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Abstract

To date, diagnosis of ADHD is exclusively clinical; recent studies focused on the search for objective biomarkers. Recently (Adriani et al., 2018), we reported selective alterations of DNA-methylation status, by measuring five CpG sites in the 5'-UTR of dopamine-transporter (DAT1) gene (in ADHD patients), including a 1CGG-2CGG-3CGG and a 5CG-6CG motif. Extending that work, we analyzed DNA-methylation levels of the CpG sites complementary-on-opposite-strand ("cos"). On these results, we have run some cross-correlations: for instance, we found M5 - M6 COS and M2 COS - M1 relationships. Our data might be of relevance to find a new method to diagnose ADHD.

Keywords	DNA Opposite Strand; Dopamine Transporter (DAT); CpG Epigenetic Marker

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Dear Editor,

please find enclosed our short communication titled **Search for an epigenetic biomarker in ADHD diagnosis, based on the DAT1 gene 5'-UTR methylation: a new possible approach** which is authored by miss Gabriella LAMBACHER, Esterina PASCALE, Mariangela PUCCI, miss Silvia MANGIAPELO, Claudio D'ADDARIO, and myself undersigned. *Note* this is a revised paper, the PSY-2020-598 draft was edited as follows.

- We now provide a table for the correlations, for ease of reading.
- We now report p-values for strong correlations; these p-values were corrected for multiple testing and the strategy we employed to this purpose has been detailed. (see blue text)
- We now refer to correlation table in text, when highlighting the main outcomes. (see blue text)
- We provide our figures with English captions only, sorry for that.

We have been dealing in recent years with the study of two particular motifs found in the 5'-UTR of the DAT gene, very well known for vulnerability to ADHD as well as many other addictive disorders, or compulsive behaviors. We have tried, and report here about, a completely new approach to methylation levels: instead of looking at CpGs individually, we think that cross-correlations may inform about which ones are methylated at the same time on the very same DNA strand. We suggest here, for the very first time, that the ***OPPOSITE STRAND*** of DNA may be as important as the strand where a candidate gene stands, as far as epigenetic regulation through CpG methylation is concerned.

There are two items for potential conflict of interest to be disclosed:

Adriani W, Laviola G, Pascale E, D'Addario C " – *Metodo per determinare il deficit di attenzione con iperattività*" (Method to determine Attention Deficit and Hyperactivity Disorder). Patent Application in ITALY at no. 102016000129938 (22-December-2016); turned into European Patent Application at no. 17830021.6 (date 21-December-2017).

Granstrem O, Adriani W, Laviola G, Porfirio MC, Curatolo P " – Biomarkers for validation of ADHD diagnosis and monitoring of therapy efficacy". Full Patent No. PN810701WO, Int. Application No. PCT/EP2013/066845, Publication International No. WO/2014/023852 (date 10-August-2013).

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Search for an epigenetic biomarker in ADHD diagnosis, based on the DAT1 gene 5’-UTR methylation: a new possible approach

Highlights

Our clinical research was aimed to provide a new biomarker for ADHD diagnosis.

We aimed to epigenetic regulation of DAT1 gene promoter, at two 5'-UTR motifs.

The more gene strand is methylated, the more COS strand will be de-methylated, or vice-versa.

Cross-correlations among CpG methylation levels are a new approach to epigenetic analysis.

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Attention Deficit/Hyperactivity Disorder (ADHD) is the most common neuro- developmental alteration in childhood, with estimated world prevalence around 5%. To date, the diagnosis of ADHD is exclusively clinical, however recent studies have focused on the search for objective biomarkers. We are presently extending our recent work (Adriani et al., 2018), where we reported a selective alteration of DNA methylation status, by measuring 5 CpG sites in the 5'-UTR region of dopamine transporter (DAT1) gene in ADHD patients (when compared to controls), including a ^{1}CGG^{2}CGG^{3}CGG and a ^{5}CG^{6}CG motif. Extending that work, we here analyzed DNA methylation levels of the same CpG sites but complementary on the opposite strand (“cos”). On the results of the present research, we have run some correlations: for instance, we found M5-M6 COS and M2 COS-M1 relationships. Our data might be of relevance in the attempt to find a new method to diagnose ADHD in affected subjects.

Search for an epigenetic biomarker in ADHD diagnosis, based on the DAT1 gene 5'-UTR methylation: a new possible approach

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Short Title: DAT1 methylation (both strands) and ADHD

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Author contribution

WA, EP, CD conceived the study; EP, MP, CD realized the original CpG-methylation study; SM and GL described the cross-correlations on original methylation data; GL wrote a first draft with close supervision by WA; EP and CD critically commented on such draft.

Contribution to the field

We suggest here for the very first time that the OPPOSITE STRAND of DNA may be as important as the strand where a candidate gene stands, as far as epigenetic regulation through CpG methylation is concerned.

Funding statement

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Abstract

Attention Deficit/Hyperactivity Disorder (ADHD) is the most common neuro- developmental alteration in childhood, with estimated world prevalence around 5%. To date, the diagnosis of ADHD is exclusively clinical, however recent studies have focused on the search for objective biomarkers. We are presently extending our recent work (Adriani et al., 2018), where we reported a selective alteration of DNA methylation status, by measuring 5 CpG sites in the 5'-UTR region of dopamine transporter (DAT1) gene in ADHD patients (when compared to controls), including a ^{1}CGG^{2}CGG^{3}CGG and a ^{5}CG^{6}CG motif. Extending that work, we here analyzed DNA methylation levels of the same CpG sites but complementary on the opposite strand ("cos"). On the results of the present research, we have run some correlations: for instance, we found M5-M6 COS and M2 COS-M1 relationships. Our data might be of relevance in the attempt to find a new method to diagnose ADHD in affected subjects.

Keywords

DNA Opposite Strand, Dopamine Transporter (DAT), CpG Epigenetic Marker, CGAS, Conner's Scales

Highlights

Our clinical research was aimed to provide a new biomarker for ADHD diagnosis.

We aimed to epigenetic regulation of DAT1 gene promoter, at two 5'-UTR motifs.

The more gene strand is methylated, the more COS strand will be de-methylated, or vice-versa.

Cross-correlations among CpG methylation levels are a new approach to epigenetic analysis.

Introduction: DAT1 gene and its epigenetic modulation

Attention Deficit/Hyperactivity Disorder (ADHD) is the most common neurodevelopmental alteration in childhood (Curatolo et al., 2008; Purper-Ouakil et al., 2011) characterized by pervasive symptoms of inattention, hyperactivity, and impulsivity, which often lead to poor academic performance and impaired social interactions (American Psychiatric Association, 2000). The estimated world prevalence for ADHD is around 5% (Polanczyk et al., 2007). In 70-80% of cases ADHD coexists with one or more other disorders which aggravate the symptoms and make diagnosis and therapy even more complex. Currently ADHD diagnosis is still based on subjective observations and it is therefore of great relevance the development of a more definitively objective diagnostic system. For the identification of genes involved in ADHD, several studies have been conducted (Li et al., 2006; Wood & Neale, 2010), but to date no conclusive determinants genetic markers have been identified.

Recent research has focused on the dopamine transporter (DAT) because modifications in the expression and/or function of this gene may well lead to ADHD symptoms (Jucaite et al., 2005; Bannon, 2005). Recently some research has focused on the epigenetic regulation of DAT1 gene promoter, and in particular on DNA methylation. This is mostly occurring at cytosine-phosphate-guanine (CpG) sites, where a methyl group (-CH_3) is added to the cytosines in a covalent manner and directly exert negative effects on the expression of genes (Bird & Wolffe 1999). DNA methylation has been recently implicated in the development of psychiatric disorders, such as bipolar disorder, depression and schizophrenia (D'Addario et al., 2012, 2013, 2017). Xu et al. (2015) examined such epigenetic marker among Chinese Han ADHD children. This study, which compared 50 ADHD patients with 50 non-ADHD control subjects, searched among19 CpG sites located in the 5'-UTR, a gene regulation area. Their results reveal three individual CpG sites, not part of any motif, that showed a significant difference in methylation compared to the control group. We similarly assessed (Adriani et al., 2018) epigenetic status of the 5'-UTR region of DAT1 gene but addressing the nearby ^{1}CGG^{2}CGG^{3}CGG and the ^{5}CG^{6}CG motif, located at +717 from TSS and onward, in the first intron. We recruited school-aged children (6-12 years old) diagnosed by routine anamnestic and cognitive evaluation, plus Conners' scales and k-SADS. By correlation with clinical scores, we found that relatively higher levels of methylation at CpG M1 correlated negatively with CGAS (Children's Global Assessment Scale) value, and slightly with some Conners' subscales, thus serving an index for severity of ADHD. In contrast, relatively higher levels at CpG M6 were correlated with rescue of ADHD symptoms after six weeks of treatment.

A new approach for methylation level

It should be considered that the fidelity for maintenance of CpG methylation within cell division has been found to be very high in hemi-methylated DNA, while on the other hand, de novo

methylation resulted to be quite low (Riggs et al., 1998). Dnmt3a and Dnmt3b methylate DNA *de novo*, and this occurs without regard to the methylation status of the complementary CpG position (Okano et al., 1998). It should also be considered that within each CpG dyad, several enzymes (i.e. DNMT-TET-TDG) could theoretically serve as many as 21 cytosine modification states, not necessarily in symmetric form (Hao & Zhang, 2014). We thus thought of relevance the evaluation of the opposite reverse strand, in order to monitor for a possible differential methylation in the two strands in ADHD subjects.
For this reason, by simply extending the data analysis of our previous work (Adriani et al., 2018), we sought at analyzing DNA methylation levels of CpG residues which are the exact *complementary on the opposite strand* ("COS"), to the previous, already assessed ones. We selected (out of the originally recruited patients) a cohort of 14 ADHD patients (half 9\10 and half 10\10 genotype), for which we assessed the other strand (Fig. 1).

We have been the first, to our knowledge, to perform all correlations among the original (i.e., on gene strand) and the newly assessed (i.e., cos) CpGs. Interestingly, the newly assessed CpGs (termed from M7-cos to M1-cos) correlated among them and also with the old CpGs (termed from M1 to M7). These results are shown elsewhere (Tonelli et al., submitted). Briefly, the M5-cos is correlated with M6-cos whereas the M1-cos, M2-cos and M3-cos are all strongly correlated one to each other. The M6-cos is negatively correlated to M6 while the M1-cos is negatively correlated to M2. The unexpected finding of a negative correlation led to the hypothesis that the opposite strand may well be de-methylated when the gene strand is methylated, and vice versa. As such, for each pair of CpG positions (e.g. for M1 and M1-cos), while in theory we have four possible situations (both methylated; both de-methylated; gene-strand methylated and cos de-methylated; gene-strand de-methylated and cos methylated), the last two may be more likely. The probability of each above situation can be calculated by simply multiplying the raw methylation level or the difference **(100 - methylation level)**. As such, the probability of the four situations is:

- both methylated, M1-M1cos: M1 x M1cos;

- both de-methylated, D1-D1cos: (100-M1) x (100-M1cos);

- gene-strand methylated & opposite de-methylated; M1-D1cos: (M1) x (100-M1cos);

- gene-strand de-methylated & opposite methylated D1-M1cos: (100-M1) x (M1cos).

For every "couple" of positions, not necessarily the "pair" of two facing CpGs, we can identify the same four possible situations. For instance, together with CpG 1 we may be considering 2 COS, or together with CpG 2 we may be considering 1 COS. We termed "OMO" those situations when one among M1-M7 is methylated and one among M1 COS - M7 COS is also methylated (or vice-versa both are demethylated). We termed "EMI" those situations when one among M1-M7 is methylated while one among the COS is demethylated (therefore termed D1 COS to D7 COS); or, one among the gene-strand CpGs is demethylated (therefore termed D1 to D7) while one among M1 COS - M7 COS is methylated. Thus, for each pair or couple of positions we have four possible products, and we have six times six i.e. 36 pairs of positions. We prepared

a matrix with all the various 144 combinations, from (de)methylation levels of six CpGs on one strand and the corresponding complementary ones on the opposite strand.
Therefore, we decided to look for all the possible correlations, between probability of a given situation in a pair or couple of positions and probability of a given situation in another pair or couple of positions, being one CpG on gene strand and one CpG on COS. In this way, we aimed at looking which setup is the most probable among four CpGs (two on base strand and two on COS), which can each be either methylated or demethylated.

Results and Discussion

By making all correlations across all the possible series of combinations (i.e., considering probability of 4 given situations in all pairs of CpGs), we have obtained a great quantity of comparisons. It is obviously impossible to take all them into account, therefore we limited the analysis tp two set-ups. In the first case (see Fig. 1), we start listing the correlations with the assumption of base strand methylated and opposite strand demethylated (one of the four possibilities); in second case (Fig. 2), all positions on the opposite strand are methylated and those on the base strand are methylated. The pairs or couples of positions are therefore the same but in the exactly opposed set-up. This assumption, namely of considering the two EMI set-ups and not the other two OMO set-ups, came from the notion of a negative correlation (if any) found between the two strands (Tonelli et al., submitted).
Within a given setup, starting from a given pair (e.g. M1 - D1 COS is the first one), the found correlations have been listed sorted according to a motif-wise criterion (see Table 1). In other words, the correlations with first motif (i.e. CpG 1, 2, 3) and with the second motif (i.e. CpG 5, 6, also including 7) have been considered separately from “hybrid” correlations (i.e. whereby one among 1\2\3 is multiplied by one among 5\6\7). This was done for statistical reasons, as the P values associated with R values had to be corrected for multiple comparisons. Within each of the three motif-wise subgroups, there is a quantity of nine pairs to correlate with (i.e. all permutations); since Bonferroni correction was applied, a significant tendency was only investigated for R>0.6411 (with 13 degrees of freedom).

First set-up: gene-strand CpG is methylated and COS is not (Fig. 1)

While a lot of correlations were found for the second set-up (see Table 1), both within and between motifs, no correlations emerged as significant for the first set-up. However, given a functional relevance of the first set-up (see Adriani et al., 2018), we reasoned that those correlations with the highest R values deserved to be shown anyway.
It is obvious that any correlation, emerging for a given pair when looking from another pair, shall be found back and emerge again for the latter pair when looking from the former pair. This is confirming that our worksheet had no calculation error. These cases are reported in “italic” in Table 1. To give just few examples:

1) when looking from M2 - D1 cos, a slight positive correlation with the pair M5 - D6 COS was found; when looking from M5 - D6 COS, the same positive correlation with the pair M2 - D1 cos was found, indeed.

2) when looking from M2 - D2 cos, a positive correlation of 0,3980886016 with the pair M5 - D5 COS was found; when looking from M5 - D5 cos, a positive correlation of 0,3980886016 with the pair M2 - D2 COS was found, ***indeed.***

Second set-up: COS is methylated and gene-strand CpG is not (Fig. 2)

The most abundant and interesting quantity of correlations emerges when looking from **M2 cos - D2** which in itself is a relevant CpG (Adriani et al., 2018). Among others:

1) A positive correlation of 0,863002086390621 with the M3 COS - M1 was found. Also, a positive correlation of 0,687787312995826 with the M3 COS - D1 was found.

2) A positive correlation of 0,762305164861466 with the M6 COS - M1 was found. Also, a positive correlation of 0,653075853880892 with the M6 COS - D1 was found.

This kind of finding may seem somewhat contradictory. How is it possible that the very same pair correlates with another pair where the CpG 1 can, at the same time, be methylated or de-methylated? To explain this, it should be took into account that the level at CpG 1 is here multiplied by another level, measured on CpG 3 cos or 6 cos. To keep correlation valid, in both cases, the only explanation is that the companions of the pairs go in opposite fashion compared to CpG 1. In other words, CpG 1 *is not* independent from CpGs 3 cos and 6 cos ! Sometimes they go in the same fashion, sometimes they go in opposite fashion, but always (as a pair) correlating with the pair formed by CpG 2 and 2 cos.
The functional implication is the following: when considering CpG 1 and CpG 6 cos, they can *both of them* covary with CpG 2, together, or *either of them* may covary while the other anti-covaries ! The same notion can be inferred for CpG 1 and CpG 3 cos. Such very unsuspected conclusion is important, in particular, since it means that, in the current set-up, the CpG 1 on the gene-strand may well turn out to get methylated not independently: either together with others on the same strand or together with those on the opposite strand.

Also, when looking either from M5 cos - D6 or from M6 cos - D6, a positive correlation with the M2 COS - M1 was found. Such a positive correlation was of 0,758999998484596 or of 0,779024617001857, respectively. This finding in particular is important since it means that, in the current set-up, the CpG 1 on the gene-strand may well turn out to get methylated.

-HERE TABLE 1 and FIGURES 1 and 2-

Possible implications drawn from this new kind of cross-correlation approach.

So, in addition to previous data about simple correlations between couples of CpGs (Tonelli et al., 2020), we presently investigated correlations between couples of situations, whereby for "situation" we defined a pair of CpG in four possible states: consequently, the diagrams (Fig. 1 and 2) show us which situations correlate with others. The results have shown that starting from one set-up, the correlating situations are quite often from that same set-up, and not from others. This was not obvious: if one given situation is in one state, another situation (if far and independent) might well be in any of its possible four states. Therefore, as a whole, the more the cos strand is methylated, the more the gene strand will be de-methylated; the vice-versa is less likely (or at least the correlations are weaker). We can observe on first set-up (Fig. 1) two special cases: M2 - D1 COS and M5 - D6 COS. Both of them are presented with two dotted lines: a blue line parallel to the black line. Therefore we can assume, from these two particular cases, that both states (methylation or de-methylation) can be considered equally probable for CpG 1 and for CpG 6 COS; in our opinion, these two specific CpGs may change of state quite frequently, compared to all the other situations.

In the previous commentary (Tonelli et al., 2020) it was found out that the CpG 6 and the CpG 6 COS are anti-correlated, when looked at individually: we propose that such an inverse correlation shall be spared in any case. Due to the fact that CpG 6 COS can be considered as a"pivot", i.e. getting frequently methylated or loosing that (see above), also CpG 6 will be either de-methylated or methylated, respectively, always opposite than CpG 6 COS.

Second set-up (Fig. 2) is similar to the preceding but carried out starting from the opposite assumption (gene strand de-methylated; cos strand methylated). As we can easily see, there are many more correlations than in the previous set-up, and they are much more stronger. For the sake of clarity, in Fig. 2 we show only correlations with R>0.70 while full results are listed in Table 1.

A noteworthy situation can be caught by regarding, again, at the black lines parallel to blue lines (which represent the OMO situations, where both CpGs in the pair are methylated). One such parallel black line was not drawn (for sake of clarity): M3 COS - D1 (see however bold lines in Table 1). In this set-up, we can notice that nearly all the OMO situations start from the CpG 1. Therefore, we can assume that there is strong ground to consider the CpG 1 as a "pivotal" one: starting from being de-methylated (as per assumption), in frequent cases it can also get to be methylated. Noteworthy, these changes of status occurring at CpG 1 are not independent from those occurring in the pair formed by 6 and 6 cos: they can covary, or they can anti-covary. Their dynamic change, rather than their instant status, may be relevant.

The opposite strand, according to our opinion, could act as a control element, because in order to allow the base strand to be methylated the opposite strand has to be de-methylated and vice-versa. The transition from first to second setup may well imply a change at the level of CpG 6 COS, transition back may well imply a change at the level of CpG 1.

In the meanwhile, the CpG M6 COS was considered being pivotal in the first situation (first set-up, Fig. 1). As such, we can assume that the two most-sided CpGs, M6 COS and M1, are pivotal for functional dynamics within the two nearby motifs, studied here. To reconcile the present findings with those published recently (Adriani et al., 2018), it is tempting to speculate the following hypothesis: there may be different subjects, or different conditions within a same subject, for which CpGs 6 (cos) and 1 are either moving their status together (i.e. covary) or in opposed fashion (i.e. anti-covary). It might be of interest to follow the very same subject for a prolonged period of time (i.e. with repeated samples collected over weeks, or at different time during the circadian rhythm), and to run similar correlations on these multiple samples. Thus, it would become possible to observe whether methylation at these residues actually changes, and to demonstrate that such changes can be coordinated in a yet unsuspected way.

Conclusion and future perspectives

Noteworthy, in ADHD patients the overall level of methylation was lower than controls (Adriani et al., 2018). Interestingly, the average level of methylation was more or less similar, around 5-10%, also in the COS strand. This notion together with the “pivot” phenomenon we hereby proposed leads to another implication. If the gene and COS strands have tendency to be in a hemi-methylated status, we may propose that 5-10% of DNA molecules, in which the gene strand motifs are methylated, are not the same 5-10% of DNA molecules, in which the COS strand is methylated; together, we may propose that up to 15-20% of all DNA molecules have methylation in either strand. The consequence is that 80-85% of all DNA molecules are likely to have no methylation at all in either strand, at least in ADHD. This is not obvious: in line of principle, if CpG methylations are entirely independent, or even mutually exclusive, we could at least hypothesize that 5-10% of DNA molecules are methylated in CpG 1, other 5-10% of DNA molecules are methylated in CpG 2, and so on. Since we are considering five positions per strand along the motifs, it would hypothetically be that nearly 100% of DNA molecules had at least one of these ten positions in a methylated status, in case it was completely casual.

Main purpose of our research was to construe novel notions about the dynamics occurring at 5’ UTR of the DAT1 gene. The first diagram (Fig. 1) describes a low number of slightly correlating situations, when base strand is methylated and COS is de-methylated; the second diagram (Fig. 2) describes a lot of strong correlations, when base strand is de-methylated and a COS strand methylated. On the basis of the said diagrams, we can deduce that a “zipper” effect may well occur at a certain point, which leads to the passage from one to the other setup of situations. The results obtained by our research data have shown that a crucial pivot transition is the methylated OMO situation, which starts in one case from the gene strand at CpG 1 and in the other case from COS strand CpG 6 COS. Therefore, in real DNA molecules, we may always have a normal setup, with methylated COS strand; this changes by position CpG 1 turning from de-to-methylated on the base strand, until the whole gene strand is methylated. In the opposite picture, we may always have an altered setup, with methylated gene strand; this changes by position CpG 6 COS turning from de-to-methylated on the COS strand, until the whole COS strand is methylated (“zipper” effect).

Further work is warranted to ascertain whether such a dynamic is actually occurring in reality. To date, diagnosis of ADHD is solely based on structured interviews or on clinical observation and questionnaires; purpose of this commentary is to provide a new possible approach to search for an epigenetic biomarker for ADHD diagnosis. Our data presented here suggest a new approach to DNA methylation analysis.

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Table 1 - see file sent separately.

Figures 1 and 2 - see files sent separately.

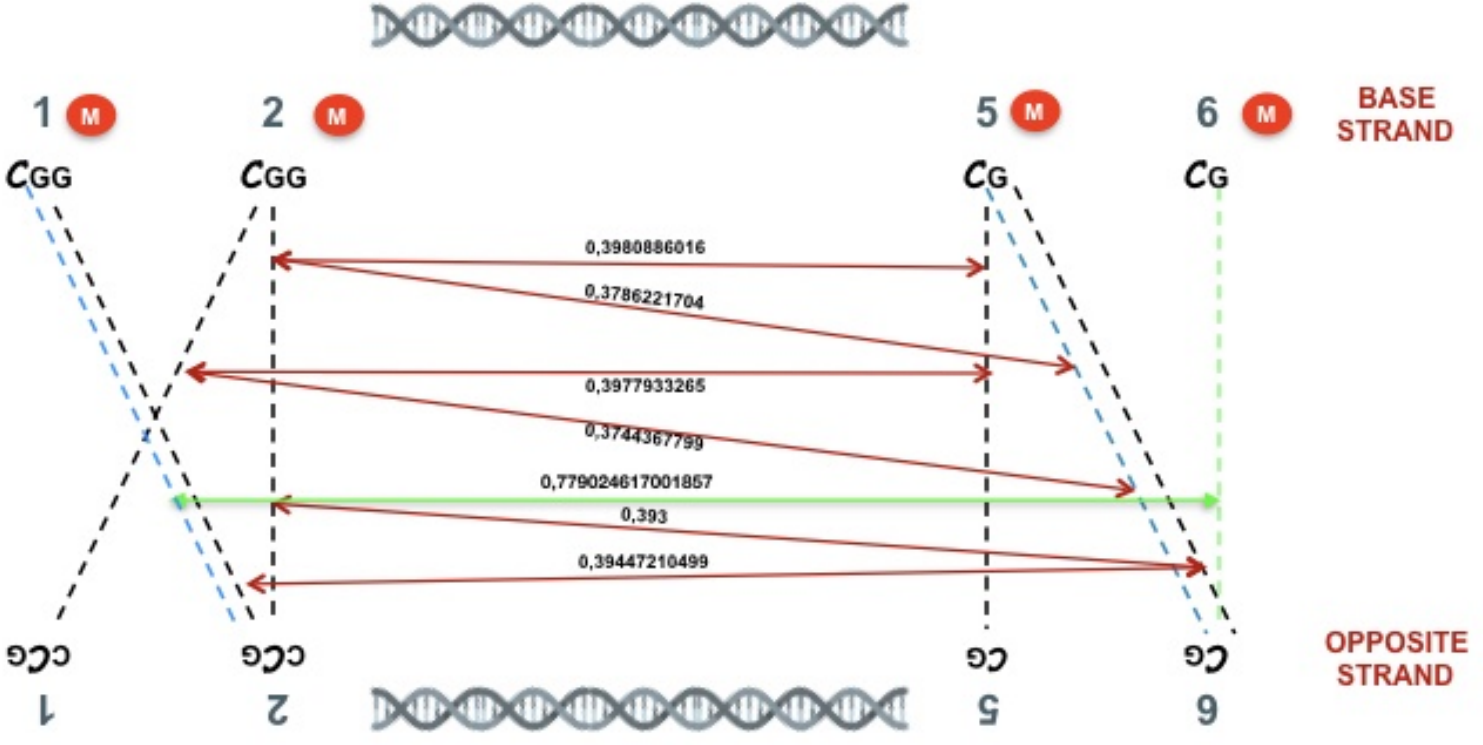
Caption of Figure 1:

Diagram representing the correlations of Table 1, first set-up. All dotted black lines represent the pairs of positions, with M on gene strand meaning methylation level and D on cos strand meaning de-methylation; the latter is estimated as (100-methylation). As just an example, first dotted line from left is M1 - D2 COS. The blue line describes an OMO situation where CpGs on both strands are methylated. Each of the red arrows in the diagram, between one dotted line and another (with R value over it), represent the correlations that we can locate between one couple of CpGs in this set-up and all other possible couples in that set-up. None of these correlations is significant, R<0.6411; P>0.10 (df=13).

Caption of Figure 2:

Diagram representing the correlations of Table 1, second set-up. All dotted black lines represent the pairs of positions, with M on cos strand meaning methylation level and D on gene strand meaning de-methylation; the latter is estimated as (100-methylation). As just an example, first dotted line from left is D1 - M2 COS (the remaining is like for Fig. 1). These correlations are highly significant for R>0.7604; P<0.01 (*) (df=13).

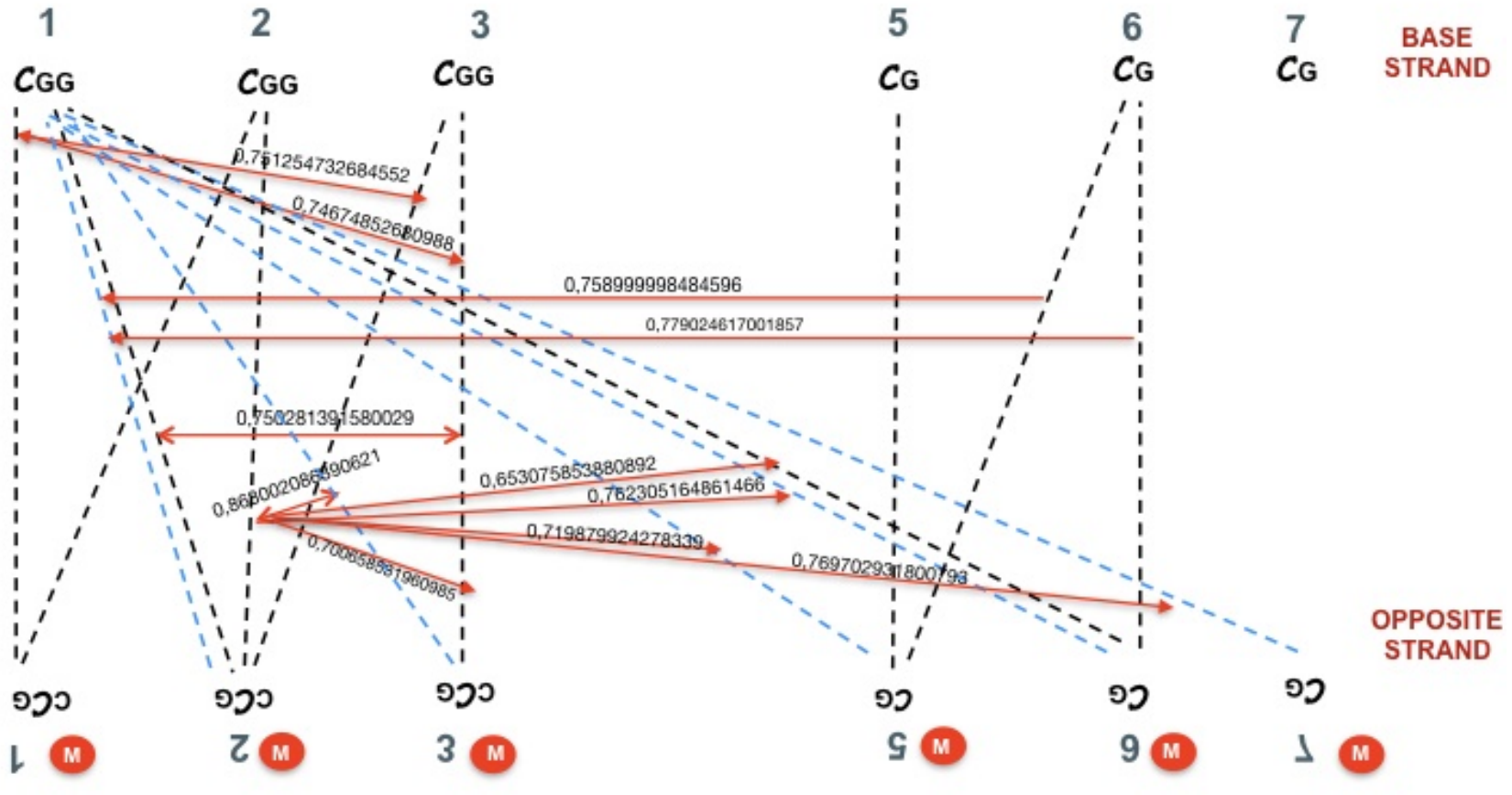
DAT GENE PROMOTER



Legend:

- Situation
- Situation EMI (base strand METH; opposite strand DEMETH)
- Situation OMO (both METH)
- Positive Correlations

DAT GENE PROMOTER



Legend:

- - - - Situation EMI (opposite strand METH; base strand DEMETH)
- - - - Situation OMO (both METH)
——— Positive Correlations

CORRELATION WITH ANY AMONG 1, 2 OR 3	HYBRID CORRELATION CpG 1/2/3 x CpG 5/6/7	CORRELATION WITH ANY AMONG 5, 6, OR 7

Gene strand Methylated; COS strand DE-methylated (Fig. 1)

None of these correlations was significant according to the Bonferroni corrected threshold.

M2 - D1 COS

	M3 - D5 COS 0,427033824	*M5 - D5 COS 0,3977333265* ***M5 - D6 COS 0,3944721049*** **M5 - M6 COS 0,3744367799** M7 - D7 COS 0,4177846334

M2 - D2 COS

	M3 - D5 COS 0,4266845347	*M5 - D5 COS 0,3980886016* ***M5 - D6 COS 0,393440450*** **M5 - M6 COS 0,378622174** M6 - D7 COS 0,3901105495

M5 - D5 COS

M2 - D1 COS 0,3977333265 *M2 - D2 COS 0,3980886016* M2 - M3 COS 0,5291183531 M3 - D2 COS 0,3890903518		

M6 – D5 COS

		M7 - D7 COS 0,5110902772

M5 – D6 COS

M2 - D1 COS 0,3944721049 *M2 - D2 COS 0,393440450* M2 - M3 COS 0,5156527803 M3 - D2 COS 0,384019804		

M6 - D6 COS

		M7 - D7 COS 0,4954077093

Gene strand DE-methylated; COS strand Methylated (Fig. 2)

M1 COS - D1

M2 COS - D2 0,67549283776 M3 COS - D3 0,74674852630 M2 COS - D3 0,75125473268		

M1 COS - D2

M3 COS - D3 0,68977713431	M3 COS – D6 0,66771637412	*M6 COS - D6 0,6660607532* *M5 COS - D6 0,6692714879*

M2 COS - D1

M3 COS - D3 0,75028139158		

M2 COS - D2

M1 COS - D1 0,67549283776 **M3 COS-D1 0,68778731299** **M3 COS-M1 0,86300208639** ***** (*these two discussed, Fig. 2*) M3 COS - D3 0,70065853196	M3 COS - D6 0,65274369907 M5 COS - M1 0,71987992427 **M6 COS - D1 0,65307585388** **M6 COS - M1 0,76230516486** ***** (*these two discussed, Fig. 2*) M7 COS-M1 0,76970293180 *	*M6 COS - D6 0,65074117747* *M5 COS - D6 0,65512958253*

M5 COS - D6

M1 COS - D2 0,66927114879 M2 COS - M1 0,7589999985 *M2 COS - D2 0,65512958253*		

M6 COS - D6

M1 COS - D2 0,6660607532 M2 COS - M1 0,7790246170 * *M2 COS - D2 0,65074117747*		

Table 1. List of correlations we found. Each box groups all pairs of situations that correlate with one given pair of situations; listing is divided between corelations with the first motif (left column), with the second motif (right column), or hybrid (middle column). Italic (blue highlight) denotes the couples of correlations found back (see text). Bold denotes couples of correlations where the very same CpG is involved both as methylated and demethylated (see text). **Bonferroni corrected, separately within nine same-motif and nine hybrid correlations: shown, P<0.10 at R>0.6411; ** P<0.01 at R>0.7604 (see text).**

Supplementary material.

M3 COS - D3

M1 COS - D1 0,74674852630* M2 COS - D1 0,75028139158* M2 COS - D2 0,70065853196* M1 COS - D2 0,68977713431*		