

## Correspondence

# Differences in DNA methylation patterns between humans and chimpanzees

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Methylation at CpG dinucleotides is important for gene regulation in mammals [1]. However, it is unknown how methylation patterns change during evolution. Here, we compare methylation patterns between humans and chimpanzees at 36 genes in the brain, the liver and in lymphocytes. We find that the extent of the change in methylation pattern is much more extensive in the brain than in the other tissues. Furthermore, of the 15 CpGs that have significantly changed methylation in the brain, 14 are more methylated in humans than in chimpanzees. This indicates that CpGs might generally be more methylated in human brains than in chimpanzee brains.

Despite considerable phenotypic differences, humans and their closest living relatives, the chimpanzees, are on average 98.8% identical in their alignable genomic DNA sequences [2,3]. It is currently unknown which genotypic differences are responsible for the phenotypic differences. One possibility to tackle this question is to compare gene expression patterns between humans and chimpanzees using functional genomic approaches [4–6]. In this respect, it may also be useful to compare methylation patterns in regulatory DNA sequences, as the methylation status can be viewed as a “footprint” of the chromatin structures that are crucial for gene regulation [7,8]. In order to take a first step toward understanding the evolution of

methylation patterns, we compared the methylation status of 145 CpGs in the presumed regulatory regions of 36 different genes between humans and chimpanzees in brain, liver and lymphocytes using a recently developed array technique [9–11]. Thereby, genomic DNA is treated with sodium bisulphite such that unmethylated CpGs are amplified as TpGs in the following PCR. For each CpG examined, the arrays contain two oligonucleotides: one complementary to a TpG, resembling a formerly unmethylated CpG and one complementary to a CpG, resembling a formerly methylated CpG.

We identified 22 CpGs in which the ratio of the intensities of these two oligonucleotides differed significantly between human and chimpanzee in at least one tissue. By contrast, zero to three differences would be expected due to random experimental and biological variation, as is shown by permutating the species labels for each tissue (see supplemental data for all methodological details). Therefore, the differences between the two species are highly significant, whereas the differences between the individuals of the same species are within the range of the experimental error (data not shown). We also do not observe a strong correlation of methylation levels with age or time *post mortem* (see supplemental data). Thus, we conclude that most of the observed methylation differences between humans and chimpanzees are neither due to random measurement errors nor due to random or systematic differences in their environment.

To exclude trivial genetic causes, we sequenced the region of the 22 CpG sites in the chimpanzee and excluded 4 CpGs that carried a sequence difference between the chimpanzee sequence and the human-based oligonucleotide sequence. The remaining 18 CpGs from 12 genes are shown in Figure 1.

Three observations from these experiments are especially noteworthy: First, despite the limited number of CpGs studied,

several significant differences in their methylation status can be found between humans and chimpanzees. Second, out of 18 differences, 15 are found between chimpanzee and human brain, whereas only six are found between the other two tissues. Third, 14 of the 15 sites differing in methylation in the brain show a higher degree of methylation in humans.

The first observation indicates that — at least in humans and chimpanzees — the methylation status of many CpG sites changes during the course of evolution. The second observation indicates that more CpG sites have changed their methylation status in the brain than in liver or lymphocytes. Notably, DNA methylation seems to be especially important for the brain, as defects in methylation lead to mental retardation in humans [8] and a mouse model for one of these diseases — Rett syndrome — indicates that the symptoms can be caused solely by a defect in postmitotic neurons [12,13]. Our third observation, namely that 14 of 15 CpG sites differently methylated in the brain show a higher degree of methylation in humans, might reflect a general up-methylation of genes in the human brain, rather than several independent, gene-specific methylation changes. Although it is unclear at this point whether this up-methylation directly translates into observable changes in gene expression (supplemental data), it is tempting to speculate that such an up-methylation was important for the evolution of the human brain. However, we cannot exclude that a general tendency towards a lower degree of methylation occurred on the chimpanzee lineage. It is furthermore unclear if the change in methylation patterns is especially pronounced in the human brain or if a rapid change in methylation patterns is typical of brain evolution in many mammals. Further work has to clarify these issues.

### Supplemental Data

Supplemental data containing experimental procedures are

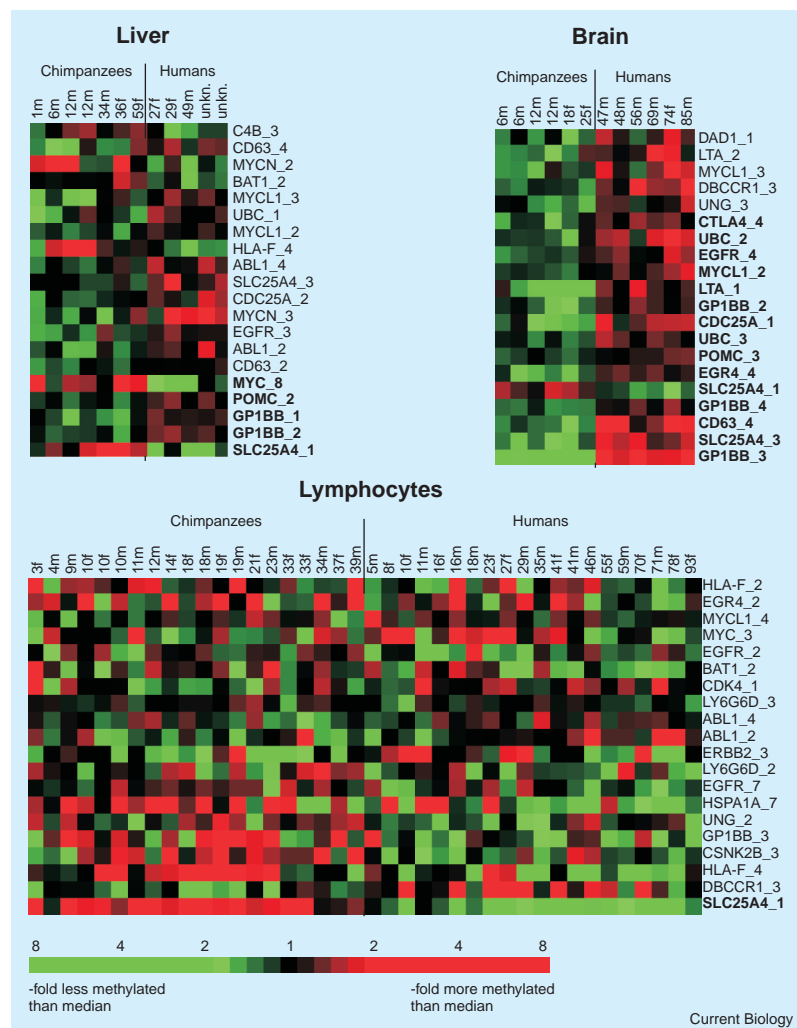


Figure 1. Methylation patterns of humans and chimpanzees in brain, liver and lymphocyte samples.

Each row corresponds to a single CpG, each column to one sample of one individual. For each cell the logarithm of the ratio of the intensity of the oligonucleotide representing the methylated CpG to the intensity of the oligonucleotide representing the unmethylated CpG is calculated and log<sub>2</sub> transformed. The color represents the methylation level calculated as the distance from the median of all values in a row. As indicated in the legend, more methylation than the median is depicted in red and less methylation in green. Each CpG is named by the official gene symbol of the associated gene and a consecutive number. For each individual the age and sex is indicated. The 20 CpG sites with the highest effect size of the *t*-statistic (*t*<sup>2</sup>) are shown, sorted by increasing *t*<sup>2</sup>-values from top to bottom. CpG sites classified as differently methylated are indicated in bold.

available at <http://www.current-biology.com/supplemental>

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