

# Genomic landscape of human allele-specific DNA methylation

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**DNA methylation mediates imprinted gene expression by passing an epigenomic state across generations and differentially marking specific regulatory regions on maternal and paternal alleles. Imprinting has been tied to the evolution of the placenta in mammals and defects of imprinting have been associated with human diseases. Although recent advances in genome sequencing have revolutionized the study of DNA methylation, existing methylome data remain largely untapped in the study of imprinting. We present a statistical model to describe allele-specific methylation (ASM) in data from high-throughput short-read bisulfite sequencing. Simulation results indicate technical specifications of existing methylome data, such as read length and coverage, are sufficient for full-genome ASM profiling based on our model. We used our model to analyze methylomes for a diverse set of human cell types, including cultured and uncultured differentiated cells, embryonic stem cells and induced pluripotent stem cells. Regions of ASM identified most consistently across methylomes are tightly connected with known imprinted genes and precisely delineate the boundaries of several known imprinting control regions. Predicted regions of ASM common to multiple cell types frequently mark noncoding RNA promoters and represent promising starting points for targeted validation. More generally, our model provides the analytical complement to cutting-edge experimental technologies for surveying ASM in specific cell types and across species.**

Genomic imprinting refers to genes that are preferentially expressed from either the maternal or paternal allele without genotype dependence (1). In mammals, such parent-of-origin gene expression is believed to have evolved along with the placenta, serving to mediate resource distribution between a mother and her offspring (2, 3), though other theories have been proposed (4–6).

The connection between imprinting and DNA methylation was uncovered shortly after the first identification of imprinted genes in mammals (7). Imprinted gene expression, in all known cases, is regulated by allele-specific methylation (ASM) of some *cis*-acting regulatory regions. We use the term allelically methylated region (AMR) in reference to any genomic interval of ASM, whether or not it is associated with imprinted regulation. Typically, an entire imprinted locus is organized as a cluster and regulated by an imprinting control region (ICR) and several other AMRs. The allelic methylation patterns of ICRs are set during gametogenesis and stably maintained throughout somatic development in the offspring (8), irrespective of gene expression levels. The remaining AMRs may be established after fertilization (9), possibly under the control of nearby ICRs or other epigenetic signals.

The identification of imprinted genes and a detailed understanding of their regulation has become increasingly important, along with the realization that aberrant genomic imprinting contributes to several complex diseases (10). Much effort has been directed toward locating imprinted genes using expression screen-based approaches (11, 12). One limitation of such approaches is that many imprinted genes may only show allele-specific expressions in particular tissues at appropriate developmental stages (13). ASM screen-based approaches might overcome the effect of temporal and spatial expression patterns because the ICRs are expected to exist through developmental

stages preceding the context in which they become active. Such methods have been successfully applied to identify unique imprinted genes (14–17).

Advances in DNA sequencing technology have been leveraged for high-throughput identification of imprinted genes. The “BS-seq” technology couples bisulfite treatment with high-throughput short-read sequencing, and has enabled genome-wide profiling of DNA methylation in mammalian genomes at single-CpG (cytosine guanine dinucleotide) resolution (18). Li et al. (19) produced a methylome from peripheral blood of a single individual and recognized the potential of using such data to profile ASM. They employed a method based on associating heterozygous SNPs with differential methylation, and identified hundreds of ASM regions. Methods such as this, however, must be applied to data from a single individual and for which matching genotypic data are available. There are two shortcomings of approaches that depend on genotype. First, they can be confounded by ASM that is associated with genotype, but which may not have any regulatory effect. The amount of ASM typically associated with genotype is not well understood, but recent reports suggest it is significant (20). More importantly, because imprinted methylation is not necessarily associated with genotypic variation, these methods will be inherently blind to some portion of ASM.

We present a probabilistic model to describe ASM based on data from BS-seq experiments. Our model is independent of genotype, and therefore has broad applicability to identify ASM in the context of imprinting. In essence, our model describes the degree to which methylation states in reads appear to reflect two distinct patterns, each pattern representing roughly half the data. We validated our method using semisimulated data in which methylation states were simulated within actual reads from BS-seq experiments. Our results indicate that technical characteristics of existing public methylomes (i.e., read length and coverage) are sufficient to accurately identify AMRs. By applying our model to 22 human methylomes, emphasizing those from uncultured cells, we identified a set of candidate AMRs involved in imprinted gene regulation. Candidates consistently identified across methylomes display remarkable concordance with known imprinted genes and allow boundaries of known AMRs to be precisely defined. Many candidates not associated with known imprinted genes mark the promoters of long noncoding RNAs (lncRNAs) and are also supported by similar analyses at orthologous regions in chimp; these provide a starting point for identifying additional imprinted genes, ICRs, and possibly imprinted clusters. Our model, therefore, is an essential analytical complement to recently emerged experimental methods for understanding the role of DNA methylation in genomic imprinting.

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Details concerning the number of simulated datasets for each parameter combination can be found in the *SI Text*.

Specificity was generally very high (approximately 99%) for all simulation parameter combinations, reflecting our conservative model selection criterion (Eqs. 4 and 5). In contrast, sensitivity showed greater dependence on properties of the datasets. Sensitivity was higher for regions of higher CpG density, as expected because our model depends on the relationships between CpG states inside a read. As shown in Fig. 1, inside CGIs sensitivity reached above 95% for all read lengths when the mean coverage was above 10 $\times$ . Sensitivity reached approximately 70% for intergenic regions but required both 10 $\times$  coverage and read length 100, which compensates for the decrease in CpG density. As expected, greater coverage and read length improved accuracy, and the effect of read length is equivalent to that of CpG density. These results indicate that methylomes with read lengths around 100 bp and mean coverage above 10 $\times$  appear sufficient for our model to accurately identify ASM. These criteria are met by most existing methylomes from BS-seq experiments.

### Properties of the Methylomes Analyzed

We analyzed 22 publicly available methylomes, including five uncultured primary cell types, eight cultured differentiated cell lines, four embryonic stem cells (ESCs), and five induced pluripotent stem cells (iPSCs) from following studies. Additional details about each of the methylomes can be found in *Dataset S1*.

Hodges et al. (24) produced four uncultured methylomes from blood cells: hematopoietic stem and progenitor cells (HSPC), B cells, neutrophils, and CD133+ cord blood cells. Because the first three samples were pooled from six unrelated individuals, ASM caused by genetic variants should not be apparent due to the effect of pooling. The last sample was generated from one individual. Li et al. (19) produced the other uncultured methylome from peripheral blood mononuclear cells (PBMC) of one individual.

The study of Laurent et al. (25) produced three methylomes: foreskin fibroblasts, H9 ESCs, and fibroblasts derived from H9 ESCs. Lister et al. (18) produced methylomes for IMR90 cells and H1 ESCs, two replicates each which we treat as distinct methylomes. In a separate study, Lister et al. (26) produced methylomes for 10 cell types. Included among these were H9 ESCs, adipose-derived stem cells (ADS), adipocytes derived from ADS cells (ADS Adipose) and foreskin fibroblasts (FF). Induced pluripotent stem cells derived from ADS, IMR90, and FF cells were also profiled, with FF iPSCs taken at three different times, the last of which were also profiled after being differentiated in the presence of bone morphogenic protein 4 (BMP4).

### Allele-Specific Methylation on the X Chromosome

Though not a form of imprinting, dosage compensation is associated with ASM differentially marking one chromosome X (chrX) in female somatic cells (27). In contrast, only a single allele from chrX is represented in male methylome data. Comparing the results of our analyses between male and female X chromosomes therefore provides a measure of specificity: AMRs identified on chrX in males are likely false-positives. In total, 12 of the analyzed methylomes are female. Although coverage on chrX in males is

reduced by half, three male methylomes approached 10 $\times$  coverage on chrX (H1 ESC rep 2, FF, and FF iPSC BMP4; refs. 18 and 26).

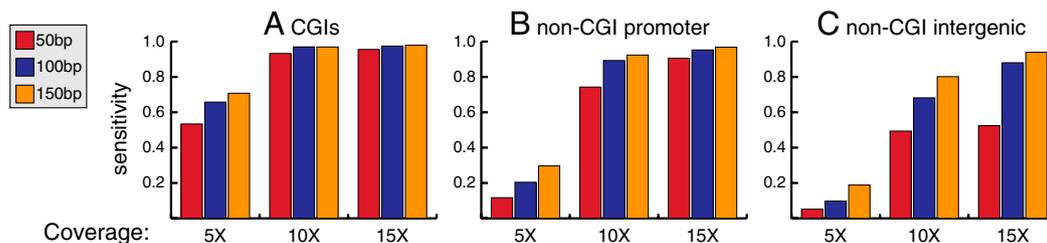
The locations of identified AMRs on chrX are presented in Fig. 2. The fraction of AMRs from chrX in female methylomes ranges from 15% to 36% with a mean of 24%. For the three male methylomes tested, the fraction is in the range of 1% to 2%. These results further support our conclusion from simulations that specificity is high in our AMR prediction. X chromosome inactivation is regulated via the X-inactive specific transcript (XIST) gene, a lncRNA with random allele-specific expression in female somatic cells. Our analyses identified an AMR at the XIST promoter in each female differentiated methylome (Fig. S1), but not in any of the ESCs, iPSCs, or male methylomes as expected (28).

### Genome-Wide AMR Identification Predicts Imprinted Genes

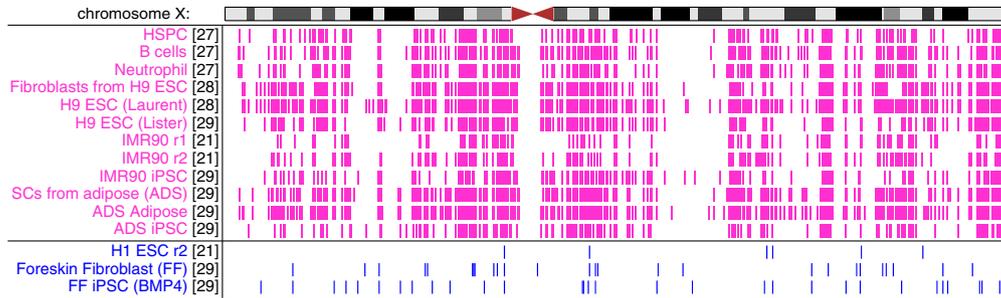
The full set of identified AMRs for each of the 22 methylomes is presented in *Dataset S1*. We emphasized the uncultured blood methylomes in compiling sets of high-confidence AMRs, and generally use the remaining methylomes to provide additional supporting evidence. We found 579 autosomal AMRs that are common to at least three of the five uncultured methylomes (HSPCs, B cells, neutrophils, CD133+ cord blood, and PBMCs), 247 common to at least four out of five, and 81 shared across all five. Table 1 presents the 39 AMRs common to all five uncultured methylomes and that are proximal to promoters ( $\pm 4$  kb of a University of California Santa Cruz KnownGene TSS). Among these, 18 overlap a known imprinted gene and 20 mark a lncRNA promoter. The high concordance between our prediction and known imprinted genes further validates our model and provides strong support for the remaining predictions as candidate imprinted genes. The regulatory activity of lncRNAs has been observed for most imprinted clusters (29), and the frequent overlap of identified AMRs and lncRNA promoters suggests these might have similar activity (30). We also identified AMRs in low-coverage chimp blood cell data from the study of Hodges et al. (24), adding additional evidence to several of our predictions.

We computed the methylation level in sperm at each of the identified AMRs using data from a previous study (31). Among the 579 autosomal AMRs common to three out of five uncultured methylomes, 146 were methylated (>50%) in sperm. As indicated in Table 1, among the 39 predicted AMRs common across all uncultured cell types, only three are methylated in sperm. Among these is the H19 ICR, which is well known to be methylated on the paternal allele (32). If we use the methylation level in sperm as an indicator of methylation on the paternal allele, these results point to an asymmetry in the paternal and maternal mechanisms of imprinting DNA methylation.

One of the central questions related to the use of iPSCs, in research or therapeutically, is the degree to which they resemble true ESCs. The landmark study of Lister et al. demonstrated significant reprogramming variability between iPSCs (26). Evidence from cloning studies suggests that imprinting might be especially difficult to reprogram (33). We assembled the union of all identified AMRs in all methylomes. For each of these AMRs, we computed average methylation in each methylome. We then clustered the methylomes hierarchically according to correlation of



**Fig. 1.** Sensitivity of AMR identification based on semisimulated data. Coverages of 5, 10, and 15 $\times$ , and read lengths of 50, 100, and 150 bp were used. CpG densities were controlled by simulating within (A) CGIs, (B) non-CGI promoter regions, and (C) non-CGI intergenic regions.



**Fig. 2.** Locations of AMRs identified on chrX. All female data (pink) was included. Only male methylomes (blue) with sufficient coverage on chrX are shown because these have coverage reduced by 50% compared with autosomes. Numbers in brackets indicate references for data sources. See text and [Dataset S1](#) for information about methylomes.

methylation levels through these intervals (Fig. S2). The iPSCs correlated better with ESCs than with the somatic cells from which they are derived, suggesting that ASM has in general been successfully reprogrammed in these iPSCs.

However, we found several examples where the iPSCs appear to diverge from the ESCs in terms of ASM (Fig. S3). An AMR was identified at the GNAS-1 promoter in 18 of the methylomes, but this interval was hypomethylated in the ADS iPSCs. Similarly,

for the AMR identified at the 3' promoter of ZNF331 gene, the ADS iPSCs are methylated at 50% and resemble differentiated cells more closely than ESCs or other iPSCs, suggesting failed reprogramming of ADS iPSCs at these locations. It has been proposed that a single imprinted cluster might be sufficient to diagnose iPSC reprogramming in mouse (34). The diversity of ASM we observe between iPSCs and even between ESC lines suggests such diagnosis will be more complex in human.

**Table 1. AMRs common to all uncultured cells that overlap gene promoters**

Gene symbols	NC	CGI	Sp	ESC	iPSC	Tot	Chimp
* GNAS,...	Y	Y		4	5	22	3
* GNAS-AS1,...	Y	Y		4	5	22	3
* MEST1/MEST,...	Y	Y		4	5	22	3
* SGCE,PEG10		Y		4	5	22	3
NHP2L1		Y		4	5	22	3
* ZNF597,NAA60		Y		4	5	22	3
* SNRPN,SNURF		Y		4	5	22	2
* AMPD3,...	Y			4	5	22	0
PMF1-BGLAP		Y		4	5	22	0
LOC554226,...	Y	Y		4	5	22	0
UNC45B				4	5	22	0
LINC00273	Y	Y		4	5	22	0
* NAP1L5				4	5	21	3
KCNQ1OT1	Y	Y		3	5	21	0
LOC284801,...	Y	Y		4	4	21	0
* PSIMCT1	Y	Y		2	5	20	0
* H19,...	Y	Y	Y	2	5	20	0
TRAPP9		Y		3	4	20	0
CR590796	Y			4	3	20	0
* DIRA53		Y		2	5	19	2
AX748049				2	5	19	2
BC028329	Y			3	4	19	2
ZNF718,ZNF595		Y		3	5	19	0
BC023516	Y	Y		3	5	18	3
LOC100130522	Y	Y		2	4	18	3
* FANK1		Y		2	5	18	0
* GNAS	Y	Y		2	3	18	0
VTRNA2-1	Y	Y		3	4	17	3
MTRNR2L3			Y	4	1	15	0
* BLCAP,NNAT		Y		2	2	14	2
LOC728024	Y			0	3	14	2
RPS2P32	Y	Y		0	3	14	2
* ZIM2,PEG3,...	Y	Y		1	0	12	0
* MEG3		Y		0	0	11	3
LOC100132167	Y			1	0	11	0
* HOXA6,HOXA5,...		Y	Y	0	0	8	2
KIAA0934,DIP2C		Y	Y	0	0	8	2
LOC440570,...		Y		0	0	5	3
LOC100335030		Y		0	0	5	1

Columns indicate whether the gene is noncoding (NC), the AMR overlaps a CGI promoter (CGI), or is hypermethylated in sperm (Sp). Counts indicate the number of ESC, iPSC, total human methylomes, and chimp methylomes in which the AMR is found. Ellipsis indicate additional gene names can be found in [Dataset S1](#). Asterisk \* indicates known imprinted gene.

### Analysis of Known Imprinting Control Regions

There are approximately 65 human genes currently validated as imprinted and these reside in 32 imprinted clusters (see [Dataset S1](#)). We asked for what proportion of these clusters do we identify an AMR shared between cells, and do these shared AMRs coincide with experimentally validated AMRs? As can be seen from Table 2, 24 of the clusters contain validated AMRs, and in 21 of those cases we correctly identify a known AMR common to four out of five uncultured cells. For the IGF2R and INPP5F clusters, we only identified AMRs shared between two and three of the uncultured cells, respectively. The AMPD3 gene has no validated AMR to our knowledge. Our algorithm finds an AMR shared across all 22 methylomes, indicating a likely candidate for validation. To our knowledge, no AMRs have yet been identified for the remaining clusters, and our algorithm fails to predict any AMRs that are shared between methylomes.

Knowledge of the location of true AMRs around several of these imprinted genes allowed us to apply a more intensive analysis to examine them with greater sensitivity and precision. We designed a dynamic programming algorithm to optimize the locations of AMR boundaries by evaluating each possible AMR size rather than joining overlapping sliding windows. This algorithm uses a scoring function based on the likelihoods of Eqs. 1 and 2 but remains too computationally demanding for genome-wide application (details are provided in the *SI Text*). We refer to AMRs identified with this algorithm as “refined” AMRs.

The imprinted cluster on chr14 consists of seven genes controlled by the maternally expressed lncRNA MEG3. The region harbors an AMR at the MEG3 promoter, and another intergenic AMR approximately 15 kb upstream of the MEG3 TSS, both paternally methylated with the upstream AMR shown to act as an ICR (35). Our genome-wide scan found the MEG3 promoter AMR in 11/13 differentiated cells. The boundaries of refined AMRs were identified in each uncultured methylome at nearly the exact same location, covering an interval that is hypomethylated in sperm (Fig. S4). A refined AMR was identified in each uncultured methylome precisely at the known ICR location, which is methylated in sperm. Interestingly, each of the ESC/iPSC methylomes shows full methylation through the ICR, suggesting possible imprinting defects in these cells.

Imprinted expression in the GNAS locus is highly complex, with maternally, paternally, and biallelically expressed transcripts sharing sets of exons (36). This locus includes four AMRs at alternative promoters (NESP55, GNAS-AS1, XLas, Exon A/B)



imal to annotated promoters, 18 are marking known imprinted genes. It appears as though the AMRs that are already known are also those identified most consistently across methylomes. This finding can be interpreted in several ways. One possibility is that a significant portion of the imprinted genes or clusters, possibly more than half, have already been identified. Estimates of the total number of imprinted genes in human hover around 100–200 (39, 40), and many parent-of-origin disease phenotypes have already been explained through known imprinted genes. Among our remaining predictions (several hundred putative AMRs) many could represent weaker ASM signals possibly without functional relevance, and these are identified with less consistency across datasets because of a lack of sensitivity in our method. Another possibility is that many genes are imprinted with cell-type specificity, and that the known AMRs are biased toward those that can be identified in a greater variety of cell types. Our top predictions were based on consistency across the available methylomes from uncultured cells, but these all happened to be from blood.

Another important finding to emerge from our analyses is the precision with which AMRs are defined across cell types. The GNAS and SGCE/PEG10 examples illustrate this strong consistency of AMR boundaries: In both of these examples, there are pairs of TSS in very close proximity, sharing CGIs, but for which one has ASM methylation and the other does not. Methods like ours that can delineate the boundaries of AMRs will assist future efforts to precisely map the elements inside these regulatory regions.

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