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Comparison of Whole-Genome DNA Methylation Patterns in Whole Blood, Saliva, and Lymphoblastoid Cell Lines

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Abstract

Epigenetic mechanisms, including DNA methylation, that underlie neuropsychiatric conditions have become a promising area of research. Most commonly used DNA sources in such studies are peripheral (whole) blood (WB), saliva (SL), and lymphoblastoid cell lines (LCLs); thus, the question of the consistency of DNA methylation patterns in those cells is of particular interest. To investigate this question we performed comparative analyses of methylation patterns in WB, SL, and LCLs derived from the same individuals, using Illumina HumanMethylation27 BeadChip arrays. Our results showed that DNA methylation patterns in SL are relatively consistent with those in WB, whereas the patterns in LCLs are similarly distinct from both WB and SL. The results indicated that due to multiple random and directed changes in DNA methylation throughout cell culturing, LCLs are not a reliable source of DNA for epigenetic studies and should be used with caution when investigating epigenetic mechanisms underlying biological processes.

Keywords

Lymphoblastoid cell lines; Saliva; Whole blood; DNA methylation; Methylation pattern

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Introduction

DNA methylation is a well-known mechanism of genes activity regulation that provides valuable information on development, typical functioning and disorders (Laird 2003), including neuropsychiatric conditions (Abdolmaleky et al. 2004, 2008; Feng and Fan 2009; Hsieh and Elisch 2010; Mill et al. 2008; Nohesara et al. 2011). Studies have provided evidence that individuals with neuropsychiatric disorders can be distinguished by epigenetic encoding disturbances found in both the brain and secondary tissues (Abdolmaleky et al. 2008; Feng and Fan 2009; Hsieh and Elisch 2010; Iwamoto et al. 2004; Mill et al. 2008). Among those "secondary tissues," peripheral blood (whole blood, WB), saliva (SL), and lymphoblastoid cell lines (LCLs) are most commonly used as sources of genomic DNA in epigenetic research (Hu et al. 2006; Kaminsky et al. 2009; Nguyen et al. 2010; Nohesara et al. 2011; Sapienza et al. 2011; Tierling et al. 2011). Consequently, the question of the consistency of the methylation profiles derived from these cells and tissues is of particular interest. One study has reported that, at least in the context of investigating genomic sex effects on DNA methylation, SL DNA has shown a pattern of methylation consistent with WB DNA (Liu et al. 2010). Yet, the degree of convergence between patterns of methylation for LCLs and WB or SL might be different as random DNA methylation pattern changes may be observed in LCLs when compared to the original B-lymphocytes from which they originated (Brennan et al. 2009; Grafodatskaya et al. 2010).

Here, we present the results of a comparison between whole genome methylation profiles of WB, LCLs, and SL samples derived from the same individuals. The aims of this study were twofold: (1) to establish the degree of differences/similarities in the DNA methylation patterns in the tissues most commonly used in epigenetic studies; and (2) to explore the scope of epigenetic alterations occurring in LCLs.

Materials and methods

Sample collection and processing

WB and SL samples were derived from 14 healthy individuals (ten females and four males ranging in age from 7 to 61 years old) from an indigenous Slavic population of Northern Russia. Informed consent was obtained from all participants who donated biological samples. Two vials of saliva were collected from each individual using BD vacutainers containing ACD (BD, Franklin Lakes, NJ) and Oragene-DNA collection kits (DNAgenotek, Ontario, Canada), respectively.

LCLs were established by transforming lymphocytes with the Epstein-Barr Virus (EBV) according to standard procedures (Neitzel 1986); these were cultured for 4 weeks, then cryopreserved prior to DNA extraction. Genomic DNA was extracted from WB samples using the FlexiGene DNA Kit, according to the manufacturer's protocol (Qiagen, Mississauga, ON); from LCLs using phenol–chloroform and ethanol precipitation; and from SL following the Oragene Laboratory protocol.

DNA methylation analysis

Analysis of DNA methylation profiles in the three sets of tissues from the same individuals was performed using Infinium HumanMethylation27 BeadChip assay (Illumina, San Diego, CA), which contains 27,578 CpG targets covering 14,495 genes. Bisulfite treatment, whole genome amplification, labeling, hybridization and scanning were performed at the Yale Center for Genomic Analysis http://medicine.yale.edu/keck/ycga/index.aspx).

The methylation data generated by the array were analyzed using the Illumina GenomeStudio software package. The methylation status of each CpG site was measured as

the ratio of signal from methylated probe to the sum of both methylated and unmethylated signals (β value, ranges from 0, unmethylated, to 1, fully methylated). All CpG sites with a detection *p* value ≥0.001 were removed from later analyses; the *p* values were obtained using a background model in the Genome Studio. Three technical replicates were run across different BeadChips; pairwise comparison of these replicates showed consistent and highly reproducible methylation level measurements (r^2 varied between 0.98 and 0.99); on average 27,566 ± 12 probes showed no significant ($p \leq 0.01$) differences in β values.

Differential methylation analysis

The Illumina methylation data were processed and analyzed using the Methylation Module v1.8 of the Illumina GenomeStudio. For comparison of whole-genome methylation profiles across the samples, correlations and hierarchical clustering were used; the results of the analysis are provided in the form of a dendrogram. To prevent variability among samples within a tissue set related to gender, CpG sites localized on sex chromosomes were excluded, leaving 26,273 sites to be analyzed.

Differential methylation analysis between sets of samples representing three tissues was performed based on differences in the mean beta value (Avgβ) of each CpG site, or Delta Avgβ (Δβ). To account for multiple testing, the Illumina Custom Error Model with the False Discovery Rate (FDR) corrections was applied; we ran 1000 permutations and included FDR up to 20 %. Targets showing significant intergroup differences in methylation levels (the methylation Difference Score, DiffScore >|30|, $p < 0.001$) more than 0.2 were considered to be differentially methylated (Grafodatskaya et al. 2010).

Functional annotation of differentially methylated genes (DMEGs)

To identify common biological processes and pathways, molecular functions, and cellular components for genes that showed differential methylation in the tissues analyzed, we applied the Database for Annotation, Visualization and Integrated Discovery (DAVID) bioinformatics software (Dennis et al. 2003; Huang et al. 2008). For this analysis the default (medium stringency) setting of the DAVID analysis tool was used, which compares the enrichment of gene ontology (GO) with the list of differentially methylated genes (DMEGs) using Fisher's exact test. Annotation clusters with enrichment scores>1.3 ($p < 0.05$) were included in the analysis. GO terms with FDRs <20 % were used to avoid reporting false positives and to reduce the large number of associations identified by DAVID in the functional annotation charts. The *p* values and the Benjamini corrections of the scores from the tool were used as inclusion criteria in the trimming of the clusters to overrepresented term lists.

Results

We examined whole genome methylation patterns in three types of biological samples—whole blood (WB), saliva (SL) and lymphoblastoid cell lines (LCLs), which are widely used as a source of genomic DNA in epigenetic studies, and performed a comparative DNA methylation analysis across these three tissues derived from the same 14 individuals. Global methylation levels of DNA from three sets of samples (SLs, LCLs and WBs) were carried out using the Illumina Infinium HumanMethylation27 BeadChip.

The resulting methylation data were analyzed by means of pairwise comparison of genome-wide DNA-methylation profiles across WB, SL and LCLs samples, and differential methylation analysis between groups of samples corresponding to different tissues. The analysis was performed based on 26,273 Illumina targets, which had passed detection quality filtering for differential methylation analysis and were localized on autosomes. To

assess the DNA methylation differences between different tissues in GO terms we performed functional annotation of genes, which showed differential methylation in pairwise comparisons across the three sets of samples, WB, SL and LCLs.

Comparison of global DNA methylation profiles

To assess the main differences in the genome-wide DNA-methylation of the three studied sets of samples, WB, SL and LCLs, we performed Euclidean hierarchical clustering analysis of the Illumina methylation data. The results of the analysis are represented in a dendrogram (Fig. 1), which leads to the following observations. First, the source of DNA is the main factor in sample differentiation: the dendrogram shows a clear separation of WB, LCLs, and SL into three distinct clusters, marked in the figure by Roman numerals. Second, according to the distances between samples within a cluster, WB samples (Fig. 1; cluster II) show minimal interindividual variability with the highest correlations ($r^2 = 0.992 \pm 0.002$) between individual methylation profiles compared to SL and LCLs. Whereas SL and LCLs (Fig. 1; clusters I and III) are characterized by average and distinctively high interindividual variability of their methylation profiles; $r^2 = 0.980 \pm 0.019$ and 0.955 ± 0.018, respectively. Third, the methylation profiles of DNA from SL and WB show a greater similarity in the comparison across all three groups, whereas LCLs cluster maximally remotely from the two others. The last observation on the distance of LCLs from SL and WB was confirmed by correlation analysis between methylation levels across these three tissues. The correlation coefficient for SL compared to WB ($r^2 = 0.967$) was much higher than those for LCLs compared to WB, and LCLs compared to SL ($r^2 = 0.880$ and 0.844, respectively). These correlations across WB, SL, and LCLs as groups of samples are consistent with the data on the correlations between the cell/tissue types within individuals (Online Resource, Table S1).

Comparison of the distributions of the methylation levels across the three sets, SL, WB and LCLs, showed that hypo and hypermethylation patterns are especially different between these three cell types. Specifically, the methylation profiles of LCLs are characterized by a lower frequency of CpG sites with high methylation levels, as well as unmethylated CpG sites compared to WB and SL (Fig. 2). This analysis shows that LCLs differ predominantly in areas of up and down regulated genes.

Differential methylation analyses and functional annotation of DMEGs

Data was then analyzed to detect differential methylation patterns of DNA obtained from three groups of samples corresponding to the three cell types. CpG sites showing significant ($p < 0.001$) intergroup differences of at least 1.2-fold change in methylation levels were considered to be differentially methylated. The results of the pair-wise comparison between sets of samples are presented in supplementary materials (Online Resource, Table S2–S4). The distribution of DMEGs across pairwise comparisons is shown in Fig. 3.

The minimal number of differentially methylated sites was found in the comparison of DNA from WB and SL: of the 27,578 CpG sites initially analyzed, 488 sites showed a significant difference in methylation level measurements (Online Resource, Table S2; Fig. 3). These 488 CpG sites are located on 431 genes, which represents ~3 % of the total number of genes (14,495) contained in the Illumina's array (Online Resource, Table S5). The majority of sites ($n = 370$; ~76 %) are downregulated in SL compared to WB. The remaining sites were characterized by upregulation ($n = 118$; ~ 24 %) in SL. This corresponds to the results in Fig. 2, where blood showed more occurrences of hypermethylated sites compared to SL.

Whereas several thousand differentially methylated CpG sites were found in the comparisons of these two cell types with LCLs, differential methylation analysis between

WB and LCLs groups revealed that 2,661 sites exhibited a methylation level difference at the same level of significance (Online Resource, Table S3; Fig. 3). These 2,661 CpG sites are located on 2,156 genes, which represent ~15 % of the total number of genes analyzed (Online Resource, Table S5). As in the WB and SL analysis, a great majority of the methylated sites (n = 2,545; ~96 %) were downregulated in LCLs compared to WB. Also evident in Fig. 2 is that LCLs exhibited lower levels of hypermethylated and hypomethylated sites when compared to WB and SL. The remaining sites were characterized by upregulation in LCLs (n = 116; ~4 %). Similar differences in methylation levels were found in the comparison between DNA from SL and LCLs: 2,513 sites (localized on 2,081 different genes) were found to be differentially methylated between these tissues, and most of them (~79 %) were hypomethylated in DNA from LCLs (Online Resource, Table S4, S5; Fig. 3).

Functional annotation of differentially methylated genes

The functional annotation of genes showed significant enrichment of the lists of DMEGs in some of the GO terms. Most of the genes differentially methylated in WB and SL are involved in the regulation of the immune response and body fluid levels, as well as genes belonging to the plasma membrane functional group (Table 1). There was a high concordance in GO terms overrepresented in the lists of DMEGs in LCLs compared to WB and SL. These genes are involved predominantly in the immune response, and the control of cellular activity and signaling systems, specifically genes coding glycoproteins, cytokines, and their receptors (Table 2).

The differences in methylation levels observed may be a result of tissue specific regulation. We performed a functional annotation of DMEGs, using the DAVID tissue expression annotation tools. For the WB versus SL comparison, this assumption was confirmed; namely, the list of DMEGs was overrepresented by genes known to be expressed in white blood cells and the salivary gland (Table 3). In contrast, the spectrum of GO terms for genes differentially methylated in WB versus LCLs was wider and included a number of tissues—from the cerebellum to the uterus, as well as cultivated cells—bone marrow CD105+ endothelial cells and Burkitt's lymphoma cell lines (Table 3).

Discussion

We compared DNA methylation profiles between WB, SL and LCLs from the same individuals using cluster and linear-regression analyses of the methylation profiles, and analysis of DMEGs. The results showed that LCLs have the most distinct methylation patterns compared to those in WB and SL. These results are not surprising and are consistent with published studies that warn researchers about the methylation changes in LCLs due to cell culturing (Brennan et al. 2009; Calıskan et al. 2011; Grafodatskaya et al. 2010; Sun et al. 2010; Sugawara et al. 2011). In contrast, WB and SL showed a relatively similar methylation pattern that is in line with a previous study (Liu et al. 2010). The DNA methylation differences found between WB and SL might be explained in terms of tissue specific methylation; thus most of the DMEGs are genes coding membrane complexes and are involved in immune response, as well as genes known to be expressed specifically in white blood cells and the salivary gland.

The distinctiveness of methylation patterns in LCLs might be caused by a complex of factors, including the mono-cellular nature of LCLs, which are composed only of B-lymphocytes, and their modifications throughout the culturing procedure. The compositional differences might be a result in the differential regulation of genes involved in the immune response, cellular activity and signaling systems, as was found in LCLs. At the same time, the EBV transformation of B-lymphocytes, preceding the cell culturing, caused uncontrolled

growth, proliferation and abnormal cell signaling. DMEGs in LCLs were found to be predominantly involved in the control of cellular activity and signaling systems, specifically genes coding signal peptides, such as cytokines and chemokines. This is consistent with a study reporting that EBV-mediated transformations rely extensively on interference with cytokine signaling networks (Mosialos 2001). Additionally it was found that the list of DMEGs in LCLs was enriched in genes found to be expressed in other cultivated cells—bone marrow CD105+ endothelial cells and Burkitt's lymphoma Raji cell line; the latter, Burkitt's lymphoma, is known to be associated with EBV infection (Fujita et al. 2004; Maeda et al. 2009). This finding provides further evidence that cell culturing procedures are responsible for the specificity of the methylation pattern in LCLs and its distance from those in WB and SL cells.

Taken together, the results of the study suggest that due to multiple random and directed changes of methylation patterns, LCLs are not a reliable source of DNA for epigenetic studies, as opposed to peripheral blood and saliva. As a result, LCLs should be used with particular caution to identify the epigenetic mechanisms underlying biological processes and their violations, due to disorders associated with DNA methylation variants.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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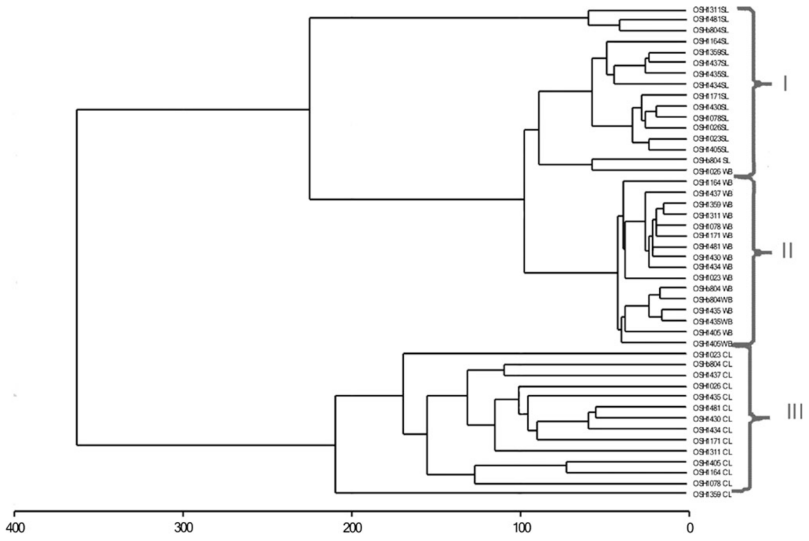

Fig. 1.

Euclidean hierarchical clustering of 14 individuals based on the pair-wise comparison of the whole genome methylation profiles in WB, SL and LCLs. The dendrogram shows a clear separation of the samples from the different tissues into three distinct clusters, those containing samples from SL, whole blood (WB), and LCLs, marked as clusters I, II, and III, respectively

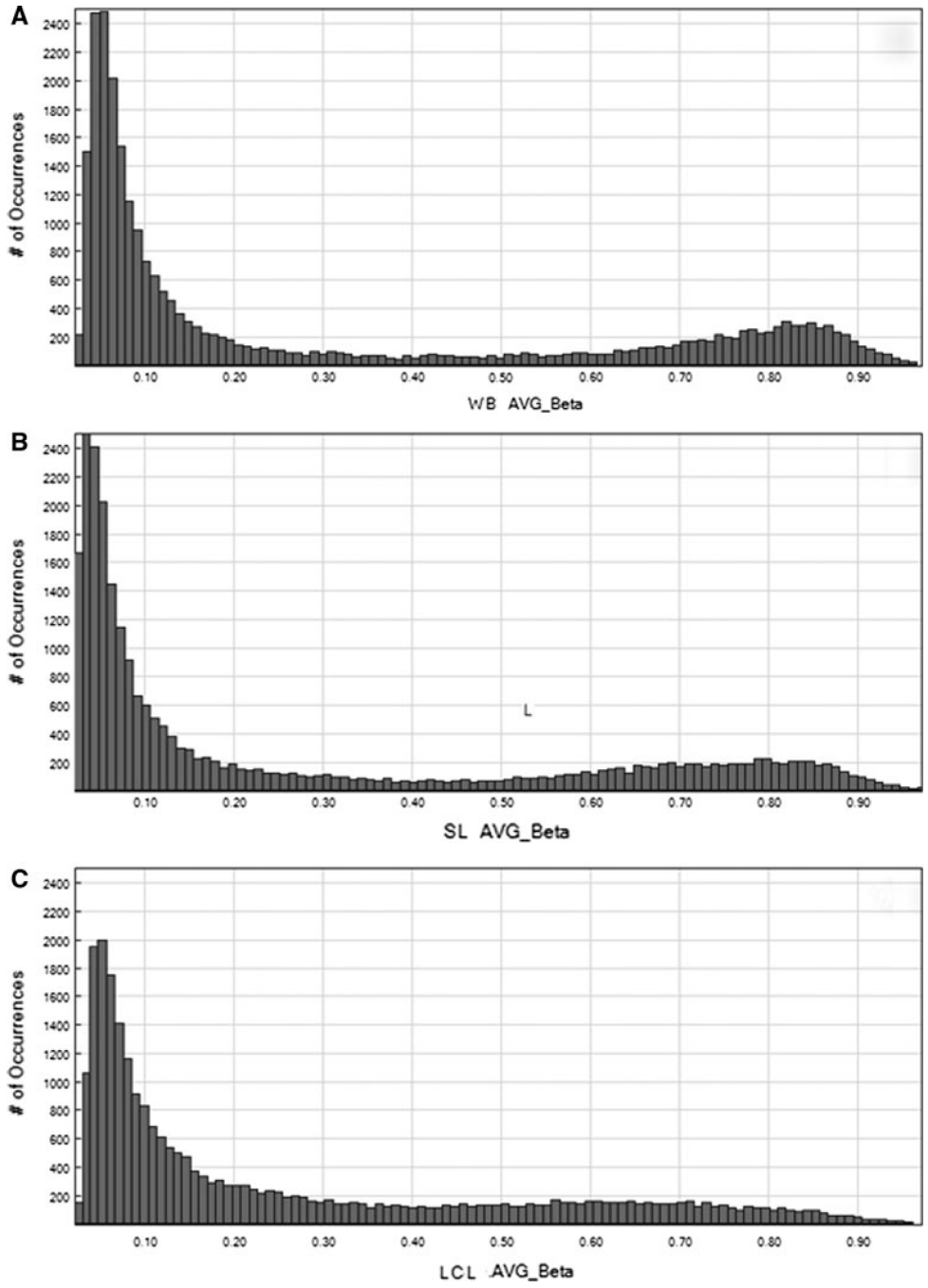

Fig. 2.

The distributions of the methylation levels (Avgβ) of 26,273 CpG sites contained in the Illumina Infinium27 array that had passed detection quality filtering and were localized on autosomes, in three groups of DNA samples derived from blood (**a**), saliva (**b**) and lymphoblastoid cell lines (**c**)

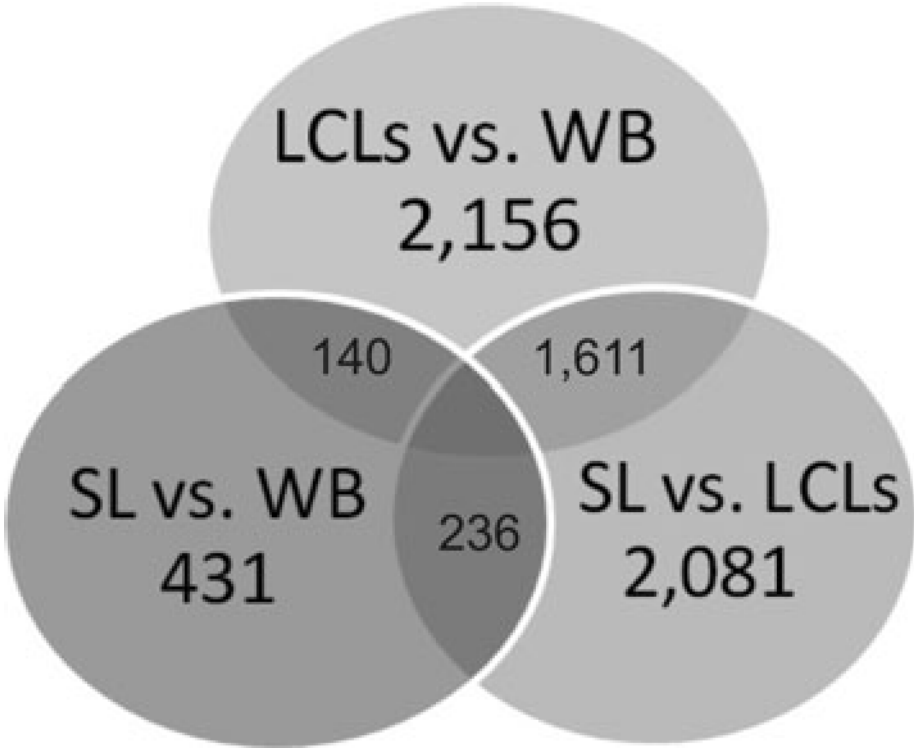

Fig. 3.
Venn diagram representing the results of differential methylation analysis across WB, SL and LCLs. The numbers in the *circles* represent both the number of differentially methylated genes detected in each pair-wise comparison and the number of overlapping genes across different comparisons

Table 1

Gene Ontology (GO) terms overrepresented in the list of genes, which showed differential methylation between DNA samples from SL and WB

GO term	Genes, count	Genes (%)	*p* value	Fold enrichment	Benjamini	FDR
GO: 0005887—integral to plasma membrane	61	16.05	5.53E–10	2.33	0.0000	0.0000
GO: 0031226—intrinsic to plasma membrane	62	16.32	4.84E–10	2.31	0.0000	0.0000
GO: 0044459—plasma membrane part	87	22.89	1.92E–08	1.79	0.0000	0.0000
GO: 0005886—plasma membrane	121	31.84	1.49E–06	1.45	0.0001	0.0020
GO: 0006955—immune response	35	9.21	1.40E–05	2.25	0.0060	0.0237
GO: 0009611—response to wounding	30	7.89	8.91E–06	2.51	0.0077	0.0151
GO: 0044421—extracellular region part	40	10.53	1.37E–04	1.89	0.0084	0.1832
GO: 0004867—serine-type endopeptidase inhibitor activity	11	2.89	3.79E–05	5.33	0.0203	0.0552
GO: 0050878—regulation of body fluid levels	13	3.42	8.02E–05	4.09	0.0273	0.1354

There were annotated 381 of 431 DMEGs. The GO terms, for which the Benjamini corrections <0.05 and FDR <0.20, are listed

Table 2

Functional annotation and clusters of genes that showed significant differences of their methylation levels in lymphoblastoid cell lines (LCLs) compared to WB and SL

Term	LCLs versus WB				LCLs versus SL			
	Cluster# (EScore)	Fold enrichment	Benjamini	FDR	Cluster# (EScore)	Fold enrichment	Benjamini	FDR
Disulfide bond	1 (75.8)	2.11	9.96E–79	2.01E–78	1 (76.8)	2.14	1.26E–80	2.65E–80
Signal		2.02	3.54E–77	2.14E–76		2.05	3.08E–79	1.29E–78
Signal peptide		2.01	4.79E–75	2.14E–75		2.04	3.58E–77	1.66E–77
Glycoprotein		1.76	2.33E–64	1.89E–63		1.82	2.18E–71	1.38E–70
Glycosylation site: N-linked (GlcNAc…)		–	–	–		1.80	6.99E–64	9.76E–64
Secreted	2 (59.0)	2.59	5.07E–79	2.05E–78	2 (53.4)	2.41	5.20E–62	4.37E–61
Signal		2.02	3.54E–77	2.14E–76		2.05	3.08E–79	1.29E–78
Signal peptide		2.01	4.79E–75	2.14E–75		2.04	3.58E–77	1.66E–77
GO: 0005576—extracellular region		1.97	8.99E–52	2.56E–51		1.89	1.13E–42	3.17E–42
GO: 0005615—extracellular space		2.46	1.05E–31	5.97E–31		2.32	1.12E–25	6.28E–25
GO: 0044421—extracellular region part		2.10	8.15E–28	6.96E–27		1.99	3.55E–22	4.99E–21
Glycoprotein	3 (48.8)	1.76	2.33E–64	1.89E–63	3 (27.9)	1.82	2.18E–71	1.38E–70
Glycosylation site: N-linked (GlcNAc…)		1.73	3.44E–56	4.61E–56		1.80	6.99E–64	9.76E–64
Topological domain: cytoplasmic		1.49	1.30E–19	3.48E–19		1.78	1.14E–35	2.12E–35
GO: 0006952—defense response	4 (20.8)	2.64	3.05E–31	1.49E–31	5 (20.1)	2.64	1.88E–31	1.81E–31
GO: 0006954—inflammatory response		2.48	1.82E–12	2.66E–12		2.41	3.26E–11	4.71E–11
GO: 0009611—response to wounding		2.05	3.20E–11	6.25E–11		2.02	1.74E–10	5.02E–10
Cytokine	5 (15.8)	3.69	2.24E–18	3.17E–17	7 (9.9)	3.49	1.18E–15	2.73E–14
GO: 0005125—cytokine activity		3.36	1.72E–16	2.35E–16		3.07	1.50E–12	1.98E–12
hsa04060: cytokine–cytokine receptor interaction		2.11	1.01E–07	1.38E–06		2.35	1.68E–10	1.17E–09
GO: 0031226—intrinsic to plasma membrane	6 (15.1)	1.71	7.98E–16	9.09E–15	4 (22.6)	1.90	4.05E–24	3.42E–23
GO: 0005887—integral to plasma membrane		1.71	6.87E–16	9.78E–15		1.89	4.04E–23	4.54E–22
GO: 0044459—plasma membrane part		1.46	4.69E–14	7.99E–13		1.57	7.11E–21	1.20E–19
GO: 0005886—plasma membrane		1.26	7.70E–10	1.53E–08		1.35	6.24E–17	1.23E–15
Topological domain: cytoplasmic	7 (12.2)	1.49	1.30E–19	3.48E–19	3 (27.9)	1.63	8.09E–31	1.88E–30
Membrane		1.25	1.13E–12	2.28E–11		1.30	6.76E–18	9.95E–17
GO: 0005886—plasma membrane		1.26	7.70E–10	1.53E–08		1.35	6.24E–17	1.23E–15

Term	LCLs versus WB				LCLs versus SL			
	Cluster# (EScore)	Fold enrichment	Benjamini	FDR	Cluster# (EScore)	Fold enrichment	Benjamini	FDR
Transmembrane region		1.25	3.14E–08	9.85E–08		1.34	1.19E–15	3.87E–15
Transmembrane		1.25	2.49E–09	1.01E–07		1.34	5.12E–16	9.68E–15
Cell membrane		1.47	3.92E–11	1.11E–09		1.57	9.79E–16	2.06E–14
Topological domain: extracellular		1.65	3.06E–26	6.85E–26		–	–	–
GO: 0008544—epidermis development	8 (9.9)	2.85	3.04E–10	8.92E–10	9 (6.6)	2.41	4.80E–06	3.23E–05
GO: 0030216—keratinocyte differentiation		4.40	3.32E–10	1.14E–09		3.58	5.81E–06	4.47E–05
GO: 0007398—ectoderm development		2.73	6.19E–10	2.42E–09		2.42	1.12E–06	6.49E–06
GO: 0009913—epidermal cell differentiation		4.17	5.74E–10	2.81E–09		3.41	8.19E–06	6.70E–05
Keratinization		5.84	2.68E–10	8.67E–09		4.90	6.57E–07	3.73E–05
GO: 0030855—epithelial cell differentiation		3.08	2.26E–09	1.33E–08		2.42	2.05E–04	2.75E–03
GO: 0031424—keratinization		4.80	6.87E–08	6.38E–07		3.96	1.08E–04	1.24E–03
IPR003267: small proline-rich		6.51	5.77E–07	1.57E–06		4.97	8.95E–04	1.38E–03
GO: 0009617—response to bacterium	9 (8.9)	2.67	4.08E–09	2.59E–08	6 (10.2)	2.89	4.59E–11	1.11E—10
Antimicrobial		4.19	2.82E–09	1.20E–07		4.60	5.17E–11	1.52E–09
GO: 0042742—defense response to bacterium		3.18	3.16E–08	2.48E–07		3.46	5.94E–10	2.00E–09
Antibiotic		4.21	4.99E–09	2.32E–07		4.32	3.15E–09	1.32E–07
GO: 0042330—taxis	10 (8.4)	3.05	1.62E–10	3.96E–10	7 (9.9)	3.13	3.54E–11	6.82E–11
GO: 0006935—chemotaxis		3.05	1.62E–10	3.96E–10		3.13	3.54E–11	6.82E–11
Chemotaxis		4.28	8.28E–10	2.85E–08		4.38	4.10E–10	1.55E–08
IPR000827: small chemokine, C–C group, conserved site		6.91	1.16E–07	1.05E–07		7.04	4.35E–08	7.92E–08
IPR001811: small chemokine, interleukin-8-like		5.21	2.12E–07	3.85E–07		5.56	3.17E–08	2.88E–08
SM00199: SCY		5.07	1.07E–07	4.59E–07		5.24	1.54E–08	6.44E–08
GO: 0008009—chemokine activity		4.68	1.48E–07	1.22E–06		4.90	5.57E–08	1.47E–07
GO: 0042379—chemokine receptor binding		4.39	3.70E–07	5.06E–06		4.60	1.73E–07	6.86E–07
PIRSF001950: small inducible chemokine, C/CC types		5.37	5.78E–06	1.17E–05		5.60	3.24E–06	6.44E–06
GO: 0007610—behavior		1.64	6.06E–04	1.29E–02		1.81	5.89E–06	4.25E–05
GO: 0007626—locomotory behavior		1.92	2.57E–04	4.65E–03		2.07	1.35E–05	1.24E–04

The top list of DAVID annotation clusters is presented

Table 3

The annotation of genes that have shown differential methylation in whole blood compared to saliva (WB vs. SL) and lymphoblastoid cell lines (WB vs. LCLs), in terms of their expression in different tissues

Term	Gene count	Gene (%)	*p* value	Fold enrichment	Benjamini
WB versus SL					
WBCs, plaque macrophage	38	10.00	1.47E–04	1.93	1.68E–02
WBCs, monocyte-depleted mononuclear cells	39	10.26	1.32E–03	1.70	3.21E–02
WBCs, monocyte	48	12.63	2.28E–03	1.55	4.83E–02
Salivary gland	247	65.00	1.85E–02	1.07	8.20E–02
WB versus LCLs					
Cerebellum	1110	60.72	5.88E–23	1.15	4.58E–21
Salivary gland	1140	62.36	4.45E–22	1.14	1.74E–20
Burkitt lymphoma (Raji)	1018	55.69	3.21E–09	1.10	3.13E–08
Tonsil	939	51.37	7.55E–05	1.07	3.68E–04
Tongue	386	21.12	5.35E–05	1.18	2.98E–04
Whole blood	479	26.20	5.37E–05	1.15	2.79E–04
BM-CD105+ endothelial cells	427	23.36	4.08E–04	1.14	1.77E–03
Uterus	293	16.03	1.74E–03	1.17	6.17E–03