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Pluripotent stem cell–specific imprinted genes identified using human uniparental-induced pluripotent stem cells

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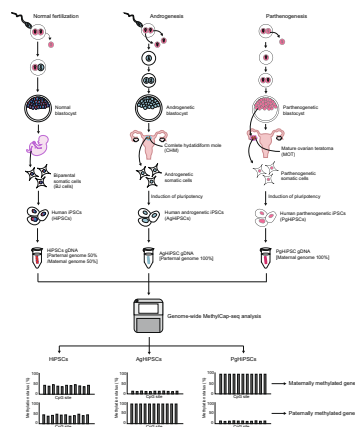
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HIGHLIGHTS

- Identified 26 novel imprinted DMRs using genome-wide methylation profiling of human uniparental iPSCs.
- Validated *DOCK4* and *LYNX1* as pluripotent stem cell–specific imprinted genes with distinct methylation patterns.
- *DOCK4* and *LYNX1* imprinting was absent in fibroblasts and adult tissues, indicating pluripotency specificity.
- *DOCK4* was highly expressed despite hypermethylation, while *LYNX1* showed an inverse methylation-expression correlation.

GRAPHICAL ABSTRACT

Experimental design for identification of imprinted genes through genome-wide methylation capture sequencing analysis. An outline of the experimental design to identify new imprinted genes using genome-wide methylation capture sequencing (MethylCap-seq) in three types of human induced pluripotent stem cells (hiPSCs): BipHiPSCs (biparental), AgHiPSCs (androgenetic), and PgHiPSCs (parthenogenetic). BipHiPSCs were derived from somatic cells acquired from the foreskin of a newborn, AgHiPSCs from somatic cells of a complete hydatidiform mole (CHM), and PgHiPSCs from somatic cells of a mature cystic teratoma (MCT). The analysis of DNA methylation patterns ensured the classification of genes with specific methylation differences across these HiPSCs as maternally or paternally imprinted.



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ABSTRACT

Genomic imprinting is an epigenetic mechanism in which gene expression is determined by the parent of origin. Imprinted genes are crucial for mammalian growth and development, and mutations at imprinting sites can lead to developmental disorders and diseases. Therefore, identifying novel imprinted differentially methylated regions (DMRs) is essential for understanding imprinting mechanisms and their implications in human biology. This study aimed to identify new imprinted DMRs using parthenogenetic and androgenetic human induced pluripotent stem cells (PgHiPSCs and AgHiPSCs), derived from mature cystic teratomas and complete hydatidiform moles, respectively. Genome-wide DNA methylation profiling via DNA methyl-capture sequencing (MethylCap-seq) was used to identify candidate imprinted DMRs. Subsequent bisulfite sequencing validated the methylation status, and comparative analyses confirmed pluripotency-specific imprinting. We identified 26 novel imprinted DMRs and excluded 6 that exhibited the absence of allelic imbalance or the presence of potential confounding by expression quantitative trait locus. Among the remaining 20, the DMRs in *DOCK4* and *LYNX1* were validated. They had distinct imprinted methylation and expression patterns unique to pluripotent stem cells and absent in fibroblasts and normal adult tissues. These findings highlight the utility of uniparental iPSCs for discovering imprinted genes specific to the pluripotent state and provide insights into the epigenetic regulation of early human development.

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Introduction

Genomic imprinting is an epigenetic phenomenon that regulates gene repression based on the parent of origin. This regulation is achieved primarily through differential DNA methylation established during germ cell development [1,2]. Many imprinted genes are crucial in fetal and placental growth, development, and adult behavior [3]. Proper fetal and placental development requires contributions from both the maternal and paternal genomes [4]. Consequently, uniparental embryos—derived exclusively from the maternal (parthenogenetic) or paternal (androgenetic) genome—fail to develop normally [5,6].

Numerous studies have investigated the methylation status of embryonic stem cells (ESCs) derived from mouse and human uniparental embryos [7–11]. Since uniparental ESCs originate from a uniparental genome, they have proven valuable in studying genomic imprinting and the roles of imprinted genes during development [12–14]. However, generating human uniparental ESCs requires embryos to be manipulated through procedures such as *in vitro* fertilization, raising ethical concerns. In rare cases, human parthenogenesis may lead to the formation of ovarian mature cystic teratomas (MCTs) [15], whereas androgenetic complete hydatidiform moles (CHMs) can form when an oocyte lacking the nucleus is fertilized by one or two sperm cells [16–18]. The CHMs used to derive AgHiPSCs in our previous study were confirmed to be of monospermic origin, resulting from the fertilization of an enucleated oocyte by a single sperm. This monospermic origin was verified in our previous study [19] through genome-wide SNP array analysis, showing over 99% homozygosity across the genome—consistent with a single paternal genome contribution and clearly distinct from dispermic fertilization, which yields allelic heterogeneity [19–21]. Previously, we also succeeded in generating human parthenogenetic induced pluripotent stem cells (PgHiPSCs) from these spontaneously occurring MCTs [22]. This approach attempts to address the ethical concerns associated with human embryo manipulation.

Although comparative methylation analyses between MCTs and CHMs have led to the discovery of novel imprinted differentially methylated regions (DMRs) [23], these tissues consist of heterogeneous cell populations, which may harbor tissue-specific methylation profiles. While uniparental embryos and PgHiPSCs have proven invaluable tools for studying genomic imprinting [24–26], previous methodologies have resulted in incomplete comparisons between parthenogenomes and androgenomes due to the use of different tis-

sue sources or the lack of an androgenetic counterpart, such as AgHiPSCs.

This study aimed to overcome these limitations by conducting a comprehensive screening for new imprinted DMRs. Using DNA methyl-capture sequencing (MethylCap-seq), we analyzed biparental human induced pluripotent stem cells (BipHiPSCs), PgHiPSCs, and AgHiPSCs. The overall experimental design is illustrated in the graphical abstract. This approach allowed for direct comparisons between parthenogenomes and androgenomes, providing a useful tool for discovering new imprinted genes and a more thorough understanding of genomic imprinting in cells of different parental origins.

Materials and methods

Cell culture

PgHiPSCs and AgHiPSCs were generated and characterized in previous studies and were cultured according to established protocols [19,22]. This study was approved by the Institutional Review Board of Konkuk University Medical Center (KUH 1040045). All fibroblastic cells—biparental somatic cells (BipSCs: BJ cells) obtained from the American Type Culture Collection, androgenetic somatic cells (AgSCs) derived from CHMs, and parthenogenetic somatic cells (PgSCs) derived from MCTs—were cultured in fibroblast medium, which consisted of Dulbecco's modified Eagle medium (DMEM; Welgene) supplemented with 10% fetal bovine serum (FBS; Hyclone), 1% non-essential amino acids (Invitrogen), and 1% penicillin–streptomycin (Welgene). All iPSC lines, including BipHiPSCs, AgHiPSCs, and PgHiPSCs, as well as the hESC line, H9, were cultured in mTeSR 1 medium (StemCell Technologies) on Matrigel (Corning)-coated plates.

RNA isolation and quantitative real-time PCR

Total RNA was isolated using a miRNeasy Mini kit (Qiagen). The RNase-Free DNase set (Qiagen) was used to remove genomic DNA from total RNA. Approximately 1 µg of total RNA was reverse transcribed with a High-Capacity cDNA Reverse Transcription kit (Applied Biosystems). Quantitative real-time PCR was performed using SYBR Green (Enzynomics) in a 7500 Real-Time PCR System (Applied Biosystems). Primer sequences are listed in Table S1.

Genomic DNA isolation and bisulfite treatment

Genomic DNA from human liver, heart, and brain was purchased from MyBioSource. DNA from cell lines was extracted using a G-spin Total DNA Extraction kit. Bisulfite conversion was performed with an EpiTect Bisulfite kit (Qiagen) using a thermal cycling protocol. The bisulfite-treated DNA was purified with an EpiTect spin column and eluted in 20 μ L of elution buffer, following the manufacturer's instructions.

Methyl-capture sequencing

Genomic libraries were prepared using the SureSelect XT Methyl-seq kit (Agilent Technologies). MethylCap-seq was conducted using an Illumina HiSeq 2500 with 2×101 bp paired-end reads following the manufacturer's instructions. The quality control of sequences was performed using FastQC.33. The bisulfite-treated reads were aligned to the reference human genome (hg19) using Bismark34 and Bowtie2.35. Duplicate read removal and methylation extraction were performed using Bismark34. The MethylCap-seq data were deposited in the GEO under the accession number GSE146202.

Rna-sequencing analysis

Total RNA quality was assessed using an Agilent 2100 Bioanalyzer. mRNA libraries were prepared using the Illumina TruSeq Stranded mRNA kit and evaluated for quality and size. Libraries were quantified by qPCR and sequenced using an Illumina NextSeq 500 with paired-end 75 bp reads.

Differentiation of pluripotent stem cell into neural stem cells

PgHiPSCs, AgHiPSCs, and BipHiPSCs were differentiated into neural stem cells (PgNSCs, AgNSCs, and BipNSCs, respectively) using our previously established protocol for iPSC-to-NSC differentiation [27]. Bisulfite sequencing was then performed on DNA extracted from differentiated NSCs to assess the methylation status of the candidate pluripotency-specific imprinted gene.

Bisulfite sequencing analysis

For each iPSC line, the same biological samples used for MethylCap-seq were used for subsequent bisulfite sequencing validation, unless otherwise stated. PCR with bisulfite treated genomic DNA was conducted using a PyroMark PCR kit (Qiagen) for 45 cycles with the primers listed in Supplementary Table S1. The PCR products were cloned using the PCR Cloning kit (Qiagen). Individual clones were isolated using the ExpRep Plasmid SV Mini kit (GeneAll Biotechnology) and sequenced using the T7 promoter primer. The sequence data were analyzed using the Quantification Tool for Methylation Analysis (QUMA; <https://quma.cdb.riken.jp>).

Additional filtration for imprinting

To avoid false imprinting caused by methylation quantitative trait loci (mQTLs), we analyzed differential methylation according to alleles of single-nucleotide polymorphisms (SNPs) near identified methylation sites. MethylCap-seq data from biparental cell lines (BipHiPSCs and H9) covering novel and known imprinted DMRs were examined to identify biallelic sequences within each cell line. The assessment of differential methylation was based on the presence or absence of CpG sites near the SNP within the same sequencing read, with Bonferroni multiple testing correction applied.

We conducted large structural variant analysis to exclude potential spurious imprinting signals that might be confounded by copy number variation (CNV) or structural rearrangements. We analyzed RNA-seq data to estimate CNV using the Control-FREEC toolkit [28]. The DMRs identified in this study were compared with previously reported structural variants (including CNVs, inversions, and insertions/deletions) available in the UCSC Genome Browser (<https://genome.ucsc.edu>), as annotated in the Human Genomic Structural Variation database (dbVar) at the National Center for Biotechnology Information (NCBI) and the Database of Genomic Variants (DGV) at the European Bioinformatics Institute (EBI).

DMRs with biallelic expression equally contributed by both parents were excluded. SNPs in genes with imprinted DMRs were identified using RNA-seq data from BipHiPSCs and H9, followed by differential expression analysis of the selected SNPs. The SNP with the highest read count, corresponding to dbSNP153, was chosen, with a minimum of 20 total reads and at least 3 reads per allele. Deviation from equivalent expression between alleles was assessed using a binomial test for heterozygous cell lines; Bonferroni correction was applied in multiple tests.

Statistical analysis

Data are presented as mean $\pm$ standard error of the mean (SEM) for each experimental group. Statistical significance was assessed using Student's *t*-test for comparisons between groups. A *p*-value of < 0.05 was considered statistically significant.

Results and discussion

Genome-wide analysis of CpG methylation profiles in human biparental and uniparental cells

To investigate imprinting dynamics in human pluripotent stem cells, we performed genome-wide DNA methylation profiling using MethylCap-seq. This approach captured methylation information at over 3 million CpG sites, including known imprinted DMRs, promoters, and other regulatory elements [29]. Our analysis included biparental somatic cells (BJ cells; BipSCs), androgenetic and parthenogenetic somatic cells (AgSCs and PgSCs), human ESCs (H9), and reprogrammed biparental and uniparental iPSCs (BipHiPSCs, AgHiPSCs, and PgHiPSCs) showing pluripotency-specific gene expression (Fig. 1A and 1B).

High Pearson correlation coefficients (> 0.9) among pluripotent stem cells (AgHiPSCs, PgHiPSCs, BipHiPSCs, and H9; Fig. 2C) indicated highly similar global methylation patterns. In hierarchical clustering and principal component analysis, these cells clustered together and were clearly distinguishable from somatic cells (Fig. 1D and 1E, respectively). Notably, somatic cells (AgSCs, PgSCs, and BipSCs) had unique methylation signatures, suggesting tissue-specific epigenetic regulation. This observation aligns with previous studies indicating that epigenetic memory in somatic cells influences their reprogramming efficiency [30]. Our findings further emphasize that while iPSC lines largely recapitulate the methylation landscape of ESCs, underlying differences in imprinting persist, potentially affecting differentiation potential.

Identification and validation of known imprinted DMRs

To identify candidate imprinted regions, we performed genome-wide DNA methylation profiling of AgHiPSCs, PgHiPSCs, and biparental BipHiPSCs. Methylation levels were categorized as hypomethylation ($\beta \leq 0.3$), hemi-methylation ($0.3 < \beta < 0.7$), and hypermethylation ($\beta \geq 0.7$). Candidate DMRs were screened based

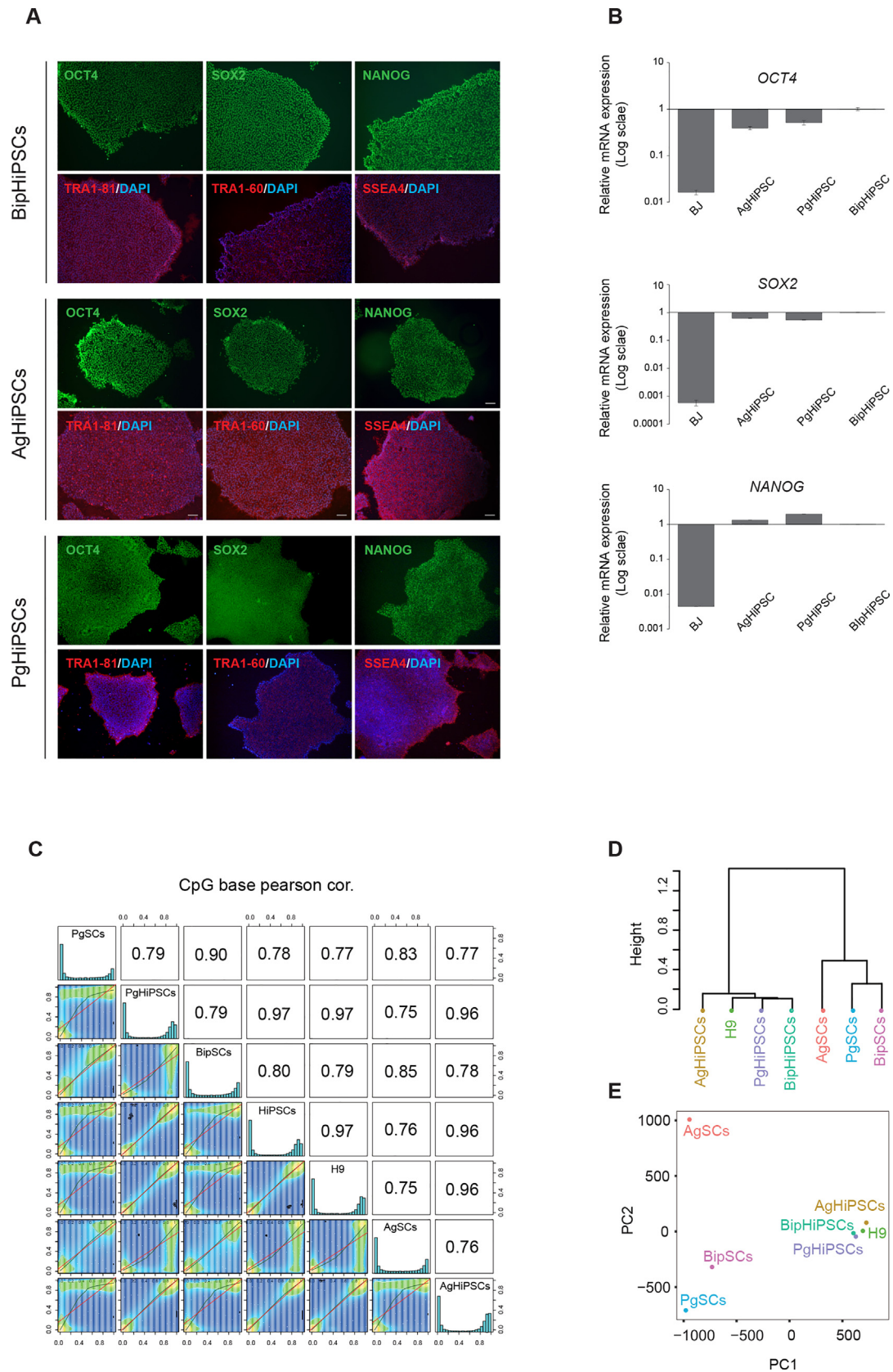


Fig. 1. Characterization and genome-wide analysis of CpG methylation profiles in human biparental and uniparental cells. (A) Immunocytochemical staining of pluripotency markers (*OCT4*, *SOX2*, *NANOG*, *TRA1-81*, *TRA1-60*, and *SSEA4*) in BipHiPSCs, AgHiPSCs, and PgHiPSCs. Scale bars = 100 μ m. (B) Quantitative RT-PCR analysis of pluripotency marker genes (*OCT4*, *SOX2*, and *NANOG*). Expression levels in BipHiPSCs were normalized to 1. Data are presented as mean $\pm$ SEM ($n = 3$). (C) Scatter plots below the diagonal, histograms on the diagonal, and the Pearson's correlation coefficients above the diagonal were calculated using Pearson's correlation analysis. (D) Unsupervised hierarchical clustering based on methylation profiles. (E) Principal component analysis (PCA) of DNA methylation profiles showing the relationships among AgSCs, PgSCs, BipSCs, and pluripotent stem cell clusters.

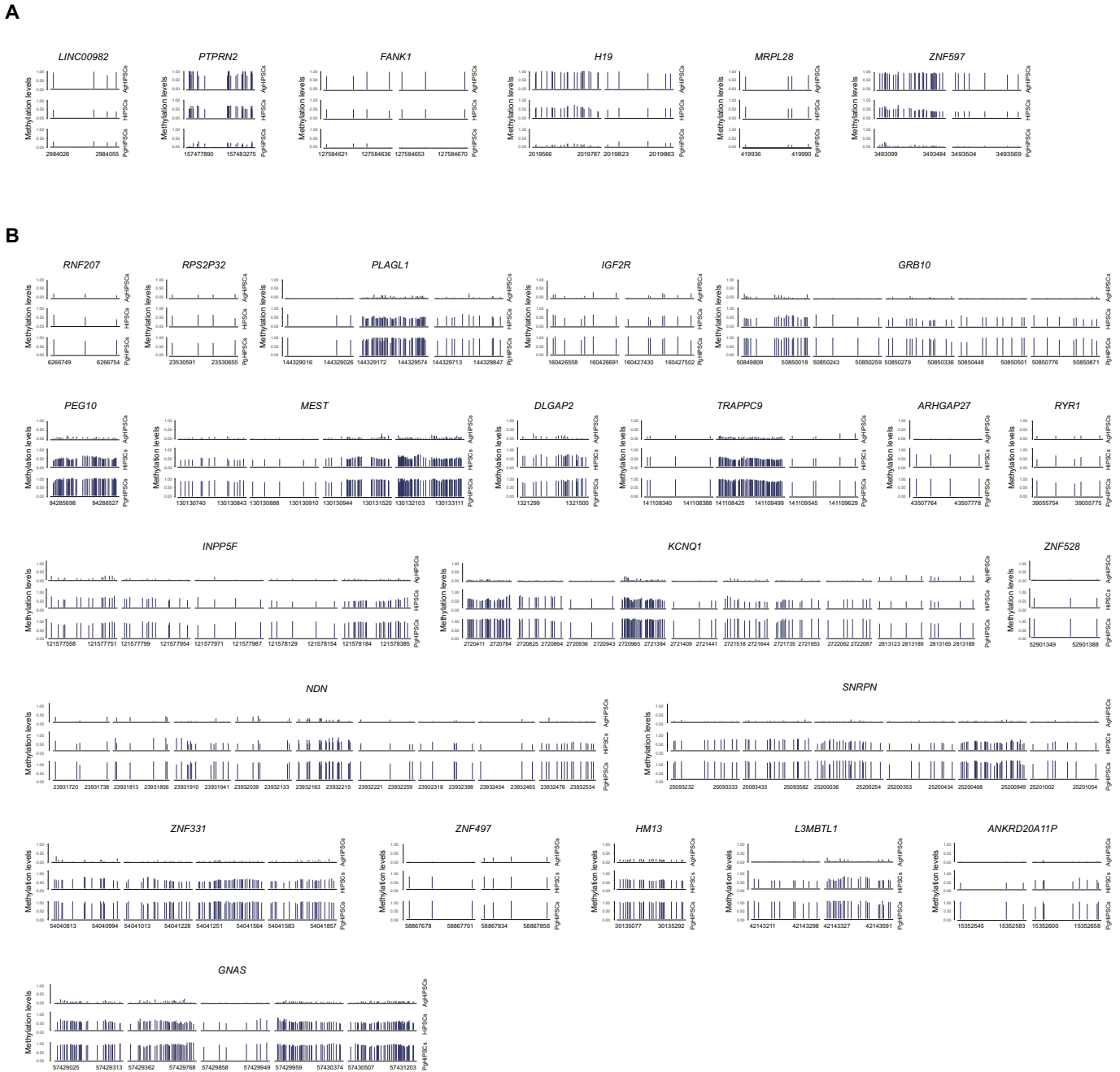


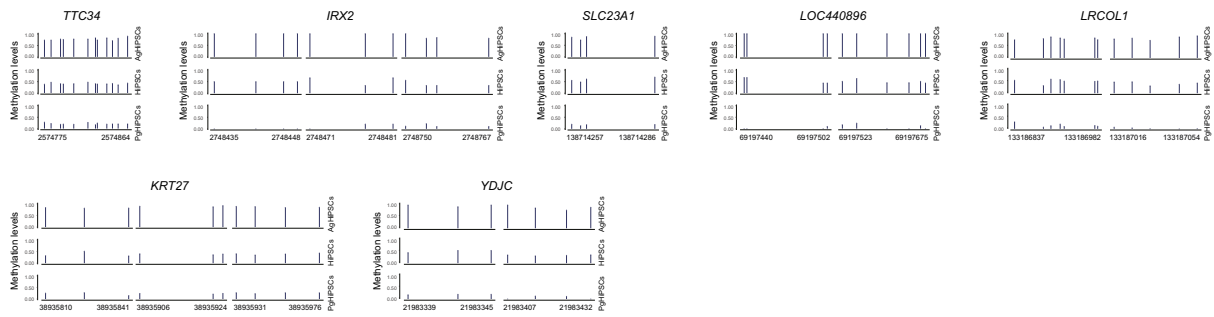
Fig. 2. Identification of known imprinted DMRs in human biparental and uniparental cells by MethyCap-Seq analysis. We compared the methylation profiles among BipHiPSCs, AgHiPSCs, and PgHiPSCs to identify the DNA methylation status of known imprinted DMRs. Information on these DMRs was obtained from the Genomic Imprinting Database (<http://www.geneimprint.com>). (A) For paternal DMRs, we set the methylation criteria to ≥ 0.7 in AgHiPSCs, $0.3-0.7$ in BipHiPSCs, and ≤ 0.3 in PgHiPSCs, and identified 6 known paternal DMRs. (B) For maternal DMRs, set the methylation criteria as ≤ 0.3 in AgHiPSCs, $0.3-0.7$ in BipHiPSCs, and ≥ 0.7 in PgHiPSCs, and identified 22 known maternal DMRs.

on combined methylation patterns across all three cell types: regions where AgHiPSCs were hypomethylated ($\beta \leq 0.3$) and PgHiPSCs were hypermethylated ($\beta \geq 0.7$) while BipHiPSCs exhibited hemi-methylation ($0.3 < \beta < 0.7$) were classified as maternal DMR candidates. Conversely, regions where PgHiPSCs were hypomethylated and AgHiPSCs were hypermethylated with hemi-methylation in BipHiPSCs were classified as paternal DMR candidates.

Using this screening strategy, a total of 54 candidate DMRs were identified, including 41 maternal and 13 paternal regions. To confirm the reliability of our approach and the iPSC models,

we first checked whether known imprinted DMRs were captured among these candidates. Among the 13 paternal candidates, 6 corresponded to previously known paternal DMRs, while among the 41 maternal candidates, 22 corresponded to previously known maternal DMRs (Fig. 2A and 2B). The detection of these known imprinted regions validates both our screening method and the suitability of the iPSC models for imprinting analysis. Importantly, the presence of these DMRs in our iPSC models confirms that imprinting is largely preserved during reprogramming, although epigenetic alteration specific to pluripotency may also occur [30].

A



B

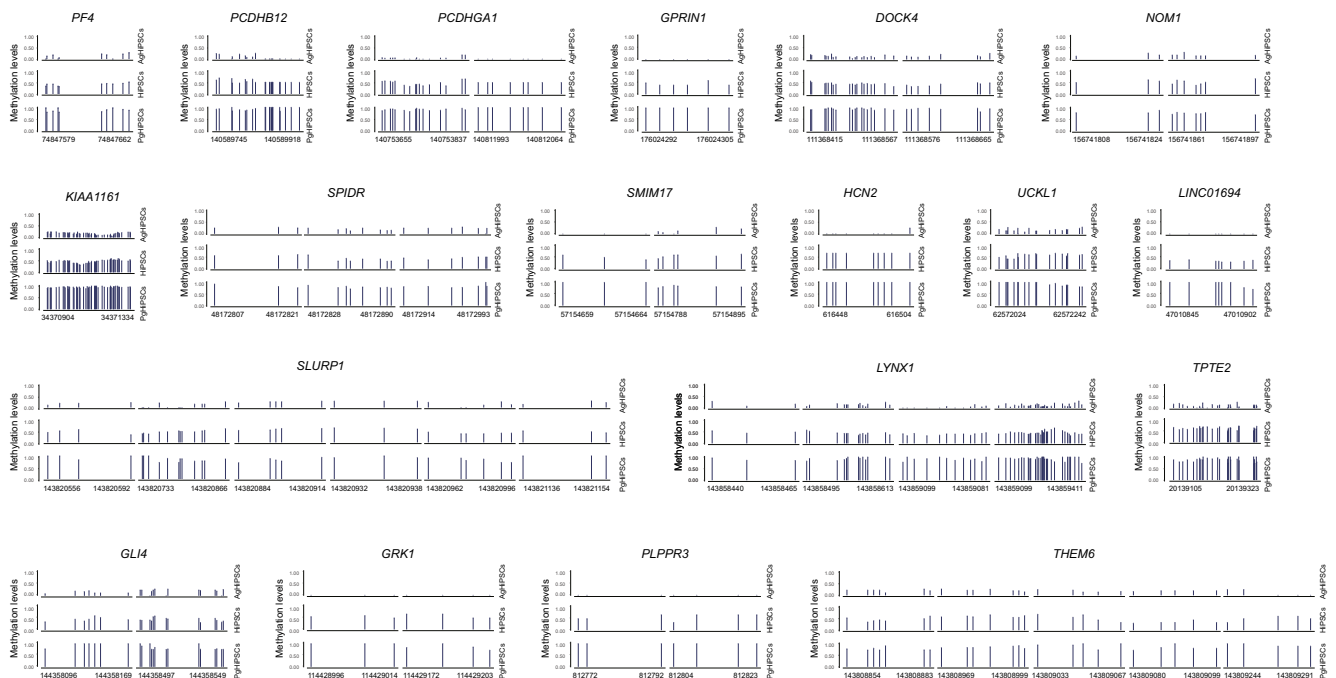


Fig. 3. Discovery of new imprinted DMRs by methyl-capture sequencing. We used the same criteria as in Fig. 2 to discover new DMRs. Our analysis revealed 7 new paternal DMRs (A) and 19 new maternal DMRs (B).

Discovery of novel imprinted DMRs

Expanding our analysis beyond known imprinted DMRs, we identified the remaining 26 novel imprinted DMRs—7 paternal and 19 maternal—on the basis of methylation profiles across biparental and uniparental iPSCs (Fig. 3A and 3B). The discovery of these previously uncharacterized imprinted regions suggests that additional layers of epigenetic regulation may be involved in maintaining pluripotency. Since imprinted genes play critical roles in embryonic development and cell fate determination, further investigation into the functions of these genes in pluripotent stem cells is warranted. Overall, we mapped the known and new imprinted DMRs onto human chromosomes (Fig. S1).

We compared the DMRs identified in this study with the imprintome dataset, which compiles candidate imprinting regulatory elements associated with DNA methylation across human germ layers and gametes [31]. Among the 54 DMRs identified in

our analysis, 19 overlapped with regions reported in the imprintome, the majority of which (18) correspond to previously known DMRs. In contrast, only one of the 26 novel DMRs overlapped, spanning chromosome 4 (74847593–74847662 bp, hg19) and representing part of the PF4 imprinted locus (ICR_271).

The greater overlap observed for known DMRs likely reflects their localization within well-characterized genomic domains that are reproducibly detected across studies. In contrast, the limited concordance with novel DMRs may result from differences in cellular context, sample composition, and/or analytical methodologies between datasets. Notably, this restricted overlap underscores the sensitivity and specificity of our analytical approach, suggesting that the newly identified regions represent previously unrecognized, pluripotency-specific imprinted loci that are not readily captured by conventional phase-unresolved or population-based imprintome analyses.

Filtering genes with uncertain imprinting status

To avoid false positive imprinting caused by different nucleotide sequence variants, we identified SNPs near 2 of the 26 DMRs (Table S2) but found no evidence for their potential mQTL effects. No differential methylation was observed ($P_t > 5.56 \times 10^{-3}$) between the A and C alleles of an intronic variant (rs28406517) in *SMIM17*. Although differential methylation was detected ($P_t < 5.56 \times 10^{-3}$)

between the C and T alleles of a 5'UTR variant (rs10870480) in *LRCOL1*, the allelic effect of rs10870480 was associated with cell lines: H9 and BipHiPSCs ($P_{\chi^2} < 1.67 \times 10^{-2}$). This suggests that the allelic effect was inconsistent across cell lines. Specifically, the allelic effect was reversed, with the T allele exhibiting a higher methylation rate in H9, whereas the C allele showed a higher methylation rate in BipHiPSCs. Therefore, the mQTL effect of rs10870480 in the imprinting of *LRCOL1* was considered negligible.

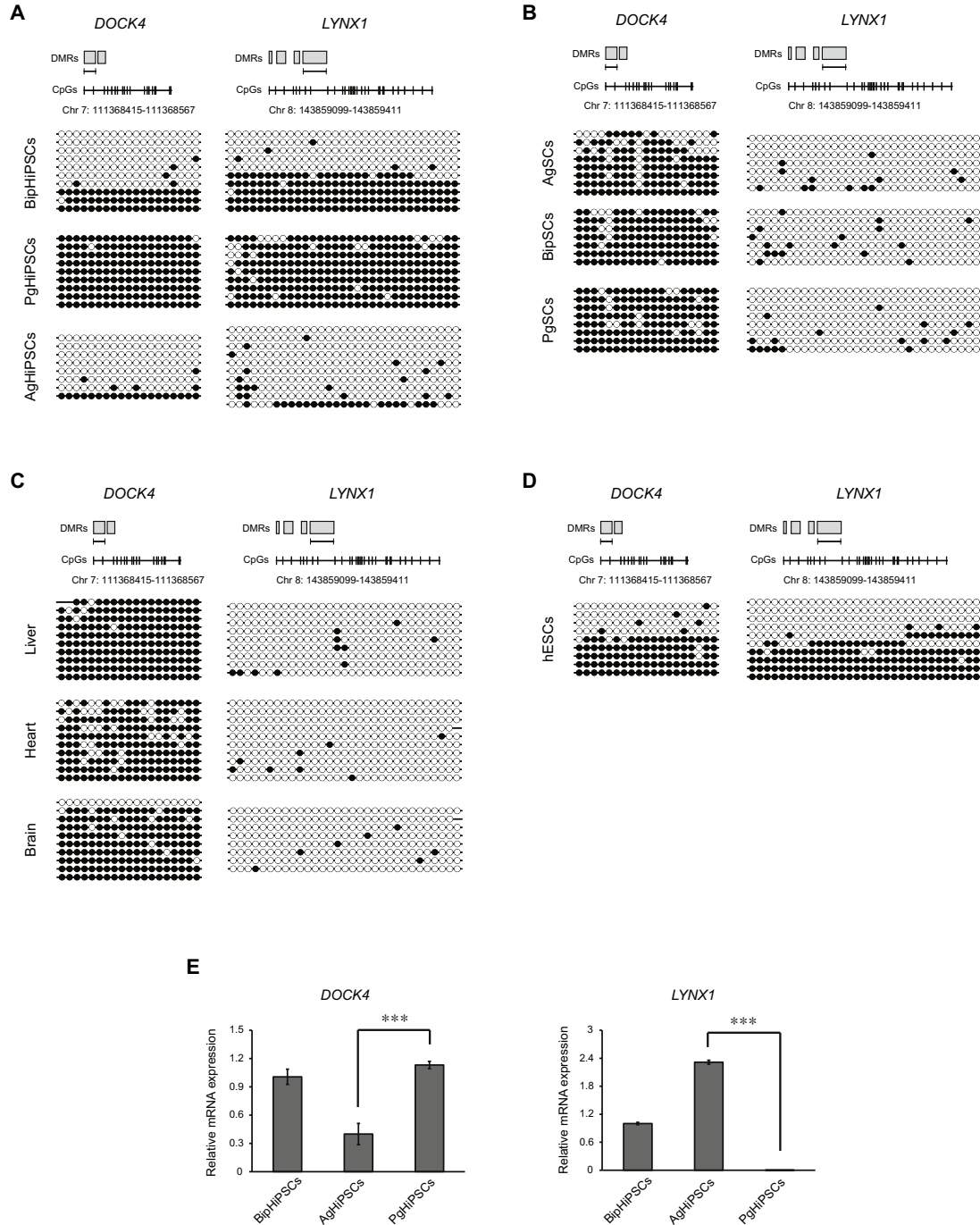


Fig. 4. Validation of DNA methylation and gene expression correlation for *DOCK4* and *LYNX1*. (A) Bisulfite sequencing confirmed the DNA methylation status of imprinted DMRs in *DOCK4* and *LYNX1*, identified by MethylCap-seq. *DOCK4* and *LYNX1* were hemi-methylated in BipHiPSCs, hypomethylated in AgHiPSCs, and hypermethylated in PgHiPSCs. (B) In somatic cells (AgSCs, BipSCs, and PgSCs), *DOCK4* was hypermethylated, while *LYNX1* lacked an imprinted methylation pattern. (C) Bisulfite sequencing of human somatic tissues (liver, heart, and brain) revealed no imprinted methylation in *DOCK4* or *LYNX1*. (D) Both DMRs of *DOCK4* and *LYNX1* were hemi-methylated in the human ESC line, H9, which confirmed their pluripotency-specific imprinting. (E) Gene expression analysis by quantitative PCR showed higher expression of *DOCK4* in hypermethylated PgHiPSCs than in hypomethylated AgHiPSCs, while *LYNX1* expression was higher in hypomethylated AgHiPSCs than in hypermethylated PgHiPSCs, indicating inverse regulation by DNA methylation. Data are mean $\pm$ SEM, *** $p < 0.001$.

Similarly, we investigated whether large structural variants might underlie or confound the identified DMRs. However, no putative large structural variants were detected or found to overlap with any of the DMRs identified in the present study. We next assessed potential false-positive DMRs using intragenic SNPs identified from RNA-seq data. Under the assumption of imprinting-associated DMRs, transcripts from heterozygous individuals are expected to exhibit extremely skewed allelic expression. Among the analyzed loci, 17 DMRs contained intragenic SNPs; of these, eight DMRs showed no significant allelic expression imbalance ($P > 2.94 \times 10^{-3}$; Table S3; Fig. S2). Additionally, the DMRs corresponding to HCN2 and MRPL28 were further excluded because their apparent imprinting effects could be confounded by expression quantitative trait loci (eQTL) encompassing the same intragenic SNPs, as identified in the GTEx dataset (Table S3). Consequently, six genes (GPRIN1, IRX2, NOM1, UCKL1, YDJC, and HCN2) were removed from the set of novel imprinted genes due to either lack of allelic imbalance or potential confounding by eQTL effects.

Pluripotency-specific imprinting of *DOCK4* and *LYNX1*

After systematically filtering genes with uncertain status by comparing methylation and expression patterns in biparental cell lines, we further validated the methylation status of newly identified imprinted DMRs by performing bisulfite sequencing. Among the candidate DMRs, we found *DOCK4* and *LYNX1*, two genes previously implicated in cancer and developmental disorders [32–34]. The analysis confirmed hemi-methylation in BipHiPSCs, hypermethylation in PgHiPSCs, and hypomethylation in AgHiPSCs, consistent with parental-origin imprinting (Fig. 4A). In contrast, these DMRs had no imprinting patterns in non-pluripotent somatic cells (AgSCs, PgSCs, and BipSCs) (Fig. 4B), suggesting that the imprinted status of these DMRs is restricted to the pluripotent state.

To further support the pluripotency-specific imprinting identified by genome-wide methylation analysis, we performed additional validation experiments using independent pluripotent stem cell lines that were not included in the initial genome-wide methylation analysis. These analyses included a mesenchymal stem cell (MSC)-derived iPSCs, an additional BipHiPSC line, derived from an alternative somatic cell origin (adipose tissue-derived MSCs), a PgHiPSC line#2 established from an independent MCT sample, and an independently reprogrammed AgHiPSC clone#2 derived from the same AgSCs used to generate the initially analyzed AgHiPSCs. Examination of *DOCK4*, one of the candidate imprinting genes, revealed consistent imprinting patterns across these independent iPSC lines (Fig. S4). Notably, analysis of an additional AgHiPSC clone suggests that the observed imprinting patterns are not attributable to clonal variation introduced during reprogramming, but rather reflect stable epigenetic characteristics associated with the pluripotent state. While these analyses provide further support for the robustness of pluripotency-specific imprinting identified in this study, future investigations using a larger number of independently derived iPSC lines will be helpful to further strengthen these findings.

To further assess whether these methylation patterns are truly pluripotency-specific, bisulfite sequencing of differentiated human tissues (liver, heart, and brain) and H9 cells reinforced this finding: *DOCK4* and *LYNX1* exhibited imprinting only in H9 and not in the differentiated tissues (Fig. 4C and 4D). These results identify *DOCK4* and *LYNX1* as pluripotency-specific imprinted genes—representing an underexplored epigenetic phenomenon that may be essential for maintaining stem cell identity.

We then directly assessed the pluripotency-specific patterns of these DMRs by differentiating PgHiPSCs, AgHiPSCs, and BipHiPSCs into neural stem cells (PgNSCs, AgNSCs, and BipNSCs, respectively). *LYNX1* was selected for further analysis because of its

reported involvement in neuronal development, making it a suitable candidate for examining methylation dynamics during neural differentiation. Bisulfite sequencing of these differentiated cells (Fig. S3) revealed marked changes in *LYNX1* methylation upon differentiation: in BipHiPSCs, the imprinting-like hemi-methylation pattern observed in pluripotent cells was lost in BipNSCs; in PgHiPSCs, the fully methylated state remained largely unchanged; and in AgHiPSCs, the fully unmethylated state in pluripotent cells shifted to a fully methylated state after differentiation.

These results demonstrate that the unique imprinting patterns of the DMRs are maintained only in pluripotent stem cells and are lost or altered upon differentiation into lineage-committed cells, reinforcing their designation as pluripotency-specific imprinted genes.

Correlation of gene expression with DNA methylation

To assess the functional importance of DNA methylation, we examined the expression levels of *DOCK4* and *LYNX1* in each iPSC line. Expression of *DOCK4* was paradoxically higher in PgHiPSCs than in AgHiPSCs (Fig. 4E), suggesting a potential context-dependent role of DNA methylation in gene activation rather than repression [35]. In contrast, *LYNX1* exhibited a classical pattern, with lower expression in PgHiPSCs and higher expression in AgHiPSCs, consistent with methylation-mediated silencing [35–37]. These findings indicate that imprinting-related methylation can have diverse regulatory effects on gene expression [35].

Conclusion

Our study highlights the utility of human androgenetic and parthenogenetic iPSCs as powerful models for identifying novel maternally and paternally imprinted genes, particularly those specific to pluripotent cells. We identified *DOCK4* and *LYNX1* as pluripotency-specific imprinted genes. However, the precise roles of the genes in pluripotent stem cells and their involvement in human embryonic development remain unclear. Therefore, future research should explore the imprinting patterns of these genes during early embryogenesis and in differentiated somatic cells such as hepatocytes, cardiomyocytes, and neurons to investigate tissue-specific imprinting mechanisms. This approach could uncover additional imprinted genes that play crucial roles in lineage-specific functions and development, ultimately advancing regenerative medicine, improving our understanding of organogenesis, and providing new insights into susceptibility to imprinting-related diseases.

Compliance with ethics requirements

All experimental procedures were approved by the Institutional Review Board of Konkuk University Medical Center (KUH 1040045) and conducted in accordance with its ethical guidelines.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.jare.2026.01.023>.

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