technique were comparable to those obtained by bis-seq, suggesting that qAMP is a reliable technique to evaluate the levels of DNA methylation of a given region or gene in epigenetic studies. The qAMP technique is a quantitative method that can be used in a larger number of samples because quantitative real-time–based assays are relatively low cost when compared with other methods, and it is an accurate and sensitive method for the detection and quantification of nucleic acids (17).

Despite the variation observed at the KvDMR1, the absence of informative single-nucleotide polymorphisms does not allow us to conclude the parent-of-origin imprinting in the KvDMR1 region and hence interpret the variation observed. A limitation of this study is also the restricted number of pregnancies analyzed and the absence of karyotype information to exclude any genetic alterations that could alter the DNA methylation results. In addition, we cannot exclude

FIGURE 2



Percentage of DNA methylation in the KvDMR1 analyzed by qAMP. (**A**) Percentage of DNA methylation at the KvDMR1 region in embryonic and extraembryonic tissues of several first-trimester (T1) pregnancies. (**B**) Percentage of DNA methylation at the KvDMR1 region in chorionic villus of several term placentas (T3). (**C**) Average (and standard deviation) of the percentage of DNA methylation at the KvDMR1 region in embryos, umbilical cords, chorionic villi, and amnions from the first-trimester (T1) and chorionic villi from the term pregnancies (T3) analyzed.

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that some of the variation observed could have resulted from preanalytical technical bias, such as the DNA quality or efficiency of the enzymatic digestion. Hence, caution is necessary in interpreting the data.

Nevertheless, the KvDMR1 maps to an important ICR associated with human placental and embryonic growth and development. Developing and applying novel techniques can facilitate the evaluation of levels of DNA methylation for important ICRs during pregnancy, potentially predicting developmental and growth disorders such as Beckwith-Wiedemann syndrome. The variation observed in the levels of DNA methylation in the KvDMR1 region in embryonic and extraembryonic tissues is an important finding in understanding the epigenetic plasticity during the first trimester.

Alterations in KvDMR1 may be related to developmental disorders and long-term adult health. Loss of imprinting at this ICR may be a cause of recurrent miscarriage, and discerning the pattern of methylation may help us better understand early pregnancy loss before 20 weeks of gestational age. As the placenta and the intrauterine environment are essential for the proper development of the embryo, new efforts must be employed to understand the importance of different DMRs during pregnancy.

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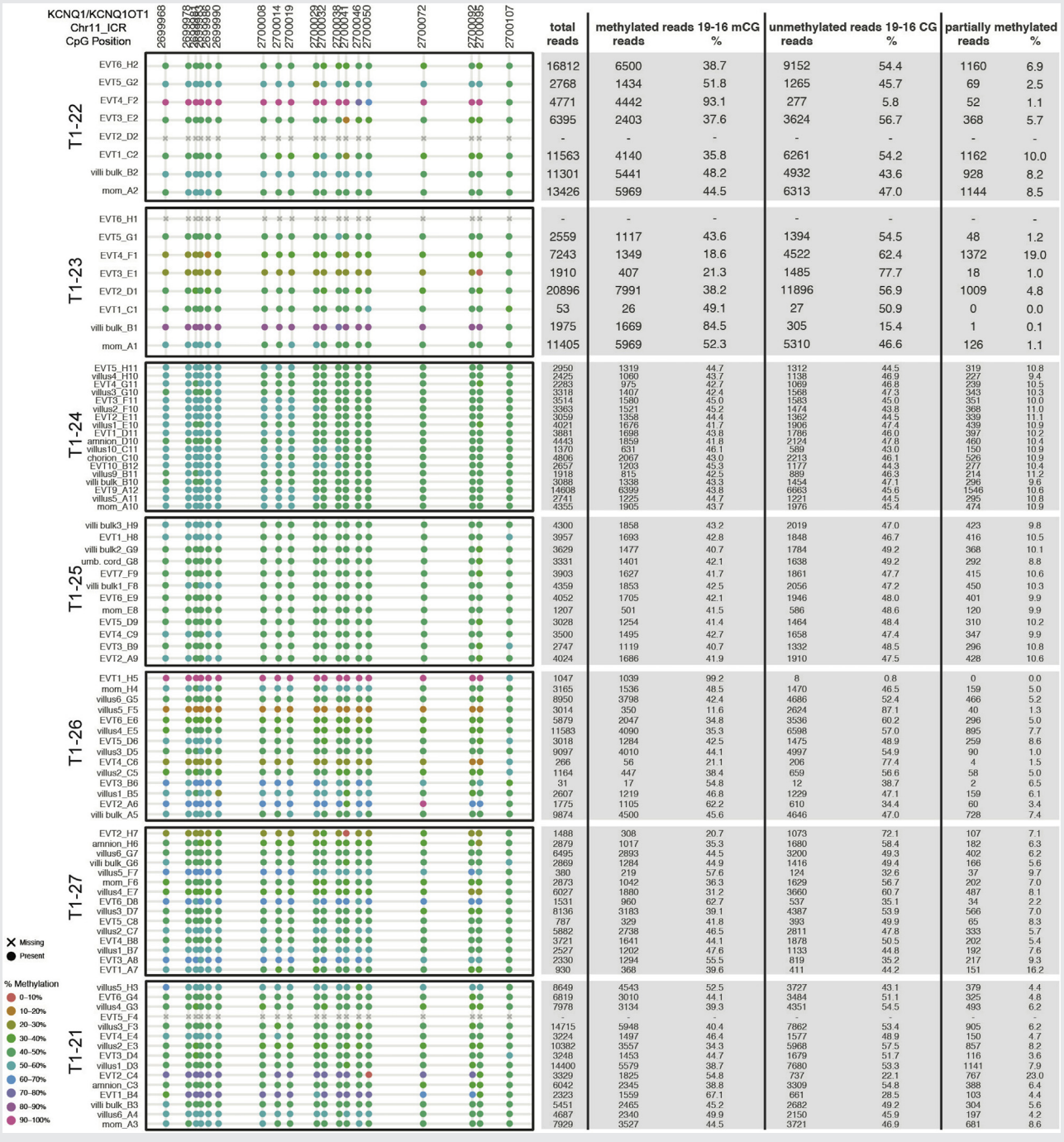
FIGURE 3



	total reads	methylated reads 19-16 mCG reads	%	unmethylated reads 19-16 CG reads	%	partially methylated reads	%
EVT6_H2	16812	6500	38.7	9152	54.4	1160	6.9
EVT5_G2	2768	1434	51.8	1265	45.7	69	2.5
EVT4_F2	4771	4442	93.1	277	5.8	52	1.1
EVT3_E2	6395	2403	37.6	3624	56.7	368	5.7
EVT2_D2	-	-	-	-	-	-	-
EVT1_C2	11563	4140	35.8	6261	54.2	1162	10.0
villi bulk_B2	11301	5441	48.2	4932	43.6	928	8.2
mom_A2	13426	5969	44.5	6313	47.0	1144	8.5
EVT6_H1	-	-	-	-	-	-	-
EVT5_G1	2559	1117	43.6	1394	54.5	48	1.2
EVT4_F1	7243	1349	18.6	4522	62.4	1372	19.0
EVT3_E1	1910	407	21.3	1485	77.7	18	1.0
EVT2_D1	20896	7991	38.2	11896	56.9	1009	4.8
EVT1_C1	53	26	49.1	27	50.9	0	0.0
villi bulk_B1	1975	1669	84.5	305	15.4	1	0.1
mom_A1	11405	5969	52.3	5310	46.6	126	1.1
EVT5_H11	2950	1319	44.7	1312	44.5	319	10.8
villus4_H10	2425	1060	43.7	1138	46.9	227	9.4
EVT4_G11	2283	975	42.7	1069	46.8	239	10.5
villus3_G10	3318	1407	42.4	1568	47.3	343	10.3
EVT3_F11	3514	1580	45.0	1583	45.0	351	10.0
villus2_F10	3363	1521	45.2	1474	43.8	368	11.0
EVT2_E11	3059	1358	44.4	1362	44.5	339	11.1
villus1_E10	4021	1676	41.7	1906	47.4	439	10.9
EVT1_D11	3881	1698	43.8	1786	46.0	397	10.2
amnion_D10	4443	1859	41.8	2124	47.8	460	10.4
villus10_C11	1370	631	46.1	589	43.0	150	10.9
chorion_C10	4806	2067	43.0	2213	46.1	526	10.9
EVT10_B12	2657	1203	45.3	1177	44.3	277	10.4
villus9_B11	1918	815	42.5	889	46.3	214	11.2
villi bulk_B10	3088	1338	43.3	1454	47.1	296	9.6
EVT9_A12	14608	6399	43.8	6663	45.6	1546	10.6
villus5_A11	2741	1225	44.7	1221	44.5	295	10.8
mom_A10	4355	1905	43.7	1976	45.4	474	10.9
villi bulk3_H9	4300	1858	43.2	2019	47.0	423	9.8
EVT1_H8	3957	1693	42.8	1848	46.7	416	10.5
villi bulk2_G9	3629	1477	40.7	1784	49.2	368	10.1
umb. cord_G8	3331	1401	42.1	1638	49.2	292	8.8
EVT7_F9	3903	1627	41.7	1861	47.7	415	10.6
villi bulk1_F8	4359	1853	42.5	2056	47.2	450	10.3
EVT6_E9	4052	1705	42.1	1946	48.0	401	9.9
mom_E8	1207	501	41.5	586	48.6	120	9.9
EVT5_D9	3028	1254	41.4	1464	48.4	310	10.2
EVT4_C9	3500	1495	42.7	1658	47.4	347	9.9
EVT3_B9	2747	1119	40.7	1332	48.5	296	10.8
EVT2_A9	4024	1686	41.9	1910	47.5	428	10.6
EVT1_H5	1047	1039	99.2	8	0.8	0	0.0
mom_H4	3165	1536	48.5	1470	46.5	159	5.0
villus6_G5	8950	3798	42.4	4686	52.4	466	5.2
villus5_F5	3014	350	11.6	2624	87.1	40	1.3
EVT6_E6	5879	2047	34.8	3536	60.2	296	5.0
villus4_E5	11583	4090	35.3	6598	57.0	895	7.7
EVT5_D6	3018	1284	42.5	1475	48.9	259	8.6
villus3_D5	9097	4010	44.1	4997	54.9	90	1.0
EVT4_C6	266	56	21.1	206	77.4	4	1.5
villus2_C5	1164	447	38.4	659	56.6	58	5.0
EVT3_B6	31	17	54.8	12	38.7	2	6.5
villus1_B5	2607	1219	46.8	1229	47.1	159	6.1
EVT2_A6	1775	1105	62.2	610	34.4	60	3.4
villi bulk_A5	9874	4500	45.6	4646	47.0	728	7.4
EVT2_H7	1488	308	20.7	1073	72.1	107	7.1
amnion_H6	2879	1017	35.3	1680	58.4	182	6.3
villus6_G7	6495	2893	44.5	3200	49.3	402	6.2
villi bulk_G6	2869	1284	44.9	1416	49.4	166	5.6
villus5_F7	380	219	57.6	124	32.6	37	9.7
mom_F6	2873	1042	36.3	1629	56.7	202	7.0
villus4_E7	6027	1880	31.2	3660	60.7	487	8.1
EVT6_D8	1531	960	62.7	537	35.1	34	2.2
villus3_D7	8136	3183	39.1	4387	53.9	566	7.0
EVT5_C8	787	329	41.8	393	49.9	65	8.3
villus2_C7	5882	2738	46.5	2811	47.8	333	5.7
EVT4_B8	3721	1641	44.1	1878	50.5	202	5.4
villus1_B7	2527	1202	47.6	1133	44.8	192	7.6
EVT3_A8	2330	1294	55.5	819	35.2	217	9.3
EVT1_A7	930	368	39.6	411	44.2	151	16.2
villus5_H3	8649	4543	52.5	3727	43.1	379	4.4
EVT6_G4	6819	3010	44.1	3484	51.1	325	4.8
villus4_G3	7978	3134	39.3	4351	54.5	493	6.2
EVT5_F4	-	-	-	-	-	-	-
villus3_F3	14715	5948	40.4	7862	53.4	905	6.2
EVT4_E4	3224	1497	46.4	1577	48.9	150	4.7
villus2_E3	10382	3557	34.3	5968	57.5	857	8.2
EVT3_D4	3248	1453	44.7	1679	51.7	116	3.6
villus1_D3	14400	5579	38.7	7680	53.3	1141	7.9
EVT2_C4	3329	1825	54.8	737	22.1	767	23.0
amnion_C3	6042	2345	38.8	3309	54.8	388	6.4
EVT1_B4	2323	1559	67.1	661	28.5	103	4.4
villi bulk_B3	5451	2465	45.2	2682	49.2	304	5.6
villus6_A4	4687	2340	49.9	2150	45.9	197	4.2
mom_A3	7929	3527	44.5	3721	46.9	681	8.6

KvDMR1 DNA methylation pattern analyzed by bisulfite sequencing (bis-seq). Extraembryonic tissues from seven different first-trimester human placentas (T1) and respective maternal decidua (mom) were analyzed. Extravillous trophoblast cells (EVTs) from individual chorionic villi, individual chorionic villi, and pooled chorionic villi (bulk) were analyzed. The average levels of DNA methylation per CpG per sample are represented by colored circles. On the right, a table shows [1] the total reads per sample; [2] methylated reads (containing between 19 and 16 methylated CpGs [mCG]) and percentage to the total reads; [3] unmethylated reads (containing between 19 and 16 unmethylated CpGs [CG]) and percentage to the total reads; and [4] partially methylated reads and percentage to the total reads.

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Variación en la metilación del ADN en la región KvDMR1 (ICR2) en el primer trimestre de embarazos humanos

Objetivo: Investigar los niveles de metilación del ADN en el KvDMR1 (KvLQT1 región 1 metilada diferencialmente) en tejidos embrionarios y extraembrionarios.

Diseño: Estudio transversal.

Entorno: Centro médico universitario y hospital clínico.

Paciente (s): Tejidos embrionarios y / o extraembrionarios (cordón umbilical, vellosidades coriónicas, corión, decidua y / o amnios) recogidos en el primer trimestre de embarazo (hasta 12 semanas de gestación, embriones únicos) de 27 abortos electivos, citotrofoblasto extravelloso (EVTs) de la parte superior de las vellosidades coriónicas individuales y vellosidades coriónicas de 10 placentas normales recogidas después del nacimiento.

Intervención (es): Ninguna.

Principales medidas de resultado: La metilación del ADN de la región KvDMR1 se evaluó mediante el análisis cuantitativo de la metilación del ADN, seguido de un análisis de la reacción en cadena de la polimerasa en tiempo real (qAMP) y el análisis de la secuenciación del bisulfito (bis-seq).

Resultado (s): Los resultados mostraron variabilidad en la metilación del ADN KvDMR1 en diferentes tejidos del mismo embarazo. El promedio de metilación del ADN no fue diferente entre el embrión, el cordón umbilical, el amnios y las vellosidades coriónicas, a pesar del nivel relativamente bajo de metilación observado en el amnios (33.50% ± 14.48%). Las vellosidades coriónicas de placentas a término mostraron un patrón de metilación normal en KvDMR1 (42.60% ± 6.08%). El patrón de metilación normal en KvDMR1 en las vellosidades coriónicas (así como en las EVTs) de las placentas del primer trimestre fue confirmado por bis-seq.

Conclusión (es): Nuestros resultados resaltan una heterogeneidad existente en la metilación del ADN de la región KvDMR1 durante el primer trimestre y una hipometilación consistente en el amnio en este período de gestación.