Cold exposure impacts DNA methylation patterns of genes involved in early embryonic and bone development

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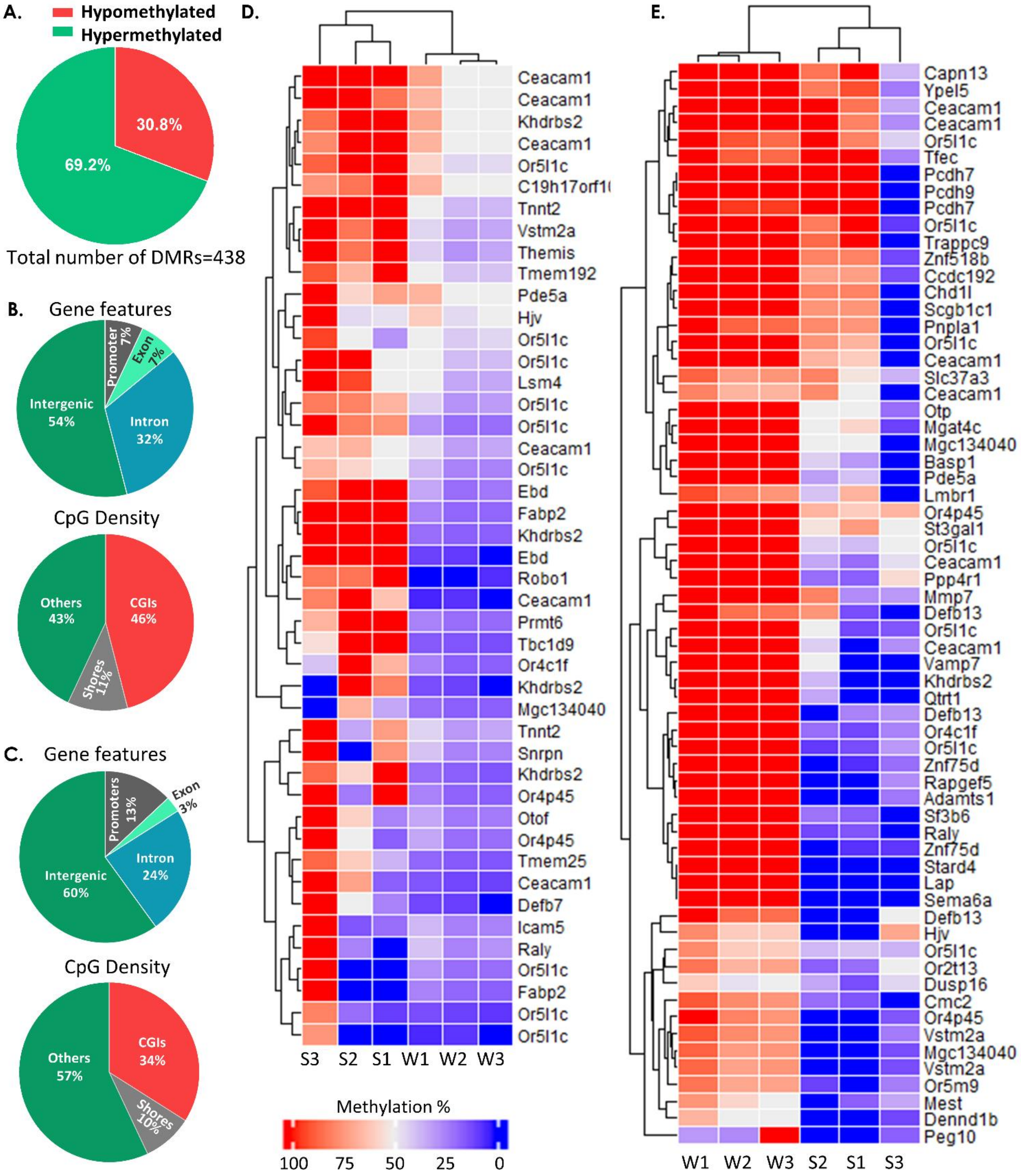

Figure 1. Differentially methylated regions (DMRs) identified among sperm samples collected during winter and late spring. (A) Percentage of hypomethylated and hypermethylated regions between late spring and winter. A total of 438 DMRs were detected among which 135 were hypermethylated and 303 were hypomethylated. (B) Distribution of hypermethylated DMRs. (C) Distribution of hypermethylated DMRs across different gene features and CpG islands (CGIs). (D) Heatmap showing methylation percentage of top 44 differentially hypomethylated DMRs had $p<0.05$ on differentially mediated regions (DMGs). (E) Heatmap showing top 65 hypermethylated DMRs had a $p<0.05$ on DMGs. Each cell in the heatmap is colored according to the methylation level in each DMR. S: Late spring, W: Winter.

Table 1. Differentially methylated genes (DMGs) related to embryonic development and osteogenic activity among sperm samples collected during winter and late spring.

Chr	DMR Location			No. of DMCs	Differential Methylation (%)	Methylation Status	Gene Name	Overlapping gene features			
	Start	End	Length					Promoter	Exon	Introns	CGI
DMGs involved in early embryonic development											
3	36311879	36312012	134	9	-30.952	Hypo	*Prmt6*	Promoter	Exon		CGI
4	118276064	118276192	129	9	-55.807	Hypo	*Lmbr1*			Introns	CGI
4	103795994	103796062	69	4	-54.878	Hypo	*Slc37a3*	Promoter		Introns	CGI
6	66716994	66717074	81	9	-30.450	Hypo	*Nipal1*	Promoter	Exon	Introns	CGI
21	23263663	23263832	170	9	-31.060	Hypo	*C21h15orf40*	Promoter	Exon		CGI
8	102791262	102791354	93	7	47.265	Hyper	*Rgs3*			Introns	CGI
13	63412501	63412667	167	5	15.075	Hyper	*Raly*			Introns	CGI
18	7969785	7969847	63	4	51.942	Hyper	*Gan*			Introns	CGI
24	43445459	43445765	307	8	20.289	Hyper	*Fam210a*			Introns	CGI
DMGs involved in osteogenesis											
3	106792147	106792226	80	6	-54.401	Hypo	*Macf1*			Introns	CGI
8	76925715	76925810	96	10	-32.290	Hypo	*Ubqln1*	Promoter			CGI shores
18	51956744	51956850	107	9	-31.247	Hypo	*Smg9*	Promoter			CGI shores
14	3359064	3359128	65	4	-52.616	Hypo	*Trappc9*			Introns	CGI
22	13968071	13968223	153	7	-52.576	Hypo	*Ctnnb1*			Introns	CGI
4	94344895	94344962	68	5	54.463	Hyper	*Mest*			Introns	CGI
7	4893727	4893808	82	4	54.057	Hyper	*Lsm4*				CGI shores
15	62586466	62586665	200	12	31.940	Hyper	*Pax6*			Introns	CGI
4	12064511	12064698	188	9	31.104	Hyper	*Peg10*	Promoter		Introns	CGI

Chr: Chromosome, DMR: Differentially methylated region, DMC: Differentially methylated cytosine, TSS: Transcription start site, CGI: CpG Island, CGI Shores: 0 to 4 kb from CpG island