

## DNA Methylation of Imprinted Loci on Autosomal Chromosomes and IGF2 are not Affected in Parkinson's Disease Patients Peripheral Blood Monocytes

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### Abstract

Genomic imprinting is an epigenetic phenomenon that results in differential expression of alleles depending on their parental origin. The functional significance of DNA methylation in genomic imprinting has been widely investigated and to date, around 100 imprinted genes have been identified in humans. To investigate, if methylation status of these "known" imprinting genes is associated with Parkinson's disease (PD), we analyzed methylation profile of all these "known" imprinting genes using an epigenome wide approach with Illumina's 450 K methylation chip. Strikingly, none of these total autosomal annotated genes show changes of DNA methylation between PD and healthy individuals. We further refined our analysis by evaluating DNA methylation for maternally imprinted human gene encoding insulin-like growth factor 2 (IGF2) by using bisulfite sequencing PCR (BSP) and by considering different dosages of L-dopa. Our results demonstrate that methylation profiles specifically at exon 8-9 genomic region of IGF2 gene in PD are neither influenced by the dosage of L-dopa treatment nor by the disease itself. Thus loss or disruption of imprinting in autosomal chromosomes seems not to be apparent in PD and is not relevant for the pathogenesis of the disease.

**Keywords:** Parkinson's disease; Imprinting; Epigenetics; DNA methylation; Autosomes

### Introduction

Genomic imprinting is an epigenetic phenomenon that results in the expression of either the maternally or paternally inherited allele of a subset of genes [1]. In humans, alterations of imprinting patterns give rise to numerous diseases with well characterized growth phenotypes (Beckwith-Wiedemann and Silver-Russell syndromes), behavioral disorders (Angelman and Prader-Willi syndromes) as well as several types of cancers [2-6].

To date, approximately 100 imprinted loci have been identified in the human genome ([www.geneimprint.com](http://www.geneimprint.com) and <http://igc.otago.ac.nz>) and epigenetic alterations in several imprinting genes have been connected with disease phenotypes [7-10]. There is, however, one report about two adults with Angelman syndrome (AS) who responded well to medication levodopa (L-DOPA) by showing dramatically improved resting tremor and rigidity signs [11]. Consequently, leading to a clinical trial of L-DOPA in individuals with AS, which is a severe neurodevelopmental imprinting disorder that results from loss of function of the maternal UBE3A allele [12]. However, in clinics levodopa (L-DOPA) has been widely used for treating Parkinson's disease (PD). Recently, our group has shown that higher dosage of L-dopa subsequently increases the  $\alpha$ -Synuclein (SNCA) methylation in sporadic PD patients [13].

In present study, we sought to investigate, if methylation status of these "known" imprinting genes also shows epigenetic modulation like SNCA methylation. We analyzed methylation profile of imprinting genes by selecting them from Illumina's 450 k arrays and created a subset of maternally and paternally imprinted genes. In addition, it has been shown that polymorphisms in the 3' untranslated region of the IGF2 gene, a homologue of IGF1 was protective against PD [14]. Loss of imprinting (LOI) of IGF2 causes human cancers, especially the Beckwith-Wiedemann syndrome. If loss of imprinting or aberrant imprinting of insulin-like growth factor 2 (IGF-2) is also associated with the onset of Parkinson's disease (PD) has not yet been investigated. Thus we focused on DNA methylation analysis of one maternally

imprinted human gene encoding (IGF2) by using bisulfite sequencing PCR (BSP) and investigated the influence of different dosages of L-dopa on its methylation.

### Material and Methods

#### Study cohort and DNA isolation for IGF2 analysis

Analysis of IGF2 imprinting locus for L-dopa was examined in age matched PD patients (n=15) and healthy individuals (n=9). Genomic DNA was isolated from peripheral blood. DNA from blood samples was extracted using commercially available kit protocols (QiaAmp DNA Blood<sup>®</sup>, Qiagen, Hilden, Germany; Oragene<sup>®</sup>, DNA Genotek, Ottawa, Canada). The Ethics Committee of the Medical Faculty of the University of Bonn approved the study (No. 51/00, 6th July 2000).

#### Study cohort and DNA isolation for epigenome-wide analysis (450 K arrays)

DNA samples derived from blood monocytes of 17 male Parkinson's disease patients vs. 21 healthy male individuals were used for a pairwise DNA methylation microarray analysis using the Illumina<sup>®</sup> 450 K methylation microarray system. PD patients were male only paired with male healthy brothers. And DNA samples derived from blood monocytes of 12 discordant MZ twin pairs aged 69.0±9.06 years were used for a pairwise DNA methylation microarray analysis using the

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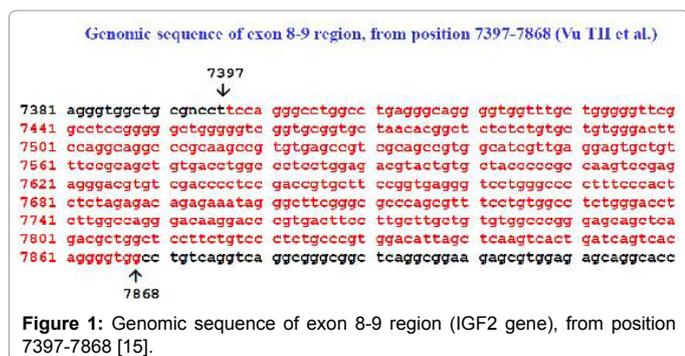
Illumina® 450 K methylation microarray system (Figure 1 and Table 1). MZ twin pairs were selected according to the following criteria: (i) European origin, (ii) both co-twins available for analysis, (iii) one twin of pairs was diagnosed Parkinson's disease.

### Profiling of CpG methylation using microarrays

400 ng of bisulfite treated DNA was analyzed using the Infinium Human Methylation 450 K bead arrays according to the manufactures instructions. For analysis of CpG methylation, the Illumina HumanMethylation 450 K BeadChip was used according to the manufacturer's instructions (Illumina, San Diego, CA, USA); BeadChip images were scanned and the data analysed using GenomeStudio (Illumina) software. The methylation values for individual CpG sites in each sample were obtained as  $\beta$ -values, calculated as a ratio of the methylated signal intensity to the sum of both methylated and unmethylated signals after background subtraction ( $\beta$ -values range from 0 (completely unmethylated) to 1 (completely methylated)).

### IGF2: DNA methylation and L-Dopa dosage

DNA extracted from human blood leukocytes was first treated with



Genomic orientation 5-3'		
IGF2	Forward	TTTAGGGTTTGGTTTGAGGGTAGGGT
IGF3	Reverse	CCACC CCTAT AACTA ATCAATAACTTAACT

Table 1: Primer sequences used for IGF2 methylation.

bisulfite conversion. Then PCR amplification was performed and PCR products of IGF-2 exon 8-9 region (GenBank Accession No. X03562) [15] sub cloned into a TOPO vector. Plasmid DNA was isolated from at least ten clones per individual and sequenced. Each group [high dopa load (1000 mg/day), low dopa load (100-400 mg/d), controls] consisted of 5 individuals. The methylation profiles of the samples were finally investigated with the Big Analyzer (BiQ), developed by Max-Planck-Institute, Saarbrücken, Germany [16]. Genomic region and primers used in the study are listed in Figure 1 and Table 1.

### Result

#### Characterization of methylation differences between maternally and paternally inherited imprinted genes

We report only the methylation profiles of autosomal imprinted genes distributed across the genome. By matching a list of all "known" imprinting genes (30 maternally imprinting, 60 paternally imprinting) to our Illumina 450 K data, we estimated the methylation differences among PD and healthy controls. In our analysis, regardless of any inheritance pattern (paternal or maternal) and of chromosomal location, we did not identify any imprinting locus showing significant differences. Apart from male PD samples (Figure 2, upper panel), we have extend our analysis on monozygotic twins PD samples as well. Our cumulative analysis on monozygotic twin males and females also confirmed the absence of any kind of methylation specific changes in PD (Figure 2, lower panel).

#### DNA methylation patterns at imprinted locus IGF2 and effects of L-dopa

To evaluate the methylation of the imprinted region of IGF-2 (human insulin-like growth factor) exon 8-9 of Parkinson's disease patients treated with (1) high dosage of L-dopa, (2) low dosage of L-dopa, in particular a predominantly agonist treatment and (3) of healthy individuals, we used bisulfite sequencing PCR (BSP) and cloning based sequencing. When compared with the controls (n=9), the patients with low L-dopa dosage (n=8) and with high L-dopa dosage (n=7) for 27 CpG sites located at genomic location in exon 8-9 (+7393 till +7868) of IGF-2 locus did not display any observable difference (Figure 3). As per analysis, the distribution of methylated and unmethylated CpG sites

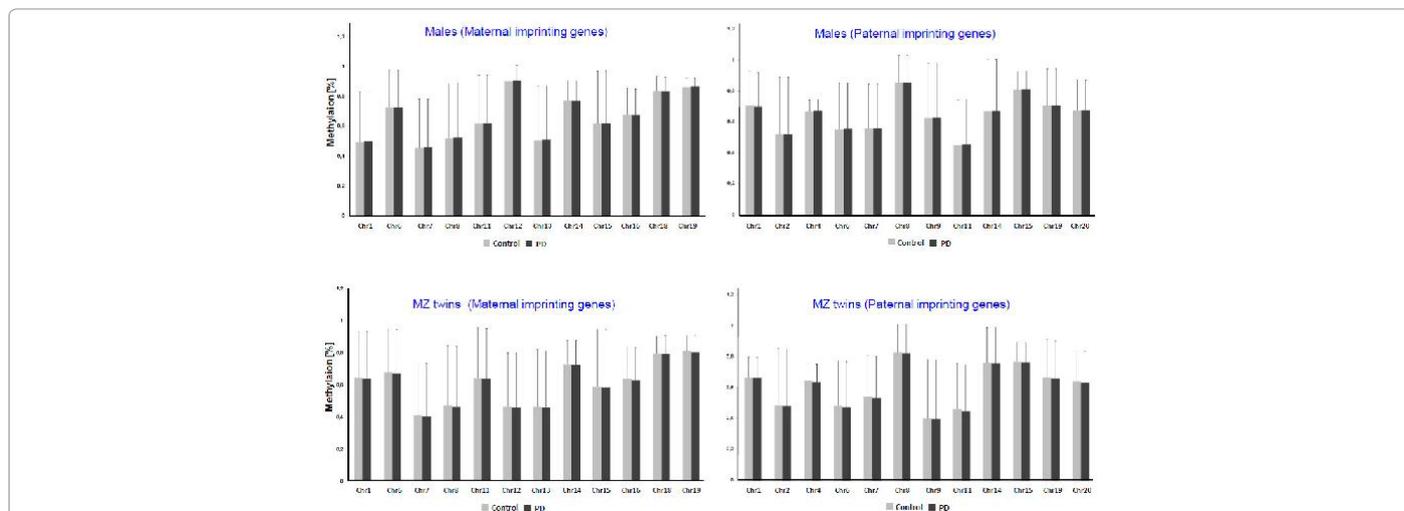
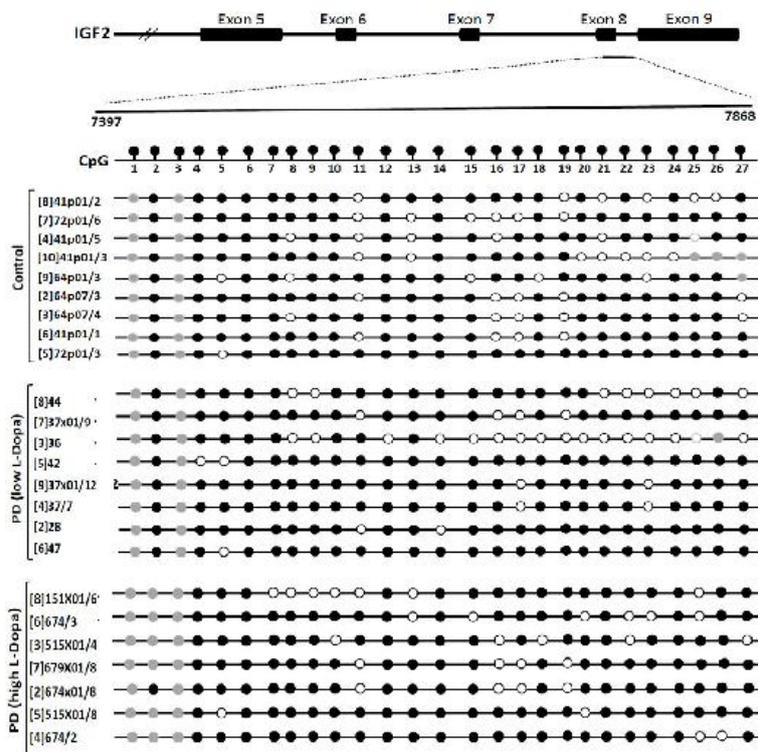


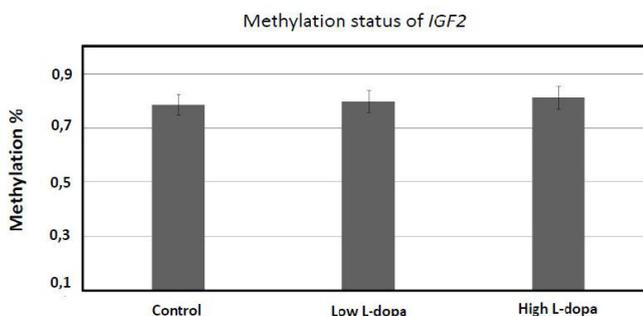
Figure 2: Methylation status of "Known" imprinting genes in PD.

Note: DNA methylation of selected imprinting genes has been demonstrated. Upper panel shows the methylation level in PD males, subgrouped as maternal (left panel) and paternal (right panel), imprinting genes with respect to each chromosomal location. Lower panel display data from monozygotic PD twins (males and females) along with healthy controls.



**Figure 3:** Structural characteristics and representative BSP methylation analysis of the human IGF2 gene.

**Note:** General map of the exon intron structure IGF2 gene is shown. All 27 CpG sites spanning the exon 8-9 region have been represented. DNA methylation status per CpG locus in healthy controls (n=9), low L-dopa (n=8) and high L-dopa patients (n=7) has been displayed. Each row of represents a single patient, and each circle represents a single CpG site (filled circle: methylated, Open circle: unmethylated cytosine).



**Figure 4:** Methylation status with L-dopa treatment.

**Note:** Graphs obtained from methylation data with healthy and PD patients with different L- dopa treatment.

revealed that methylation of IGF2 of Parkinson's disease patients was as stable as that of unaffected individuals (Figure 4). Moreover, evaluation of patients treated with high dosage of L-dopa, low dosage of L-dopa and healthy individuals shows no statistic significance (Figure 4).

## Discussion

DNA methylation plays a crucial role in imprinting, a phenomenon in which the expression of a gene is dependent on its parent of origin. Several human phenotypes have been associated with imprinting genes and to date approximately 100 autosomal human imprinted genes have been reported. IGF2 (human insulin-like growth factor) is located on chromosome 11p15.5, a region which contains numerous imprinted genes. As reviewed in Cui et al. [17] loss of imprinting (LOI) of IGF2 is

an association with a number of tumor types, including prostate cancer, breast cancer, lung cancer, colon cancer, and Beckwith-Wiedemann syndrome [17]. If loss of imprinting or aberrant imprinting is also associated with the onset of Parkinson's disease (PD) has not yet been investigated. DNA methylation analyses of PD brains revealed that the SNCA gene encoding alpha synuclein could be subjected to epigenetic regulation [18,19]. Also, decreased DNA methylation in the gene region was linked to increased expression of CYP2E1 in the brains of Parkinson's disease patients [20]. To investigate, if DNA methylation of "known" imprinting gene is associated with PD, we select them manually from our Illumina methylation 450 data and visualize their epigenetic status. Our results clearly demonstrate that regardless of maternal or paternally inherited status, no significant methylation differences between PD and controls were observed.

Our analysis on IGF2 imprinted region in Parkinson's disease patients treated with (1) high dosage of L-dopa, (2) low dosage of L-dopa, in particular a predominantly agonist treatment and (3) of healthy individuals, showed no difference of DNA methylation between any of the three groups. Our observation of a high percentage of methylated CpG islands of IGF2 in healthy humans is consistent with previous studies in mice and humans. Thus loss or disruption of imprinting seems not to exist in Parkinson's disease and therefore seems to be irrelevant for the pathogenesis of PD.

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