CROSS-CORRELATIONS BETWEEN MOTIFS IN THE 5'-UTR OF DAT1 GENE: FINDINGS FROM PARKINSON'S DISEASE

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Author contribution

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

ABSTRACT

The Parkinson's disease (PD) is a neuro-degenerative disorder affecting the striatal motor system, caused by the loss of neuronal cells in the mid-brain. The reduced amounts of dopamine in the mid-brain cause involuntary movements and others symptoms. Alterations of the methylome have been reported in PD epigenomic studies. The human dopamine transporter gene (DAT1, *SLC6A3)* is considered as a candidate risk factor for PD. Since the DNA methylation on DAT promoter may have a role in the development of this disease, we aimed to further assess the epigenetic control, by focus on specific CpG sites located in the 5'-untranslated region (5'-UTR) of the DAT1 gene. Significant changes in DAT 5'-UTR methylation were ~~already~~ found in peripheral blood mononuclear cells (PBMCs) of PD subjects (Rubino et al., 2020). Of note, methylation values at the CpG 5 were increased. We run on same data a novel statistical approach: cross-correlation between pairs of loci. CpG 5 was the only always-differing variable but, alternatively, CpGs 2 and 6 or CpGs 1 and 3 were also significantly correlated with CpG 5. Interestingly, this picture emerged for those patients whose M2xM6 index was above-median; loci were rather independent for below-median patients. Present data may shed light into dynamics occurring at 5'-UTR of DAT1, a gene involved in PD but also in many psycho-physiological pathologies.

KEYWORDS: CpG Epigenetic Marker, Gene Promoter, Neuro-psychiatric Disease, Parkinson disease.

HIGHLIGHTS

1) DAT1 gene expression is very susceptible to epigenetic modifications, also in ADHD

2) We aimed to ~~find~~ a correlation, in PD, between two motifs in DAT1 gene's 5'-UTR

3) Combination of (de)methylations, in pairs of CpGs of two specific motifs, was studied

4) CpG 5 in 5'-UTR of DAT1 gene can be used as marker in the diagnostics of Parkinson's

1. INTRODUCTION

The Parkinson's Disease (PD) is a neuro-degenerative disorder that affects the extra-pyramidal tract of the midbrain ~~motor system of patients~~. The exact cause is still unknown but most of the research points to a combination of genetic and environmental factors. ~~genetic involvement: if someone had a family member with this disease, there is a high possibility that she/he will develop the disease him/herself.~~ Parkinson's manifests with a loss of neural cells in a well-known part of the midbrain, the substantia nigra, responsible for producing dopamine. The reduced amount of dopamine released in the dorso-striatal forebrain, when the midbrain cells are damaged or dead, causes the involuntary movements and the other symptoms like tremor, slowness of movements and rigidity.

The human dopamine transporter gene (DAT1, symbol *SLC6A3*) is considered as a candidate risk factor for Parkinson's Disease (Zhai et al., 2014). Synaptic DA dysfunction may represent an early stage results of degeneration of the substantia nigra pars compacta. Some modifications in PD were reported to occur early on, before the extensive neuronal death has occurred. Interestingly, a trend for reduced expression of DAT1 was detected on idiopathic PD patients in DA neurons from the substantia nigra (Fazio et al., 2018; CIT). The dopamine-transporter protein accumulates otherwise cytotoxic dopamine within dopaminergic terminals of neurons. These dopamine levels cannot be measured by imaging techniques: an indirect way to measure them is by looking for the DAT protein as the marker. The DAT-scan technique is used for the confirmation of a Parkinson's diagnosis: it is a specific type of single-photon emission computed tomography (SPECT), used to visualize dopamine-transporter levels in the brain (Costa et al., 2011). The DAT-scan can be used to differentiate PD symptoms from essential tremors or drug-induced PD: in the last two cases, patients don't have any loss of dopamine-transporter sites within their nigro-striatal system. The possible cytotoxic effects, related to reduced reuptake, may be conferred by genetic factors like a polymorphism in the DAT gene. The human DAT1 gene contains a 40-bp variable number of tandem repeats (VNTR) polymorphism, found in the 3'-untranslated region (3'-UTR) of the gene. Several groups have studied the associations between this VNTR polymorphism and some neuro-psychiatric disorders such as PD, as well as ADHD, schizophrenia and alcoholism (Vandenbergh et al., 2000; Hahn et al., 2011). Being the VNTR polymorphism located outside the open reading frame, it cannot affect the protein structure but it may affect its expression level, by modulating the structure or degradation of mRNA (Fuke et al., 2001).

Beyond genetics, the promoter-specific DNA methylation may have a role in the development of these diseases: epigenetic mechanisms include any process regulating gene expression without affecting the genome sequence. In most of the cases, a higher methylation leads to repression of a gene (Domcke et al., 2015). 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). Of note, epigenetic control likely involves the promoter region, well before the start site of mRNA transcription, or the 5'-untranslated region (5'-UTR), namely the portion of the gene which is at beginning of mRNA but before the start of the protein. This portion of DAT has been recently investigated by others, for instance in the frame of ADHD (Wiers et al., 2018). We similarly have assessed (Adriani et al., 2018) the epigenetic status of the 5'-UTR region of DAT1 gene, but we have been addressing the ^{1}CGG^{2}CGG^{3}CGG and the ^{5}CG^{6}CG motifs. We found that higher levels of methylation at CpG 1 were serving an index for severity of ADHD; in contrast, higher levels at CpG 6 were correlated with rescue of ADHD symptoms after six weeks of treatment. As a whole, the ADHD patients were characterized by greatly reduced methylation, compared to controls. It was yet to be ascertained what would happen in the case of elevated overall methylation.

Alterations of the methylome have been reported in PD epigenomic studies, and altered pattern of methylation in some genes have been found in both sporadic and familial PD (Erylmaz et al., 2017; Kaut et al., 2017; CIT). Even so, there is no clear report about which methylations do change during the progress of the disease (Wüllner et al., 2016). Therefore, we aimed to assess the epigenetic control of DAT1 in PD (Rubino et al., 2020), by focus on the same specific CpG sites (in the 5'-UTR of DAT1), previously shown to be differentially methylated in ADHD subjects (Tonelli et al., 2020; Adriani et al., 2018). A difference between PD patients and controls appeared only when considering the age as factor: methylation values at the CpG sites 2, 3, and 5 were different between the age groups. Whereas CpG 5 was the only clearly different variable, and can be considered as hyper-methylated, CpG 2 and 3 also changed significantly, depending on the stage of the disease, and were somewhat correlated with CpG5. However, we recently developed a new approach to study dynamic relationships for changes of methylation status (Carpentieri et al., 2020). Presently, we aimed at applying such approach on our PD data, because they gave us the unique opportunity to monitor the dynamics of change, in DAT1 5'UTR, when a situation of overall hyper-methylation could unveil unsuspected dynamics for interaction among the loci.

2. METHOD

The original study group (Rubino et al., 2020) consisted of 101 unrelated sporadic PD outpatients, exclusion criteria where the following: signs of atypical parkinsonism; history of neurological diseases other than idiopathic PD. Following initial recruitment, alcohol and substance abuse, family history of movements disorder, any other neurogical disorder were also added to the exclusion criteria. Methylation status of the DAT1 5'-UTR sequence was determined using pyro-

sequencing of bisulfite-converted genomic DNA (isolated by standard method from blood cells). The full details of this quantification are reported elsewhere (Rubino et al., 2020).

Out of the original study group, we extracted a subset of 19 patients with "advanced stage of illness" (see Rubino et al., 2020). For these, we re-considered all data, not only as individual loci for each CpG site, but also in terms of covariation among them. In first place, we considered not only the level of methylation, calculated by the methylation percentage **(mC/(mC + C))=M** (mC is methylated cytosine and C is unmethylated cytosine), but also de-methylation **(C/(mC + C))=D** i.e. 100-methylation. In second place, for each couple of loci, we considered the four possible situations: both methylated, M1xM2; both demethylated, D1xD2; one methylated and one not, M1xD2 or D1xM2. The latter two situations are particularly important for this new kind of approach (see Carpentieri et al., 2020). Indeed, if a correlation with such an index is found, this sheds innovative insights because some loci loose methylation when other loci get new methylation. Cross-correlations analyses were performed in such PD population, by means of Pearson's index between pairs of situations. Being it impossible to consider all permutations of the six CpGs methylations, we restricted our interest to some "quadrants" (this term denotes all the correlations emerging out from one single given situation, where the two loci are consecutive and in opposite setup, namely: M1D2, D1M2, M5D6, D5M6). Accordingly, the same loci were then also looked for correlations individually: M1, M2, M5, M6.

Simple correlations. We ... BOH

3. RESULTS

3.1 Cross-correlations among situations

The cross-correlations found in the analysis are too many to be described one by one. Just as an example of how the analysis was conducted, we display the following ones: these are few cases of "repetitive", overlapping and apparently contradictory findings. In these examples, a same locus is reported twice (i.e. appearing in two situations, both second term of a correlation), either in pair with two different other loci (which are then never returned as pair), or as methylated and de-methylated at once. This is only apparently a strange situation: it has always allowed us to draw interesting insights (Lambacher et al., 2020). All correlations that reach statistical significance can be found in **figure 1** and are discussed below **(paragraph 4).**

Quadrant M1-D2

-There is a positive correlation of (R=0,70768633251) with the situation M3-M5.

-There is a negative anti-correlation of (R=-0,77401026034) with the situation M3-D5.

Quadrant M2-D1

-There is a positive correlation of (R=0,8042906728906) with the situation M6-M5.

-There is a positive correlation of (R=0,7229847130409) with the situation M6-M7.

Quadrant M5-D6

-There is a positive correlation of (R=0,766470087161101) with the situation M1-M2.

-There is a negative anti-correlation of (R=-0,776450428877) with the situation M1-D2.

Quadrant M6-D5

Nothing (in terms of repetitive findings).

3.2 Cross-correlations between loci and situations

The situation is more clear when looking at pairwise correlations between a given CpG and all the possible situations (i.e. pairs of loci, which can be either methylated or demethylated).

Locus M1

-There is a positive correlation of (R=0,747724742839) on the locus M1 with locus M5; this means very simply that both get methylated together.

-There is an anti-correlation of (R=-0, 76103771049) on the locus M1 with the situation M3-D5; in further specification, it never can happen that CpG 1 gets methylated together with the position CpG 3 whenever there is a trend towards a de-methylated position CpG 5 ! In other words, there is *an apparent covariation* among CpGs 1, 3, and 5.

-There is a positive correlation of (R=0,765) with the quadrant M5D6; in further specification, it can happen that CpG 1 gets methylated together with the position CpG 5 whenever there is a trend

towards a de-methylated position CpG 6! As a *quick clue to this situation: CpGs 1 and 6 do go in an opposite fashion !*

Locus M2

-There is a positive correlation of (R=0,7748382776567) on the locus M2 with the locus M6; this means very simply that both get methylated together.

-There is a positive correlation of (R=0,8182810209905) with the situation M6-M5; in further specification, it can happen that CpG 2 gets methylated together with the position CpG 6 *whenever there is a trend towards a methylated position CpG 5!*

-There is a positive correlation of (R=0,7153623793399) with the situation M6-M7.

Locus M5

- There is a positive correlation of (R=0,777392841288213) with the situation M1-M2.

-There is a positive correlation of (R=0,756259284125) with the quadrant M1-D2; as further specification, it can happen that CpG 1 gets methylated together with the position CpG 5 *whenever there is a trend towards a de-methylated position CpG 2!* In other words, there is an apparent *opposite* covariation among CpGs 1 and 5 on the one side, and CpG 2 on the other hand. As a *quick clue to this situation: CpG 5 may covary either with CpG 1 or CpG 2, not both !*

-There is a positive correlation of (R=0,705073855117) with the situation M2-M7.

-There is a positive correlation of (R=0,881486951638) with the situation M1-M7.

Locus M6

-There is a positive correlation of (R=0,7968824627297) with the quadrant M2D1; in further specification, it well can happen that CpG 6 gets methylated together with the position CpG 2 *whenever there is a trend towards a de-methylated position CpG 1 !* As a *quick clue to this situation: CpGs 1 and 6 do go in an opposite fashion !*

3.3 Simple-correlations between loci BOH

4. DATA INTERPRETATION

First of all, in order to get a clear insight into our results, we shall underline that there are four "important" CpGs (i.e. 1, 2, 5, 6): with them, ten pairs can be formed. In this pair, each locus can enter as either methylated or de-methylated (a total of 4 situations x 10 pairs = 40 permutations). Therefore, pairwise correlations of all 40 permutations become 40x40=1600 ! Note that the **figure 1** shows significance for just a small portion of all theoric relationships.

If however these CpGs varied independently one from each other, there would be no specific relationship emerging as significant from our analysis. Thus, the fact that just some among all these relationships reached significance tells us that specific dynamics exist among the CpGs, and the two motifs, when getting methylated.

If we look well the graphic, we can see that there is no arrow pointing directly between situations M1-M2 and M5-M6; this is a clear indication of the fact that we cannot ever have any dynamic situation with all these 4 positions that are getting methylated together.

4.1 Dynamics excluded due to negative correlations

If we look the graphic of **figure 1** from the up right, we have **D5** who points with a blue arrow to situation **M1-D2** with R-value of *-0,77401026034*: this is an anti-correlation. Thus, it can *never* happen that CpG 2 gets *de-methylated together with the position CpG 5 whenever there is a trend towards a methylated position CpG 1 !* In other words, the dynamic situation whereby CpG *1 gets methylated*, *2 de-methylated* and *5 de-methylated* is impossible.

The same happens with the locus **D5** who points to **M1-M3** with a blue arrow, R-value of *-0,76103771048*: here is another anti-correlation. If the CpG 5 gets de-methylated, this cannot happen when the positions CpG *1 and 3 are getting methylated.* In other words, CpGs 1 and/or 3 *shall get methylated*, if CpG 5 is getting methylated; we don't have any other possible situation.

The situation **M5-D6** that points with a blue arrow to **M1-D2** with R-value of *-0,776450428877* is also an anti-correlation (see above). Here, we have a crucial notion about PD patients: there, overall, CpG 5 gets hyper-methylated (Rubino et al., 2020). Even whereby the positions CpG 1 and 5 get together methylated, anyway it *cannot happen* that CpGs 2 and 6 are getting *de-methylated.*

4.2 Dynamics involving correlations of CpG 5 with CpG 1

If we look the red arrows that start from **M1-D2** and point to **M5** with R value of *0,70768633251* plus to **M5-M3** with R value of *0,756259284125* (*the little arrow means that CpG 3 is methylated*),

we will appreciate similarly the locus **M1** who points to **M5-M7** (*see the little arrow to CpG 7*), with the R value of *0,881486951638* (all positive correlations). This is a confirmation of the dynamics depicted above: it can happen that the positions CpG 1 and 5 are methylated together, and also CpG 7 *may get methylated*. Moreover, if we have CpGs 1 and 3 methylated together with the CpG *5 hyper-methylated,* then it can happen that CpG *2 alone is getting de-methylated.*

4.3 Dynamics involving correlations of CpG 5 with CpG 6

If we look to the situation **D1-M2** who points to **M6** with R value of *0,7968824627297* and to **M5-M6** with R value of *0,8042906728906* (all positive correlations), we can see how dynamics change when CpG 6 is involved. This can happen whereby CpG *5 is hyper-methylated*, but the CpGs 2 and 6 are now *methylated together!* Interestingly, in such case, we will have the position *CpG 1* who is getting *de-methylated.*

Accordingly, if we look to the position **M1** who points to **M5-D6**, with R value of *0,765* (see above), a dynamic profile that can happen is whereby we have CpG 1 methylated together with *5 hyper-methylated* but now CpG *6 is getting de-methylated*. Once again, CpGs 1 and 6 apparently follow their reciprocally opposite dynamics.

Conversely, we shall note the quadrant **M1-M2** which points to position **M5** alone with the R value of *0,777392841288213* and which points to **M5-D6** with the R value of *0,766470087161101* (all positive correlations). This tell us that a dynamic situation that still can happen is whereby we have CpGs *1 and 2 methylated together*, when CpG *5 is hyper-methylated;* and moreover this happens when CpG *6 gets de-methylated.*

However, in apparent contradiction, position **M2** points to **M5** with R value of *0,705073855117,* to **M5-M6** with R value of *0,8182810209905* and to **M6** with R value of *0,7748382776567* (all positive correlations). There is however no contradiction. By looking all data as a whole, it can happen to find two confirmed dynamics: the position CpG *1 methylated*, when CpGs 2 or 3 also get methylated and "as opposite" CpG *6 gets de-methylated;* or, alternatively, CpG *1 de-methylated*, when CpGs 2 and 6 *get togheter methylated.*

5. DISCUSSION

Significant changes in DNA methylation were found in peripheral blood mononuclear cells (PBMCs) and brain of PD subjects (Wüllner et al., 2016; Kaut et al., 2017; Eryilmaz et al.,

2017). Together with other genes, DAT1 expression is very susceptible to epigenetic modifications which have indeed proposed for diagnostic and therapeutic approaches (Renani et al., 2019; Hegarty et al., 2016). The role of DNA methylation and its link to sporadic PD is not completely defined nor clearly characterized (Schulze et al., 2018; Schmitt et al., 2015). By comparing the DAT1 methylation level at individual CpGs within the 5'-UTR in PD, we recently observed an increased methylation at site CpG 5 (Rubino et al., 2020). On those data, a further analysis was run in the present study: the most important finding is the correlation between the increased methylation of CpG 5 and the dynamics of corresponding change in all other CpGs of the same island.

The increased methylation of CpG5 seems to come along with corresponding de-methylation either at CpG1 or at CpG6. This suggest that two patterns, for dynamic changes of methylation, may emerge during the progression of the disease. The role of DNA methylation as a possible mediator that drives or accompanies PD development is starting to be explored. The alternative patterns of cytosine methylation, either CpG 5 with 2 and 6 or CpG5 with 3 and 1, likely can regulate gene expression by affecting the ability of transcription factors to access and bind specific regions in the promoter sequence. DNA hyper-methylation could inhibit DAT1 transcription, which in turn may lead to reduced DA uptake: interestingly, however to allow such increased new methylation at CpG5, either CpG1 or 6 appear somewhat to loose methylation. This kind of dynamics confirms similar findings we had on ADHD patients (Lambacher et al., 2020) as wells on healthy controls (Carpenteri et al., 2020).

Thought these dynamics, PD patients may turn out to have reduced level of DAT protein in the pre-synaptic membrane, as also evidenced in vivo by DAT-scan techniques. The small sample here examined cannot allow conclusive evaluations, yet the results support the theory that epigenetic modifications in the DAT1 5'UTR could take part in PD progression. The potential methylation dynamics of DAT1 gene as a biomarker in PD, especially during disease progression, warrants further investigations in a larger group of PD patients. The present discovery can be a potential step towards better understanding the role of epigenetic modifications underlying PD pathogenesis.

5.1 Methodological remark

The correlations within first motif (i.e CpGs 1, 2, 3) and within second motif (i.e CpGs 5, 6, 7) have been considered separately form "hybrid" correlations **(figure 1).** Any correlation emerging for a given pair when looking from another pair was interpreted, in a cross-correlation strategy. The simplest approach would be to look for correlations between couples of individual CpGs (Tonelli et al., 2020); this was already done in the original paper (Rubino et al., 2020) but no clear dynamics

were evidenced. As recently proposed (Lambacher et al., 2020), we rather investigated pairwise correlations between couples of situations, considering not only methylation but also corresponding de-methylation levels. The correlating situations are quite the same set-up and not from others. If one given pair is in one state, another pair might be in any of its possible four states: this innovative approach amplifies the extracted information. Specific CpGs may display changes of state enough frequently, compared to other more stable situations.

5.2 Concluding remarks

To reconcile the present findings with those published recently on ADHD (Adriani et al., 2018), the simplest observation is that ADHD patients display overall hypo-methylated states, while PD ones do show CpG 5 hyper-methylation. Accordingly, ADHD data almost never point to CpG 5 (Adriani et al., 2018). Opposite trends for CpGs 1 and 6, also evident in ADHD, are presently receiving further information: CpG 1 appears related to CpG 5 "through" CpG 3 while CpG 6 appears related to CpG 5 "through" CpG 2 ! Of these two dynamic profiles, interestingly, the second one turned out to be useful as a biomarker in ADHD, while its role in PD deserves further study.

In the end, it is tempting to speculate the hypothesis that present profiles, emerged across different subjects, may be even more evident if looking to a same individual. It may be of interest to follow the same subject repeatedly, for a prolonged period of time, and run similar cross-correlations on these multiple samples. It would become possible to observe whether methylation at these residues would show similar changes, and demonstrate that such dynamic changes can be coordinated in a yet unsuspected way.

Aggiungere di valutare l' espressione genica?

EXTRA

It is hypothesized that depletion of DA nigrostriatal neurons get increased during PD progression. Thus, modulation of DAT1 gene expression that leads to a reduction in protein levels could occur in the remaining neurons, in order to ensure stable dopamine level in the synaptic gap.

REFERENCES

Adriani, W., Romano, E., Pucci, M., Pascale, E., Cerniglia, L., Cimino, S., ... D'Addario, C. (2018). Potential for diagnosis versus therapy monitoring of attention deficit hyperactivity disorder: A new epigenetic biomarker interacting with both genotype and auto-immunity. *European Child & Adolescent Psychiatry.* 27: 241–252.

Carpentieri et al., (submitted)

Costa A, Riedel M, Müller U, Möller HJ, Ettinger U. (2011) Relationship between SLC6A3 genotype and striatal dopamine transporter availability: a meta-analysis of human single photon emission computed tomography studies, *Synapse.* 65: 998-1005

D'Addario, C., Dell'Osso, B., Galimberti, D., Palazzo, M.C., Benatti, B., Di Francesco, A., ... Maccarrone, M. (2013). Epigenetic modulation of BDNF gene in patients with major depressive disorder. *Biological Psychiatry.* 73: e6-7.

D'Addario, C., Dell'Osso, B., Palazzo, M.C., Benatti, B., Lietti, L., Cattaneo, E., Altamura, A.C. (2012). Selective DNA methylation of BDNF promoter in bipolar disorder: Differences among patients with BDI and BDII. *Neuropsychopharmacology.* 37: 1647–1655.

Domcke S, Bardet AF, Ginno PA, Hartl D, Burger L, Schubeler D. (2015) Competition between DNA methylation and transcription factors determines binding of NRF1. *Nature.* 528: 575–579.

Eryilmaz IE, Cecener G, Erer S, Egeli U, Tunca B, Zarifoglu M, Elibol B, Bora Tokcaer A, Saka E, Demirkiran M, Akbostanci C, Dogu O, Colakoglu B, Kenangil G, Kaleagasi H. (2017) Epigenetic approach to early-onset Parkinson's disease: low methylation status of SNCA and PARK2 promoter regions. *Neurol Res.* 39(11): 965-972.

Fazio P, Svenningsson P, Cselényi Z, Halldin C, Farde L, Varrone A. (2018) Nigrostriatal DAT dopamine transporter availability in early Parkinson's disease. *Mov Disord.* 33(4): 592-599.

Fuke S, Suo S, Takahashi N, Koike H, Sasagawa N, Ishiura S. (2001) The VNTR polymorphism of the human dopamine transporter (DAT1) gene affects gene expression. *Pharmacogenomics J.* 1: 152-156

Hahn T, Heinzel S, Dresler T, Plichta MM, Renner TJ, Markulin F, Jakob PM, Lesch KP, Fallgatter AJ. (2011) Association between reward-related activation in ventral striatum and trait reward sensitivity is moderated by dopamine transporter genotype. *Hum Brain Mapp.* 32: 1557-1565

Hegarty SV, Sullivan AM, O'Keeffe GW. (2016) The Epigenome as a therapeutic target for Parkinson's disease. *Neural Regen Res.* 11(11): 1735-1738.

Kaut O, Schmitt I, Tost J, Busato F, Liu Y, Hofmann P, Witt SH, Rietschel M, Fröhlich H, Wüllner U. (2017) Epigenome-wide DNA methylation analysis in siblings and monozygotic twins discordant for sporadic Parkinson's disease revealed different epigenetic patterns in peripheral blood mononuclear cells. *Neurogenetics.* 18(1): 7-22.

Lambacher G, Pascale E, Pucci M, Mangiapelo S, D'Addario C, Adriani W. (2020) Search for an epigenetic biomarker in ADHD diagnosis, based on the DAT1 gene 5'-UTR methylation: a new possible approach. *Psy Res.,* (submitted)

Renani PG, Taheri F, Rostami D, Farahani N, Abdolkarimi H, Abdollahi E, Taghizadeh E, Gheibi Hayat SM. (2019) Involvement of aberrant regulation of epigenetic mechanisms in the pathogenesis of Parkinson's disease and epigenetic-based therapies. *J Cell Physiol.* 234(11): 19307-19319.

Rubino A, D'Addario C, Di Bartolomeno M, Salamone E, Locuratolo N, Fattapposta F, Vanacore N, Pascale E. (2020) DNA methylation of the 5'-UTR DAT1 gene in Parkinson's disease patients. *Annals of Neurology*, in press.

Schmitt I, Kaut O, Khazneh H, deBoni L, Ahmad A, Berg D, Klein C, Fröhlich H, Wüllner U. (2015) L-dopa increases α-synuclein DNA methylation in Parkinson's disease patients in vivo and in vitro. *Mov Disord.* 30(13): 1794-801.

Schulze M, Sommer A, Plötz S, Farrell M, Winner B, Grosch J, Winkler J, Riemenschneider MJ. (2018) Sporadic Parkinson's disease derived neuronal cells show disease-specific mRNA and small RNA signatures with abundant deregulation of piRNAs. *Acta Neuropathol Commun.* 6(1): 58.

Tonelli E, Pascale E, Troianiello M, D'Addario C, Adriani W. (2020) DAT1 gene methylation as an epigenetic biomarker in Attention Deficit Hyperactivity Disorder: a commentary. *Opinion Article,* section Epigenomics and Epigenetics*. Frontiers in Genetics.* 11: 444 !

Vandenbergh DJ, Thompson MD, Cook EH, Bendahhou E, Nguyen T, Krasowski MD, Zarrabian D, Comings D, Sellers EM, Tyndale RF, George SR, O'Dowd BF, Uhl GR. (2000) Human dopamine transporter gene: coding region conservation among normal, Tourette's disorder, alcohol dependence and attention-deficit hyperactivitydisorder populations. *Mol Psychiatry.* 5(3): 283-92.

Wiers CE, Lohoff FW, Lee J, Muench C, Freeman C, Zehra A, Marenco S, Lipska BK, Auluck PK, Feng N, Sun H, Goldman D, Swanson JM, Wang G-J, Volkow ND (2018) Methylation of the Dopamine Transporter gene in blood is associated with striatal Dopamine Transporter availability in ADHD: A preliminary study. *Eur J Neurosci.* 48(3): 1884-1895. doi: 10.1111/ejn.14067.

Wüllner U, Kaut O, deBoni L, Piston D, Schmitt I. (2016) DNA methylation in Parkinson's disease. *J Neurochem.* 139 Suppl 1: 108-120.

Zhai D, Li S, Zhao Y, Lin Z. (2014) SLC6A3 is a risk factor for Parkinson's disease: A meta-analysis of sixteen years' studies. *Neurosci Lett.* 3: 564-599

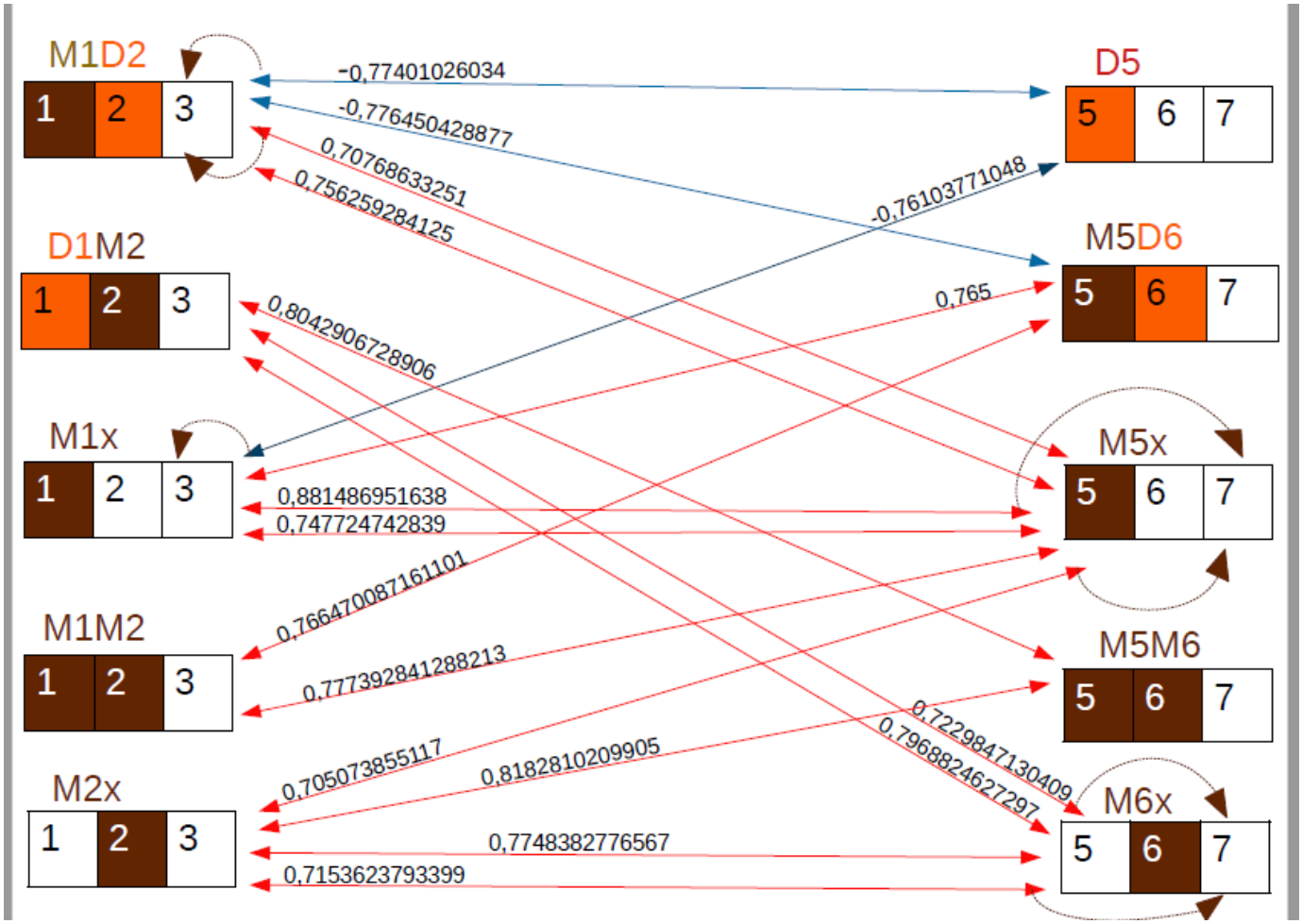

FIGURE 1

Figure 1 - we present data from cross-correlations in a graphic form. Brown-colour boxes mean the CpGs getting methylated, the orange-colour boxes mean the CpGs getting de-methylated, and the white colours means not comprised in the pair. *Example: M1-D2 means that position CpG 1 is getting methylated and position CpG 2 is getting de-methylated.* The red arrows stand for any positive correlation and the blue ones for any negative correlation. The little brown arrow, prolonging from a red arrow and indicating the white square, means that CpG (locus number 3 or number 7) is getting methylated.