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FACULDADE DE MEDICINA

Utilidade dos Exames de Ressonância Magnética no Diagnóstico da Doença de Parkinson

Revisão Sistemática e Meta Análise

José Luís Brito de Almeida Gominho

Curso de Mestrado em Neurociências

Lisboa, Ano 2013

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Resumo:

Com a atual tendência para o envelhecimento da população e sendo a doença de Parkinson (DP) uma patologia que atinge cerca de 2% da população acima dos 65 anos, podemos prever um aumento da sua prevalência. Por outro lado devido às questões éticas e à morbilidade que implicariam as biopsias cerebrais como método de diagnóstico definitivo, a necessidade de encontrar métodos de diagnóstico precoce e não invasivos são de extrema importância. Mesmo nos melhores centros de diagnóstico há uma percentagem importante de desacordo entre o diagnóstico efetuado em vida, de base clínica e o diagnóstico pós-morte, que é histopatológico. A Ressonância Magnética, nas suas diferentes modalidades, proporciona-nos um meio de investigar "in vivo" as regiões corticais e subcorticais que se sabem estarem afetadas na DP.

Diversos estudos recentes procuram demonstrar a utilidade destes testes como bio marcadores de diagnóstico e progressão da doença de Parkinson. Procurei efetuar uma revisão sistemática e meta análise em relação aos exames de Imagens de Ressonância Magnética (IRM) de modo a verificar a sua utilidade no diagnóstico da doença de Parkinson e se poderão ter potencial para serem considerados bio marcadores de diagnóstico e seguimento dos doentes.

Objetivo

Revisão sistemática dos estudos que compararem, a precisão das diferentes modalidades de ressonância magnética no diagnóstico da doença de Parkinson, com diagnóstico clínico e controlos saudáveis, explorando suas potencialidades como bio marcadores.

Material e métodos

Procedeu-se a uma pesquisa na literatura publicada sobre a temática, recorrendo à base de dados PubMed/MEDLINE, Embase, B-on, Google Scholar e ainda na bibliografia dos estudos considerados relevantes. Foram utilizadas as palavras-chave "Parkinson", "Magnetic resonance imaging", "MRI", "DTI", "Diffusion tensor imaging", "Spectroscopy", "MRI of Iron", "fMRI", "bold", “Neuromelanin”, combinados com operadores booleanos apropriados para cada pesquisa. Foram selecionados estudos redigidos em Inglês, Francês, Espanhol e Português.

Critérios de inclusão: Estudos neurorradiológicos, com mais de cinco pacientes, até a presente data, que envolvam Imagens de ressonância magnética (MRI) de diferentes modalidades tais como: Exames Estruturais (T1, T2, Neuromelanina, Ferro e outras técnicas); Diffusion Tensor Imaging (DTI); Espectroscopia em Ressonância Magnética (MRSI); Ressonância Magnética Funcional (fMRI) no diagnóstico da Doença de Parkinson e que comparem a precisão do teste com diagnóstico clínico e controlos saudáveis, explorando suas potencialidades como bio marcadores.

Foram excluídos, estudos que não usaram critérios formais de diagnóstico clínico, estudos incluindo pacientes submetidos a estimulação cerebral profunda, parkinsonismo idiopático ou vascular, casos relatados, editoriais, comentários, cartas, estudos em animais, estudos de diagnóstico diferencial com outras síndromes parkinsonianos e demências da DP.

Resultados

Dos 834 estudos identificados e atendendo aos critérios de seleção foram separados 109 estudos que após a leitura dos "A*bstracts*" verificou-se que apenas 52 preenchiam os requisitos dos critérios de inclusão. Destes estudos, foram obtidas as versões integrais publicadas, que foram integralmente lidas e sempre que existentes registados por mim, os seguintes dados: a referência, o ano, o título, o número de pacientes e controlos, a idade média, o estádio Hoehn & Yahr, a medicação, a modalidade de IRM, a região estudada, a intensidade do campo magnético do sistema, as conclusões e ainda se possível, a especificidade, a sensibilidade, a área sob a curva ROC e valores-p (Sigma) do teste t-Student (t-test) da comparação entre os valores obtidos dos exames dos pacientes com DP e a dos controlos saudáveis.

Os resultados obtidos foram divididos em quatro grupos, em função da modalidade de estudo de IRM (imagens de ressonância magnética), para avaliação: 1º exames estruturais utilizando os métodos clássicos e IRM do ferro e da Neuromelanina; 2º exames utilizando DTI (Diffusion Tensor Imaging); 3º exames de espectroscopia de RMN; 4º fMRI (ressonância magnética funcional) incluindo a de em estado de repouso (RS-fMRI).

1º Exames estruturais utilizando os métodos clássicos e IRM, do ferro e Neuromelanina.

Neste grupo de estudos podemos verificar que, utilizando T1 imagens Inversão de recuperação (a área) [24]; T2W (o volume) [33]; T1p (sensível á perda neuronal) [46]; MRI sensível á Neuromelanina (medição do volume) [23], encontramos uma

diminuição significativa na SN (substância nigra) dos pacientes com DP, quando comparados com controlos saudáveis pareados por idade, e um aumento significativo dos valores do R2 * (= 1/T2 *) e T2p, (sensível à deposição de ferro) em pacientes com DP [41, 27], quando comparados com controlos saudáveis. É de salientar que estas alterações se mantem ainda que tenhamos valores de sistemas com diferentes intensidades dos campos magnéticos 3T; 4T; 7T.

2º Exames utilizando DTI (Diffusion Tensor Imaging).

Com este tipo de exames podemos detetar em pacientes com DP, alterações na AF (anisotropia fracionada) e DM (difusibilidade média) em todo o cérebro [74], mas que são mais pronunciadas na substância branca frontal e parietal refletindo deste modo um dano microestrutural generalizado. Estas alterações ocorrem nos estádios iniciais da PD[75], em fibras de projeção do tálamo [11]. Os valores da MK (mean kurtosis) e da AF foram significativamente menores no cíngulo anterior [22], na área motora, na pré-motora e motora suplementar do córtex [64],nas áreas de substância branca próximas das áreas motoras suplementares, cápsulas externa e interna, tálamo direito, putamen esquerdo [65] e como se demonstra na meta-análise há uma redução significativa da AF na SN.

3º Exames de espectroscopia de RMN

Na espectroscopia dos metabolitos Substância Nigra na doença de Parkinson foram observadas diferenças significativas entre doentes PD e controlos saudáveis nas razões, NAA / Cr, NAA / Cho, NAA / (Co + Cr) [66, 76]. Com estes exames podemos obter um in perfil neuro-químico "in *vivo",* incluindo neurotransmissores (Glu e GABA) e os níveis de antioxidantes (GSH), que estão em excelente concordância com a literatura neuro química [70]. Na pré-SMA, a

razão NAA / Cr diminuiu seletivamente, em paralelo com disfunção neuronal nos DP (P = 0,045) [73]. No putamen e mesencéfalo foi encontrada uma redução bilateral de fosfatos de alta energia, como adenosina trifosfato e fosfocreatina como recetores finais da energia da fosforilação oxidativa mitocondrial [71].

4º Exames de fMRI ressonância magnética funcional incluindo os de em estado de repouso (RS-fMRI).

Usando diferentes paradigmas e comparando pacientes com DP, com controlos saudáveis, encontramos nos diferentes estudos uma redução da percentagem de mudança de sinal em todos os núcleos dos gânglios da base contra lateral e ipsilateral, tálamo lateral e medial, M1 (córtex motor primário) e área motora suplementar. Foram detetadas correlações negativas significativas entre a UPDRS e a ativação BOLD bilateralmente nos núcleos, caudado e putamen, segmento externo contra lateral do globo pálido, bilateralmente nos núcleos sub-talâmicos, substantia nigra e tálamo contra lateral.

A bradicinesia é o sintoma que mais consistentemente previu a ativação BOLD nos gânglios da base e tálamo. Além disso, a ativação BOLD no globo pálido interno contra lateral, estava relacionada com tremor.

A atividade cortical reduzida no córtex motor primário e na área motora suplementar nos pacientes com DP recém-diagnosticada, não se relacionam com sintomas motores [58].

Durante a execução de movimentos automáticos, os pacientes com doença de Parkinson em comparação com os controlos saudáveis, necessitam de mais atividade cerebral no cerebelo, na área pré-motora, no córtex parietal, no

precuneus e córtex pré-frontal para compensar a disfunção dos gânglios basais [51].

Usando RS-fMRI para estudar a conectividade funcional (CF), verificou-se que os pacientes PD apresentam uma disrupção da rede motora. O aumento CF em estado de repouso entre os núcleos sub-talâmicos (NST) e áreas motoras corticais e os sintomas de rigidez e tremor na PD podem estar relacionados a um acoplamento anormal dessas áreas.

Com estudos selecionados foram efetuadas meta análises ponderando o efeito de tamanho da amostra nos 1º e 2º grupo, tendo-se verificado que neles há diferenças significativas (em t-test utilizando *p-value*) no que respeita á redução de volume e da anisotropia fracionada (AF) da Substância Nigra (SN) entre os doentes de Parkinson e os controlos saudáveis.

Foi detetada uma redução média de volume da SN, estimada pela tamanho do efeito das IRM Estruturais de (-0,877, 95% intervalo de confiança de -1,049 a -0.705, p <0.0001) apresentando os estudos um baixo nível de heterogeneidade (Q [12] =14,598 p =0,264 I^2 =17,795).

Na AF da SN em DTI a redução média dos valores da AF estimada atendendo ao efeito tamanho dos estudos foi de (-0,811, 95% intervalo de confiança de -1,036 a -0,586, p <0,0001) com um baixo nível de heterogeneidade entre os estudos (Q [6] =7,327, p =0,396 I^2 = 4,465).

Conclusões

Estes resultados são encorajadores pois pode-se concluir que os exames de imagem de ressonância magnética possuem uma boa capacidade discriminativa dos doentes de Parkinson em relação aos controlos saudáveis e poderão

desempenhar um papel importante na deteção, na monitorização da progressão e no impacto terapêutico na DP. Entretanto, serão necessários estudos longitudinais e prospetivos com um número mais elevado de doentes utilizando as várias modalidades, isoladamente ou em associação, para melhorar a acuidade diagnóstica e confirmar a sua utilização como bio marcadores.

Palavras-chave:

Doença de Parkinson, Imagens de Ressonância Magnética, DTI (Diffusion Tensor Imaging), fMRI ressonância magnética funcional, Espectroscopia de RMN

Key Words:

MRI; substantia nigra; Parkinson's disease; DTI; diffusion tensor imaging; fMRI; spectroscopy

Índex

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GLOSSARY

1H-MRS: Proton Magnetic Resonance Spectroscopy

3D: Three-Dimensional

AD: Alzheimer's disease

ADC: Apparent Diffusion Coefficient

ASL: Arterial Spin Labeling

AUROC: Area under the ROC

BOLD: Blood Oxygen Level Dependent

CBD: Corticobasal Degeneration

CC: Corpus Callosum

CDA: Coefficient of Diffusibility Apparent

Cho: choline-containing compounds

Cr: Creatine + phosphocreatine

DESPOT: Driven Equilibrium Single Pulse Observation of T1/T2

DKI: Diffusional Kurtosis Imaging

DTI: Diffusion Tensor Imaging;

DWI: Diffusion- Weighted Imaging;

FA: Fractional Anisotropy

FC: Functional Connectivity.

fMRI: functional MRI

GP: Globus Palidus

HC: Healthy Controls

IDP: Idiopathic Parkinson´s disease

LB: Lewy Bodies

LBD: Lewy Body Dementia

LC: Locus Coeruleus

MA: Meta-Analysis

MD: Mean Diffusivity

MRI: Conventional MRI

MRI: Magnetic Resonance Imaging

MRS: Magnetic Resonance Spectroscopy

MSA: Multiple System Atrophy;

MTR: Magnetization Transfer Ratio

NAA: N-acetyl aspartate

PD: Parkinson Disease

PSP: Progressive Supra nuclear Palsy

RF: Radio Frequencies

RN: Red Nucleus

ROC: Receiver Operating Characteristic

ROI: Region of Interest

RS-fMRI: Resting State Functional MRI

RSNs: Resting-State Networks

SIRRIM: Segmented Inversion Recovery Ratio Imaging

SMA: Supplementary Motor Area;

SN: Substantia Nigra

SNc: Substantia Nigra pars compacta

TFE: Turbo Field Echo

T-test: t-Student test

UPDRS: Unified Parkinson Disease Rating Scale

VBA: Voxel-Based Analysis.

NA: Not Available

Usefulness of Magnetic Resonance Imaging Examinations in the Diagnosis of Parkinson's disease

A systematic review and Meta-Analysis

Abstract

Objectives:

We performed a systematic review of the studies comparing the accuracy of the different modalities of magnetic resonance imaging in the diagnosis of Parkinson's disease with clinical diagnosis and healthy controls, exploring its potentials as biomarkers.

Methods:

We searched for studies and research reviews in, the MEDLINE, EMBASE, B-on (the online knowledge Library) databases, and in bibliography cited in relevant studies, comparing the MRI differences between Parkinson's disease patients and healthy controls to access the accuracy of the different methods, the results were extracted and estimates were pooled by random-effects meta-analysis.

Results:

834 studies were identified using MRI in PD but only 48 studies were eligible for inclusion, with a total of 1362 Parkinson's disease patients and 1023 healthy

controls, whose results were divided into four groups: 1st- Structural, Iron and Neuromelanin MRI; 2nd- DTI (diffusion tensor imaging) with FA and MD; 3rd- Spectroscopy (MRS); 4th- fMRI that includes RS-fMRI (resting state fMRI). It was found changes in basal ganglia, thalamus, white and gray matter in the different MRI modalities. In the 1st and 2nd group we performed a meta-analysis for the Volume and Fractional Anisotropy (FA) of the Substantia Nigra (SN) respectively. A good effect size of the reduction was found for both in the PD patients versus controls in structural MRI (-0,877, 95% confidence interval -1,049 to -0.705, p < 0.0001) and in DTI (-0,811, 95% confidence interval -1,036 to -0,586, p < 0,0001). With a low level of heterogeneity.

Conclusions:

Magnetic Resonance Imaging has a good accuracy in separate PD patients from Healthy Controls, and could have a role in detecting pre manifest disease, monitoring progression and drug therapeutic impact. Larger prospective and longitudinal studies using DTI, Spectroscopy, fMRI, RS-fMRI and other modalities of MRI on larger cohorts of patients with Parkinson´s disease are needed to investigate some of the actual encouraging preliminary findings. Standardization of protocols is a need and will be a reality in the future and that will help us to get better and comparable results. Combination of modalities could improve the diagnostic accuracy.

Key Words: MRI; substantia nigra; Parkinson's disease; DTI; diffusion tensor imaging; fMRI; spectroscopy

Background

Parkinson's disease (PD) is a progressive neurodegenerative disease that affects negatively the motor control in approximately 2% of the population above 65 years [30]. In the pathophysiology of motor changes in the DP a selective loss of dopaminergic neurons in the caudal and ventral lateral layers in the substantia nigra pars compacts (SNc) is involved, which project to the striatum[57], with the appearance of intracellular inclusions known as Lewy bodies (LB), aggregation of neurofilaments, lipids, iron, hyper phosphorylated proteins, ubiquitin and Alpha-synuclein [55] that are located in the axonal processes of neurons, indicating the presence of neurodegeneration in Parkinson's patients [5].

Neuronal loss and the Lewy bodies are not restricted to the SNc they are also found in the dorsal nucleus of the Vagus, basal nucleus of Meynert, locus coeruleus and cortical neurons [48]. The loss of dopaminergic neurons in substantia nigra causes the depletion of dopamine in the striatum, in the caudolateral sensorimotor putamen and also the loss of neurons from other dopaminergic and non-dopaminergic areas of the brain which leads to the onset of clinical manifestations of PD.

Another characteristic of SN projection neurons is that they contain a pigment called neuromelanin. Neuromelanin-laden neurons are therefore also present in the locus coeruleus (LC). Neuromelanin exhibits ferrous properties that can affect the MRI signal in the presence of other metals found in the brain stem, such as iron and copper

Although PD is frequent, it may be difficult to diagnose clinically, particularly in the early stages and approximately 5 to 10% of patients diagnosed with PD are misdiagnosed. Early symptoms are subtle and often attributed to aging. The main signs of Parkinson's disease are, bradykinesia, resting tremor, rigidity and postural instability. However a diagnoses accuracy (specificity) of 90% is the best we can achieve with the evaluation based on clinical diagnostic criteria. On the other side, 15 to 25% of the patients diagnosed with PD by clinical criteria, present at the autopsy other diagnoses such as, multiple system atrophy (MSA) Progressive Supranuclear Palsy (PSP) Alzheimer's disease (AD), cerebrovascular disease, essential tremor, senile tremor, tremor by lesion of Red nucleus.

The improvement in diagnostic accuracy and the ability to predict the rate of progression are of the most importance to the development of neuroprotective treatments.

The need to find a non-invasive methods of diagnosis is paramount, due the morbidity and ethical issues that would arise, with brain biopsies as a method of definitive diagnosis. Even in the best centers there is an important proportion of disagreement between the clinical-based diagnosis and the post-mortem diagnosis, which is obtained by histopathology.

Magnetic resonance imaging in its different modalities provides us with a tool to investigate "in Vivo" the cortical and sub cortical regions that we know to be affected in the PD, a methodology currently very studied. Several recent reports have demonstrated the usefulness of these techniques as potentials biomarkers of diagnostic and progression of Parkinson's disease.

In This systematic review we will look at advanced MR techniques that can provide SN Visualization: Volumetry, magnetization transfer, Diffusion tensor imaging (DTI), neuromelanin imaging, Spectroscopy, fMRI and resting-state fMRI (RS-fMRI) that have also been used to investigate respectively SN anatomy and functional connectivity.

Basic principles of Magnetic Resonance.

On MRI the magnetic properties of the hydrogen nuclei of the human body are used. The hydrogen nucleus has only a proton which provides a good NMR signal, in addition, it is the most abundant element in the human body. We use their properties and their interaction with a strong external magnetic field and radio frequencies (RF) to produce a detailed image of the human body. This magnetic field is measured in Tesla units. One (1) Tesla unit is equivalent to 10.000 Gauss. The Earth's magnetic field is about 0.5 Gauss, which implies that when we are using a 1 T magnet, it will generate around the individual a magnetic field 20,000 times stronger than the Earth [16]. Currently equipment's capable of generating 3, 4 and 7T already exists for clinical use, and there are 14 T systems that are used for research but that will soon be used in "in Vivo" diagnostics.

Structural or conventional magnetic resonance imaging

Contrast T1

The T1 relaxation time is the time it takes for 63% of the protons to align with the external magnetization field, after a RF pulse stimulation. A sampling of the NMR signals in a T1 relaxation gives T1 weighted images. T1 time is tissue class dependent, for example in lipids protons will relax faster than protons in water, or in molecules that are much larger, which is one of the reasons why MR images have good tissue contrast. In the brain, T1-weighting make that the nerve

connections of white matter to appear white, and the congregations of neurons of grey matter to appear grey, while cerebrospinal fluid appears dark [2].

Contrast T2

The T2 weighted images are obtained after a refocusing of the spins in the x-y direction following a 180º RF pulse. T2 relaxation is caused by reduction in magnetization in the X -Y plane, also called transverse relaxation. This is caused by in-homogeneity of the magnetic field on a molecular level, leading to a dephasing of the protons with decay of the transverse magnetization. T2 relaxation time describes how fast the decay of the NMR is because of T2 relaxation. T2 time is longer in pure water than tissues or liquids containing protein. The contrast of "white matter," "grey matter"' and "cerebrospinal fluid" is reversed using T2 imaging compared to T1 imaging. See figure 1, below [2].

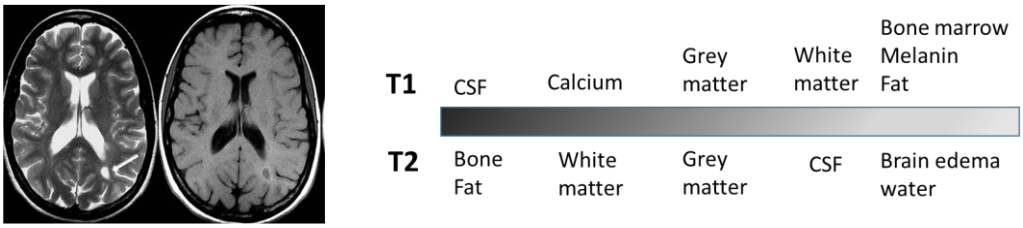

Figure 1 - T1 and T2 MRI Images

Volumetric MRI

The ability to acquire three-dimensional high resolution imaging, (3D) MRI is the result of technical improvements and analytical techniques. "Low flip angle gradient echo Imaging" is a method that enables this analysis. Through this method may be obtained "thin slices", with size of voxel of 1 x 1 x 1 mm or smaller depending on the intensity of magnetic fields generated by the system. An advantage the MRI 3D is the ability to get detailed images of complex anatomical structures as the brain.

Diffusion Tensor Imaging (DTI).

This powerful concept goes beyond conventional magnetic resonance imaging because it gives us the image of movement of molecules at a microscopic scale. Diffusion is a 3D process that can perform identically in all directions (isotropic) as in the cerebral cortex, or in an aligned direction (anisotropic) as in the white matter, where the fibers influence the movement of the molecules. The latest application of this technique is the follow-up of the nerve fibers inside the brain, the tractography, which, in combination with functional magnetic resonance imaging, can open a new window in the study of connectivity of brain structures[4].

During 50 msec water molecules move in the brain an average distance of 10 µm crossing and interacting with many structures such as tissue, cell membranes, fibers or macromolecules. The overall effect of diffusion in an image voxel of a few mm3, reflects statistically the movement and distribution of water molecules present in the voxel. The observation of this movement gives us the visualization of the geometric organization of the tissue. This is the only non-invasive way to observe this diffusion "in vivo". Diffusion is an intrinsic physical process and totally independent of the effect of MR or magnetic field. It's not the case with most parameters accessible on RM such as T1 or T2 [4]. The magnitude (diffusibility) and directionality (anisotropy) of water molecules in the brain can be quantified by its coefficient of diffusibility apparent (CDA) and fractional anisotropy (FA) respectively [7].

Magnetic Resonance Spectroscopy (MRS).

MRS provides a non-invasive method to quantify the concentration of metabolites visible on the MRI of the brain. The technique is based on the general principle,

which the resonant frequency of a metabolite is specific and depends on the chemical environment. Most of the studies of clinical MRS have been concentrated in metabolites visible on a proton spectroscopy (1H-MRS), measured in a given volume (Voxel) of individualized tissue and located in the brain. The metabolites of interest and that can be more easily studied with 1H-MRS in long echo periods are, the N-acetyl-aspartate (NAA), creatine / phosphocreatine (Cr) and choline (Cho). The synthesis and degradation of the membrane in neuronal cells can produce changes in Cho [61]. The lactate is an indicator of anaerobic Glycolysis. In addition, several other compounds and neurotransmitters can also be studied, such as glutamate, glutamine, GABA, myoinositol [13]. The NAA is contained almost exclusively within the neurons [56] and, therefore, it is considered that it can act as an “in vivo” marker of neuronal loss or dysfunction. In most cases, measurements of NAA were based on regional ratio NAA /Cr.

The justification for the use of the resonance of Cr as the denominator is based on the concept that the creatine and phosphocreatine are in chemical equilibrium and the concentration of both compounds should remain unchanged in neurodegenerative disease processes. Alternative methods are now available for the measurement of concentrations of metabolites, such as those that use water as an internal standard for calibrating [36] and those involving calibration to an external standard [34], which should allow a significant improvement on quantitative accuracy of 1H-MRS [37]. It is now possible, to quantify in absolute the millimolar concentrations of metabolites present.

Functional magnetic resonance imaging (fMRI)

Functional magnetic resonance imaging (fMRI) is a technique that allows us to view the activated brain areas in relation to a specific task or stimulus driven paradigms. The most common clinical use is to evaluate and identify critical functional areas of the brain in pre-surgery, in order to facilitate the implementation of a surgery as functional as possible. The fMRI is also being used in the study of normal functional anatomy, to investigate the phenomenon of neuroplasticity in healthy individuals and in patients with various neurological or psychiatric processes, including multiple sclerosis [42] and the PD.

When evaluating changes in brain metabolism (i.e., fluctuations in oxygenated blood in relation to the de-oxygenated), fMRI measures the increases and decreases of activity of regional brain over time. Specifically, fMRI allows measurements of tissue perfusion, changes of the blood volume or changes in oxygen concentration levels. The amount of oxygenated blood delivered to specific areas of the brain increases following the increased metabolic activity. The contrast on the blood oxygen level dependent is usually referred as BOLD. However another fMRI technique was developed the Arterial Spin Labeling (ASL) which is a method that measures brain blood flow quantitatively per unit of tissue mass [13] and thus assess perfusion changes at the brain level.

The main advantages of fMRI as a technique for imaging measurement of brain activity related to a specific motor or sensorial task are, for obtaining the signal it does not require injections of radioactive isotopes, and the resolution in terms of functional image is usually about 1.5 x 1.5 mm, although resolutions less than 1 mm are already possible. The fMRI has been widely used to explore the

functional neuroanatomy of cognitive and motor functions of many diseases, as in the case of PD [47].

In recent years, there is an increasing interest in the application of the technique in resting state, called “Resting State fMRI” (RS-fMRI) or functional connectivity magnetic resonance imaging. RS-fMRI investigates synchronous activations between regions that are spatially distinct, that occur in the absence of a stimulus or task, to identify the links in a rest state (RSNs) "resting-state networks”. The focus of RS-fMRI are the spontaneous fluctuations of low frequency (0.1 Hz <) in BOLD signal. The functional significance of these fluctuations was presented for the first time by Biswal and colleagues in 1995. In this study, subjects were told not to perform any cognitive, language or motor tasks [26].

A systematic review that will be performed is to analyze the studies carried out and published up to the present date, looking for evidence that justify the use of these diagnostic tests for Parkinson's disease.

Objectives

Primary: Systematic review of the studies comparing the accuracy of the different modalities of magnetic resonance imaging in the diagnosis of Parkinson's disease with clinical diagnosis and healthy controls, exploring its potentials as biomarkers.

Material and Methods

Literature search and data extraction.

A search for papers published, until August 2013, was done in MEDLINE, EMBASE, B-on (the online knowledge Library). We also carried out research in

the bibliography cited in relevant studies, and on Google Scholar (http://scholar.google.com/). Some bibliography was asked to the companies related to this type of diagnostic technology. We used for search terms including, "Parkinson", "Magnetic resonance imaging", "MRI", "DTI", "Diffusion tensor imaging", "Spectroscopy", "MRI of Iron", "fMRI", "bold", “Neuromelanin”, combined with Boolean operators suitable for each search. We restricted our search to the languages English, French, Spanish and Portuguese. All titles and abstracts from the retrieved articles were screened by a single reviewer and the full text of those that may be eligible was obtained. Reference lists of identified studies were searched for additional studies. We reviewed all articles and extracted the data. We included the studies if they, had more than 5 patients, were published as full text articles and used MRI, DTI, fMRI, RS-fMRI, Spectroscopy, Iron and neuromelanin MRI of PD patients confirmed with clinical diagnostic criteria and comparing them with a healthy control group.

We excluded studies that did not use formal clinical diagnostic criteria, studies including patients undergoing deep brain stimulation, studies analyzing mixed patient groups jointly, for example, including both idiopathic and vascular Parkinsonism, as well as duplicate publications, reported cases, editorials, comments, letters, animal studies, studies of differential diagnosis with other parkinsonian syndromes and PD dementias.

From each study a single reviewer recorded the following data when available: reference, year, title, number of patients and controls, the mean age, the Hoehn & Yahr stage, the medication, the modality, the studied region, the magnetic field strength of the MRI scanner, conclusions and if possible the specificity, sensitivity, area under the ROC, p-values of the t-test (Table 2 and Table 7).

Due to the heterogeneity of the studies designs and the missing data in relation with Sensitivity and Specificity and area under the ROC curve, we decided to use for the Meta-Analysis of the different region the brain, those presenting p-values of the t-test results comparing patients with PD and healthy controls.

Results

We identified 834 studies using MRI in PD, but when we started the rejection due to duplications and some of the other exclusion criteria we ended up 109. By reading the abstracts we concluded that 61 were not eligible for inclusion due to the exclusion criteria. 52 studies were eligible for inclusion, with a total of 1440 Parkinson's disease patients and 1095 healthy controls, as we can see in the Table 1 studies selection process. The key details of eligible studies are provided in the Table 2 and Table 7.

The studies were divided into 4 groups:

1st -Structural and Iron and Neuromelanin MRI; 2nd - DTI (diffusion tensor imaging) with FA and MD; 3rd-Spectroscopy (MRS); 4th- fMRI that includes RS-fMRI (resting state fMRI).

Table 1 - Studies selection process

	Identified	Rejected	Selected at Abstract	Excluded	Included	Nº-PD Patients	Nº-Healthy Controls
Structural	665	623	42	23	19	566	366
Diffusion T.I.	38	20	18	6	12	244	242
Spectroscopy	90	59	31	23	8	326	220
functional MRI	41	23	18	5	13	304	267
Total	834	725	109	57	52	1440	1095

1st - Structural and Iron and Neuromelanin MRI

Structural Changes:

Reviewing the published literature selected up to date, we found that several studies began investigating the substantia nigra degeneration on disease of Parkinson's through magnetic resonance imaging (MRI), using different approaches. Some used the images of contrast T2W as Pujol et al. (1992), Stern et al. (1989) but M. Hutchinson et al. claimed in the study published in 1999, with 6 PD patients with mean age of 58 years old and 6 age-matched Healthy Controls (HC) that they have shown for the first time, the potential effectiveness of sequences of the Inversion recovery images, in substantia nigra, to separate Parkinson's patients and healthy control. However, more work will be needed to refine the technique, in particular the use of thinner slices, faster sequences, and the use of automated image segmentation techniques [33].

M. Hutchinson et al. in 2006 in another study with 12 PD and 12 age-matched HC, introduced an approach that using the SIRRIM (Segmented Inversion Recovery Ratio Imaging) technique based on two IR imaging sequences that were designed to suppress white and gray matter to assess loss of neural cells in situ by means of a ratio image (white matter suppressed image to gray matter suppressed image) and conclude that these technique Is sensitive enough to identify patients in early-stage of IPD (idiopathic Parkinson's Disease) and that the radiological index correlated with the Unified Parkinson Disease Rating Scale (UPDRS) [40].

Anik Yonca et al. in 2007 using a new technique MTR (magnetization transfer ratio imaging) in a group of 33 early period of PD (in the first year of diagnostic)

with a mean age of 66.9 years old patients and 30 HC, concluded that these can be a useful technique for assessing PD because the decrease in MTR is more prominent in the SN *pars compacta* and probably begins before the onset of clinical disease. This reduction of MTR was also found in the *pars reticulata,* pons and Red nucleus of PD patients [63].

Deoni et al., in 2005 describes a quantitative Imaging of high resolution technique, using the balance of single pulse driven of T1 (DESPOT1), which proved to allow a better visual discrimination between the main nuclei of the thalamus [12].

R. Menke et al., in 2009 combining the Volumetry of SN, T1 (DESPOT1) and their connectivity with the thalamus by DTI in a group of 10 PD patients with mean age of 63,7 and 10 age-matched HC, achieved in the detection of PD patients a sensitivity of 100% and a specificity of 80% [30].

Shalom Michaeli et al., in their study in 2007 using a 4Tesla equipment and 8 PD patients with a mean age of 61 and 8 age-matched HC, demonstrates that a new magnetic resonance method "rotating transverse frame (T2p) and longitudinal (T1p) T2 MRI methods" are sensitive to iron and neuronal loss respectively, that there is a statistically significant difference between the Parkinson's patients and controls [46].

Maija Rossi et al. in 2013, using a 3T equipment conducted a study, with 36 PD patients with mean age of 71 and 21 age-matched HC, and acquired the sequences Malpt, SWI and T2W. The string Malpt was used to perform T2* the mapping that allowed the calculation of R2* (R2 = 1/* T2*). After the execution of statistical calculations she conclude that R2* and SWI can detect the differences

between the DP and controls, but not with T2W. Clinically the results of R2* were stronger than the other two sequences because they reflected clinical observations and that these changes were probably caused by iron deposits that not only were present in SN but also in GP (Globus Pallidus) [41].

Mechelle Lewis et al., 2013, using the same sequence and calculation R2* in a 38 recruited PD patients, mean age 60.6 and 23 age-matched HC, found that the values obtained in the Red Nucleus (RN) correlated with the UPDRS in those patients in whom they had suspended the L-dopa medication, but not with the duration of the illness or medication dosage. That the values of R2* in RN were significantly higher in patients with dyskinesia when compared to those without dyskinesia, and that the values of these, are not different from healthy controls. It was found the association between high iron content in RN with dyskinesia associated with PD [27].

K. Kudo et al., 2013, using a 3D TFE (Turbo Field Echo) technique with off-resonance magnetization transfer pulse for neuromelanin-sensitive MRI on a 3T scanner in 18 PD patients with mean age 68.8 and 27 age-matched HC, stated that these semi-automated method of volume measurement of the substantia nigra pars compacta, can distinguish the PD group from the control group with high sensitivity (83%) and specificity (85%), especially for early stage of PD [23].

Chigumi Ohtsuka et al., 2013 using a 3T scanner in a 61 selected PD patients divided in 2 groups 37 consecutive patients with suspected early stage PD that had not received any medical or surgical treatment for Parkinsonism and were at Hoehn & Yahr (H&Y) stage 1–2. And 31 consecutive patients with advanced PD having an H&Y stage of 3–5, who were hospitalized for deep brain stimulation or

medication adjustment and 22 age-matched healthy subjects. They concluded that Neuromelanin-sensitive MR imaging is able to detect significant signal attenuation in the lateral part of the SNc (AUROC 0.86) and in the LC (locus coeruleus) (AUROC 0.88), even in patients with early PD. The results suggested that neuromelanin-sensitive MR imaging can discriminate between PD patients, even at the early stage, and healthy individuals with high sensitivity and specificity[67].

Zang-Hee Cho et al., 2011, using a 7.0T MRI, in an attempt to directly visualize the SN and quantify the differences in shape and boundaries of SN between PD subjects in comparison with the normal control subjects, uses a group of 10 PD patients with a mean age of 58.3 for stage H&Y 1 and 59 for the stage H&Y 3, and conclude that it appears to be the clear visualization and eventual quantitation of PDs and normal controls based on the difference in the gross anatomical shape and the quantitative undulation values between the controls and PDs. he shown significant differences between the two groups (P = 0.0002) [69].

P.A. Gowland et al., 2013, Using a high-resolution 7 Tesla scanner, 10 PD patients with mean age of 64 and 8 age and sex-matched HC, assessed to a direct in vivo visualization of nigrosome, substructures of the substantia nigra pars compacta (SNc), they concluded that the absence of nigrosome 1 in the SNc on MRI scans might prove useful in developing a neuroimaging diagnostic test for PD [68].

Table 2 - Structural MRI Selected studies

Author (year)	Title	PD	HC	Hoehn & Yahr Stage	Diag. Criteria	Mean Age	Medication	Modality	Studied Region	Tesla	Conclusions
Structural Changes											
M. Hutchinson, et al. (1999)	Parkinson's disease: a novel MRI method for determining structural changes in the substantia nigra	6	6	1; 2 ; 3	clinical diag	58	yes	T2 weighted images	SN	1.5T	Substantia nigra degenerates from lateral to medial and in a rostral to caudal direction
C. Haegelen, et al. (2013)	Automated segmentation of basal ganglia and deep brain structures in Parkinsons's disease	57	10	NA	clinical diag	58.9 (8)	NA	T1-weighted and T2-weighted MRI	BG	3.0T	The construction of an MR image template specific to PD, and also evaluated a method of achieving the accurate segmentation of the basal ganglia and deep brain structures
Jan Linder,et al. (2009)	Degenerative changes were common in brain magnetic resonance imaging in patients with newly diagnosed Parkinson's disease	66	30	UPDRS-III	UKBB	68 (9.6)	NA	T1 W and T2 W covering the whole brain.	All brain	1.5T	Therefore, they are of limited value in distinguishing among subgroups at clinical presentation and do not change daily practice
L. Minati,et al. (2007)	Imaging Degeneration of the Substantia Nigra in Parkinson Disease with Inversion-Recovery MR Imaging	8	8	All - 2	clinical diag	66.5 (5.0)	yes	T 1 contrate (Inversion-recovery imaging)	SN	1.5T	A way to visualize nigral degeneration. SN
M. Hutchinson, et al. (2006)	Inversion Recovery MRI in Idiopathic Parkinson Disease Is a Very Sensitive Tool to Assess Neurodegeneration in the Substantia Nigra	12	12	UPDRS	clinical diag		NA	Segmented inversion recovery (IR) ratio imaging (SIRRIM). ie, WMS/GMS.	SN	1.5T	Is sensitive enough to identify patients with early-stage IPD. All patients with IPD were identified correctly, and full dichotomization between healthy volunteers and patients was obtained with our database
Yonca Anik, et al. (2007)	Magnetization Transfer Ratio in Early Period of Parkinson Disease	33	30	1st year early	clinical diag	66.9 (8.49)	yes	Magnetization transfer ratio (MTR) imaging	SNpc SNpr RN Pons	1.5T	MTR analysis is a useful technique for initial PD assessment. MTR decreases in all PD
N. D. Forkert,et al. (2011)	Image-based Classification of Parkinsonian Syndromes Using T2'-Atlases	33	24	?	clin diagosed	61.5 (10.4)	NA	1/T2' = 1/qT2*-1/qT2 a Selectively measured using T2' MR imaging		1.5T	Automatic classification using the generated atlases.
R. A. Menke , et al. (2009)	MRI characteristics of the substantia nigra in Parkinson's disease: A combined quantitative T1 and DTI study	10	10	1; 2; 3	clinical diag	63,7 (6,7)		Driven equilibrium single pulse observation of T1 (DESPOT1) and (DTI)	SN-Put SN-Tal VCDR	3.0T	Combining SN volumetry and its connectivity with the thalamus improved the classification sensitivity to 100% and specificity to 80% for PD
I. Nestrasil , et al. (2010)	T1q and T2q MRI in the evaluation of Parkinson's disease	9	9	UPDRS and HY	clinical diag	59.0 (7.1)		T1p and T2p	SN	4.0T	Was demonstrated the ability of MRI methods to separate PD from controls. T1q may be a useful marker of asymmetry in mild-moderate PD
O. Monchi , et al. (2011)	Patterns of cortical thickness and surface area in early Parkinson's disease	49	33	UPDRS and HY 1 to 2,5	UK PDSBB	63.3 (7.3)	yes	MRI volumes / Voxel based morphometry (VBM)	Cortex	3.0T	Mean cortical surface area was significantly larger in the PD than in the HC group (2.30±0.18 mm2 vs. 2.19±0.16 mm2; p<0.005)
H. Oikawa,et al. (2002)	The Substantia Nigra in Parkinson Disease: Proton Density–Weighted Spin-Echo and Fast Short Inversion Time Inversion-Recovery MR Findings	22	22	1; 2; 3	clinically established diagnosis	59.8 (9.2)	NA	T2-weighted -- STIR sequence	SN	1.5T	Was demonstrated the ability of MRI methods to separate PD from controls. T1q may be a useful marker of asymmetry in mild-moderate PD
S. Michaeli,et al. (2007)	Assessment of Brain Iron and Neuronal Integrity in Patients with Parkinson's Disease Using Novel MRI Contrasts	8	8	2	(UPDRS)	61 (16)	yes (7)	Rotating frame transverse (T2ρ) and longitudinal (T1ρ) relaxation MRI methods	SN	4.0T	novel adiabatic T2 and T1 MRI relaxation methods used here for the measurement of the load and distribution of iron and neuronal loss may provide unique information on the pathogenesis of PD.
Z.-H. Cho, et al. (2011)	Direct Visualization of Parkinson's Disease by In Vivo Human Brain Imaging Using 7.0T Magnetic Resonance Imaging	10	9	1 and 3	(UPDRS)	58.3 (8.5) & 59 (16)	?	7.0T T2*-weighted MR images of the SN	SN	7.0T	1- This study has demonstrated that by using 7.0T MRI, one can visualize the pathologic features of PD within the SN. 2- Clear image of SNc and surroundings such as CC
M. Rossi,et al. (2013)	Clinical MRI for iron detection in Parkinson's disease	36	21	na	(UPDRS)	71	yes (4)	MapIt, SWI, and T2-weighted sequences T2* (R2*=1/T2*)	BG SNpc	3.0T	In conclusion, both R2* and SWI detected differences between patients and controls
M.M. Lewis et al. (2013)	Higher iron in the red nucleus marks Parkinson's dyskinesia	38	23	1; 2; 3	(UPDRS) (21)	60.6	yes	Iron content was estimated from bilateral RN and SN transverse relaxation rates (R2*)	RN SN	3.0T	RN R2* values correlated with off-drug Unified Parkinson's Disease Rating Scale-motor scores RN R2* values were significantly higher in PDþDYS compared with control and PD DYS
	Total	397	255								
Neuromelanin											
Kenichi Kashihara, et al. (2011)	Neuromelanin magnetic resonance imaging of nigral volume loss in patients with Parkinson's disease	80	54	HY	UKBB	70.9 (8.2)	yes	Axial T1-weighted MRI (volume)	SNc	3.0T	The mean volumes for the left and right SNc were significantly reduced in patients with PD compared to the controls. Volume loss became marked in parallel with disease severity and duration. Neuromelanin MRI may be considered as a biomarker of nigral degeneration in patients with PD.
Kohsuke Kudo, et al. (2013)	3D neuromelanin-sensitive magnetic resonance imaging with semi-automated volume measurement of the substantia nigra pars compacta for diagnosis	18	27	1; 2; 3; 4	UKBB	68.8 (6.4)	NA	3D turbo field echo (TFE) sequence for neuromelaninsensitive MRI	SNc	3.0T	method can distinguish the PD group from the control group with high sensitivity and specificity, especially for early stage of PD
Penny A. Gowland, et al. (2013)	Visualization of nigrosome 1 and its loss in PD Pathoanatomical correlation and in vivo 7 T MRI	10	8		UKBB	64 (5)	NA	with high-resolution T2*-weighted MRI scans	SNpc	7.0T	high-resolution 7 T MRI can directly visualize nigrosome 1. The absence of nigrosome 1 in the SNpc onMRI scans might prove useful in developing a neuroimaging diagnostic test for PD
Chigumi Ohtsuka, et al. (2013)	Changes in substantia nigra and locus coeruleus in patients with early-stage Parkinson's disease using neuromelanin-sensitive MR imaging	61	22	1–2 and 3-5	UKBB	50–78 (66.5)	nou	we obtained oblique-axial fast spin-echo T1- weighted images	SN LC	3.0T	Neuromelanin-sensitive MR imaging is able to detect significant signal attenuation in the lateral part of the SNc and in the LC, even in patients with early PD
	Total	169	111								

Quality Assessment of the Structural MRI studies:

Table 3 - Quality assessment of the structural MRI studies

Looking separately at the groups we concluded that we only had data to do a Meta-Analysis (MA), using the sigma (p-value) of the t- Student test to compare (using Hedge´s g, effect size calculation) the differences between the PD patients and healthy controls, in the 1st and 2nd groups.

In the first group we analyzed the differences of the SN regarding to the volume or size measured using different methodologies and in the second we measured FA in the SN.

In the first group of 19 selected studies in the table 2, 6 were not included in the MA, table 4: the 1st was a basal ganglia method segmentation where the differences in volume of SN between PD and HC were not referred (C Haegelen, et al.2013); a 2nd where no p-value was found (M Hutchinson et al. 2006); a 3rd with a good sensitivity (0.94)and a good specificity (0.91) in the diagnostic accuracy but which did not present the t-test (Forkert 2011); a 4th where they presented the excellent capacity to differentiate the nigrosome in a 7Tesla system but did not present the results of the t-test (Gowland2013); 5th in this study the t-test has a very good result but the differences found were in the cortex surface (Monchi 2011) and 6th where they found changes but not referent to the SN (Linder2009).

Author (year)	Title	PD	HC	Modality	Studied Region	Tesla	Info.	t-test p-value
Structural Changes								
C. Haegelen, et al. (2013)	Automated segmentation of basal ganglia and deep brain structures in Parkinsons's disease	57	10	T1-weighted and T2-weighted MRI	BG	3.0T	NA	0.05
Jan Linder,et al. (2009)	Degenerative changes were common in brain magnetic resonance imaging in patients with newly diagnosed Parkinson's disease	66	30	T1 W and T2 W covering the whole brain.	All brain	1.5T	NA	NA
M. Hutchinson, et al. (2006)	Inversion Recovery MRI in Idiopathic Parkinson Disease Is a Very Sensitive Tool to Assess Neurodegeneration in the Substantia Nigra	12	12	Segmented inversion recovery (IR) ratio imaging (SIRRIM). ie, WMS/GMS.	SN	1.5T	NA	
N. D. Forkert,et al. (2011)	Image-based Classification of Parkinsonian Syndromes Using T2'-Atlases	33	24	1/T2' = 1/qT2*-1/qT2 a Selectively measured using T2' MR imaging		1.5T	NA	NA
O. Monchi , et al. (2011)	Patterns of cortical thickness and surface area in early Parkinson's disease	49	33	MRI volumes / Voxel based morphometry (VBM)	Cortex	3.0T	NA	0.005
	Total	217	109					
Neuromelanin								
Penny A. Gowland, et al. (2013)	Visualization of nigrosome 1 and its loss in PD Pathoanatomical correlation and in vivo 7 T MRI	10	8	with high-resolution T2*-weighted MRI scans	SNpc	7.0T	NA	NA
	Total	10	8					

Table 4 - Studies of Structural MRI not included in the Meta-Analysis

The 13 studies selected for the Meta-Analysis are represented in the table 5 with the Author, the year, the title, number of patients and controls the MRI modality, the studied region, the magnetic field of the scanner, the info (information of the studied region, p-value) and the p-value (sigma) of the t-Student test (t-test) , table 5.

Author (year)	Title	PD	HC	Modality	Studied Region	Tesla	Info.	t-test p-value
Structural Changes								
M. Hutchinson, et al. (1999)	Parkinson's disease: a novel MRI method for determining structural changes in the substantia nigra	6	6	T2 w eighted images	SN	1.5T	NA	0.001
L. Minati,et al. (2007)	Imaging Degeneration of the Substantia Nigra in Parkinson Disease w ith Inversion-Recovery MR Imaging	8	8	T 1 contrate (Inversion-recovery imaging)	SN	1.5T	NA	0.04
Yonca Anik, et al. (2007)	Magnetization Transfer Ratio in Early Period of Parkinson Disease	33	30	Magnetization transfer ratio (MTR) imaging	SNpc SNpr RN Pons	1.5T	SNpc SNpr RN Pons	0.001 0.006 0.037 0.046
R. A. Menke , et al. (2009)	MRI characteristics of the substantia nigra in Parkinson's disease: A combined quantitative T1 and DTI study	10	10	Driven equilibrium single pulse observation of T1 (DESPOT1) and (DTI)	SN-Put SN-Tal VCDR	3.0T	NA	0.05
I. Nestrasil , et al. (2010)	T1q and T2q MRI in the evaluation of Parkinson's disease	9	9	T1p and T2p	SN	4.0T	Tiq T2q both	0.001
H. Oikaw a,et al. (2002)	The Substantia Nigra in Parkinson Disease: Proton Density–Weighted Spin-Echo and Fast Short Inversion Time Inversion-Recovery MR Findings	22	22	T2-w eighted -- STIR sequence	SN	1.5T	NA	>0,05
S. Michaeli,et al. (2007)	Assessment of Brain Iron and Neuronal Integrity in Patients w ith Parkinson's Disease Using Novel MRI Contrasts	8	8	Rotating frame transverse (T2p) and longitudinal (T1p) relaxation MRI methods	SN	4.0T	T2p T1p	0.01 0.036
Z.-H. Cho, et al. (2011)	Direct Visualization of Parkinson's Disease by In Vivo Human Brain Imaging Using 7.0T Magnetic Resonance Imaging	10	9	7.0T T2*-w eighted MR images of the SN	SN	7.0T	NA	0.0002
M. Rossi,et al. (2013)	Clinical MRI for iron detection in Parkinson's disease	36	21	MapIt, SWI, and T2-w eighted sequences T2* (R2*=1/T2*)	BG SNpc	3.0T	NA	0.001
M.M. Lew is et al. (2013)	Higher iron in the red nucleus marks Parkinson's dyskinesia	38	23	Iron content w as estimated from bilateral RN and SN transverse relaxation rates (R2*)	RN SN	3.0T	NA	0.019 0.002
	Total	180	146					
Neuromelanin								
Kenichi Kashihara, et al. (2011)	Neuromelanin magnetic resonance imaging of nigral volume loss in patients w ith Parkinson's disease	80	54	Axial T1-w eighted MRI (volume)	SNc	3.0T	NA	0.001 0.05
Kohsuke Kudo, et al. (2013)	3D neuromelanin-sensitive magnetic resonance imaging w ith semi-automated volume measurement of the substantia nigra pars compacta for diagnosis	18	27	3D turbo field echo (TFE) sequence for neuromelaninsensitive MRI	SNc	3.0T	NA	0.01
Chigumi Ohtsuka, et al. (2013)	Changes in substantia nigra and locus coeruleus in patients w ith early-stage Parkinson's disease using neuromelanin-sensitive MR imaging	61	22	w e obtained oblique-axial fast spin-echo T1- w eighted images	SN LC	3.0T	NA	0.0001
	Total	159	103					

Table 5 - Studies Selected for the Meta-Analysis of Structural MRI

Meta-Analysis of structural MRI.

Out of 13 studies that we do a MA (*), there was one study in which the SN volume loss was not found in PD (H.Oikawa et al., 2002) but in the remaining, a significant reduction of the volume was found. The effect size for the reduction was pooled from all (13) with a total of 339 PD and 249 HC showing a large mean effect size (-0,877, 95% confidence interval -1,049 to -0.705, $p < 0.0001$). Studies have a low heterogeneous although low-level heterogeneity was detected (Q [12] =14,598 p =0,264 I^2 =17,795) (Table 6) (*).

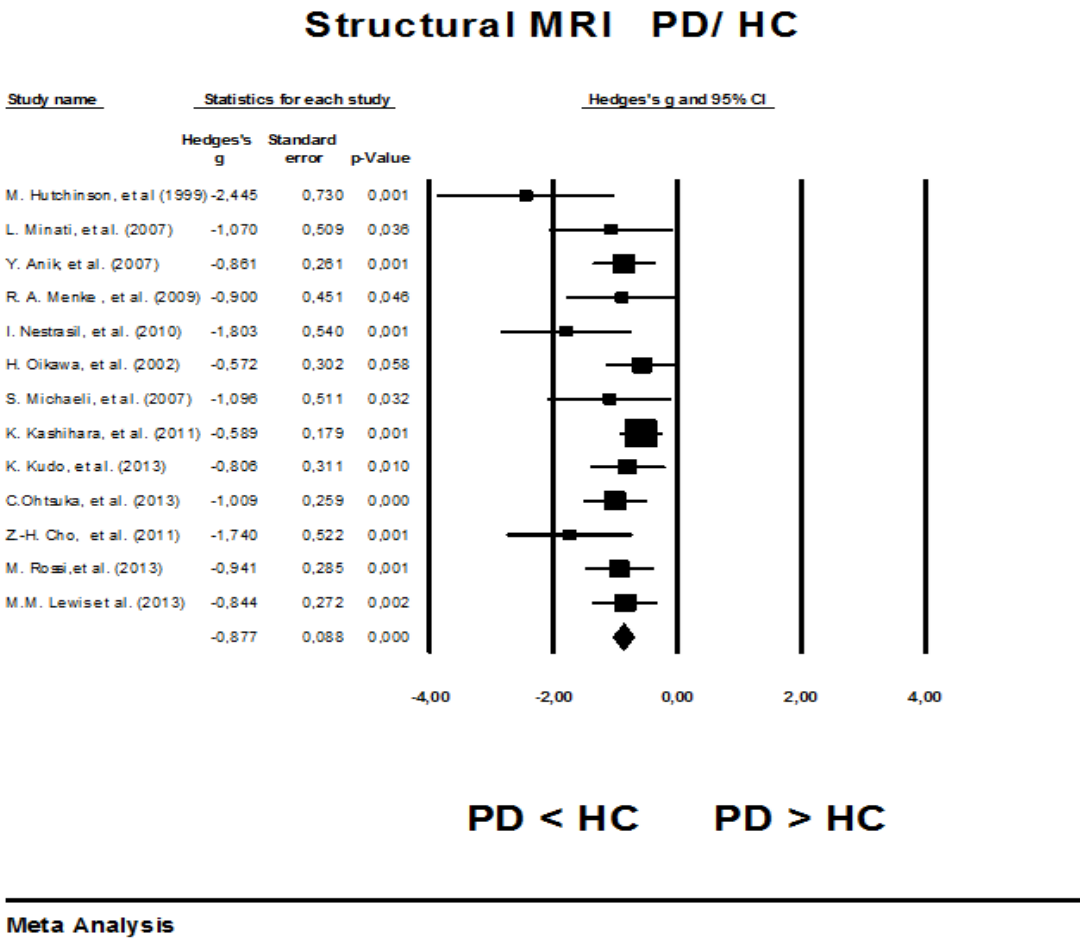

Table 6 - Meta-Analysis of p-values (Hedge's g) of the volume's differences of SN between PD and HC in Structural MRI

(*) For the statistic calculations was used the software "Comprehensive Meta-Analysis" of Biostat,Englewood, USA version 2.2.064 of July 27, 2011 licensed to Jose Gominho

2nd - DTI (diffusion tensor imaging) with FA and MD

Diffusion changes in Substantia Nigra

F. J. Maijer et al., 2013, in the paper “Update on diffusion MRI in Parkinson’s disease and atypical parkinsonism”, says that changes of FA can be detected in SN and on its projection nigro-striatum in the early stages of the disease and the values correlates in a reverse way with the severity of Parkinson's disease [29].

D.E. Vaillancourt, et al., 2009 in a study, with 14 PD patients with a mean age of 57 years old, in Stages H&Y 1 and 2 and 14 HC, using high-resolution DTI protocol at 3 Tesla to study specific segments of degeneration in the SN (FA) found that the reduction is greater in the caudal region than in the rostral of SN and can be observed with a sensitivity and specificity of 100% on discrimination of patients with early-stage, non-treated PD and healthy controls with p-value of 0.001 in t-test [60].

A multimodal approach to diffusion MRI which can be combined with other sequences such as R2* ((R2 = 1/* T2*), proton transverse relaxation rate which shows the increase of iron in the tissues, to improve the diagnostic value in the identification of DP. In studies of Péran et al. [38] reached 95% accuracy (area under the ROC curve) using combinations of R2* on SN, FA on SN and MD in Striatum, on discrimination of DP and healthy controls.

Menke, R.,A. et al, 2009 and 2010, used a new approach combining the Volumetry, driven equilibrium single pulse observation of T1/T2 (DESPOT), of SN

and its connectivity (DT) with the Thalamus in a group of 10 PD patients with a mean age of 63,7 and 10 age-matched (HC), provide 100% sensitivity and 80% of specificity [30]. Based on the connectivity profiles were identified two regions an intern corresponding to SNc (pars compacta) and an external corresponding to SNr (reticulata). However these studies found no significant differences in diffusibility determinations between patients and healthy controls [32].

Guangwei Du et al., 2011, in another multimodal imaging study combining the transverse relaxation rate (R2*) and fractional anisotropy (FA) on SN of a 16 Parkinson´s disease patients, with a mean age of 59.2 and 16 age-matched (HC), had a high accuracy in the differentiation of patients compared with controls, (t-test with a p-value of 0.05 for FA and 0,001 for R2*) [14].

L-L Chan, et al., 2007, using DTI in 73 PD patients with a mean age of 63.6 and 78 age-matched HC, found that FA value in the substantia nigra on DTI was lower in PD compared with healthy controls, and correlated inversely with the clinical severity of PD. Because of the overlap of FA values between PD and controls, no single FA value had both a high positive and negative predictive power [7].

Wang Zhan et al., 2011, using DTI in 12 PD patients with a mean age of 67.4 and 20 age-matched HC, found a reduced fractional anisotropy ($P < 0.05$, corrected) in PD subjects in regions related to the pre-central gyrus, substantia nigra, putamen, posterior striatum, frontal lobe, and the supplementary motor areas. In SN, reduced FA ($p< 0.0001$) in relation to HC, correlated with increased total UPDRS scores [65].

Diffusion changes in circuit of the striatum, cortex and white matter.

C. Tessa et al., 2008, in a study with 27 PD patients mean age of 60.9 y and 16 age-matched HC in stages 1 and 2 of H&Y, detected an increase of FA values in novo PD, more pronounced in patients with the akinetic-rigid type, probably reflecting diffuse subtle GM loss. Those changes in values FA in the brain of PD patients are observed in the initial phase of the disease, even before there was significant atrophy [52].

K Yoshikawa, et al., 2004 demonstrated a Reduction of FA values are observed in the motor cortex, motor, pre-motor and supplementary motor in PD, when compared to the control population. He studies 12 PD patients with mean age of 71.3 y and 8 HC [64].

Zhan et al. showed a good correlation between the increase in FA in post central gyros, somatosensory cortex and the increasing deterioration of the disease [65]. The increase of the values of the FA in the early stages of PD were also described by Tessa, C. et al. being interpreted as likely diffuse loss of grey matter [52].

Gattellaro et al., 2009, in a case control study with 10 PD patients, mean age 63.8 and 10 HC, found increased MD and decrease the values of FA in the genu of the *Corpus Callosum* in the superior longitudinal fasciculus and cingulum, already in the early stages of PD. Changes of diffusion in the genu of Corpus Callosum may indicate the degeneration of the axonal links inter hemispherical between front zones.

D.E. Vaillancourt, et al., 2013, More recently shown in a study with 20 PD patients and 20 HC (mean age 57.9), that the nucleus involved in the motor and cognitive

processes are affectively disrupted, whereas those who are involved in sensory processes are relatively spared. In addition, this study also showed that the current methodology has utility to explore the microstructural integrity in PD [59].

In a recent case control study (to be published) with 15 PD patients and 15 HC, Koji Kamagata, et al., 2013, using a new interpretation of the diffusion images, the "Diffusional kurtosis imaging", the author refers that, it allows a greater sensitivity to detect changes of the cingulate, earlier than the traditional method of DTI, and has an area under the ROC curve of 0.91, with a 0.94 specificity and sensitivity of 0.87 [22].

The 12 studies selected for the DTI Meta-Analysis are represented in the table 7 with the Author, the year, the title, number of patients and controls, the Hoehn & Yahr stage, the diagnostic criteria, the medication, the MRI modality, the studied region, the magnetic field of the scanner and the conclusion. In the table 8 are the quality assessment of the studies.

Author (Year)	Title	PD	HC	Hoehn & Yahr Stage	Diag. Criteria	Mean Age	Medication	Modality	Studied Region	M.F. Tesla	Conclusions
DTI											
Kuncheng Li, et al. (2011)	Voxel-based analysis of diffusion tensor indices in the brain in patients with Parkinson's disease	25	25	1; 2; 3	UKBB	58.4 (9.8)	yes	Voxel-based analysis (VBA) is a technique that can identify the changes of diffusion indices in any part of the whole brain (FA) and (MD)	WB	3.0 T	Abnormal diffusivity in the white matter of bilateral cerebellar hemispheres The positive correlation of the FA values with the TOI and negative correlation of the MD values with the TOI in cerebellum indicated that the cerebellar hemispheres may play an
L-L Chan, et al. (2007)	Case control study of diffusion tensor imaging in Parkinson's disease	73	78	1; 2; 3; 4	UKBB	63.6 (9.8)	yes	DTI = Difusion Tensor imaging (AF), (ADC)	SN	1.5T	1-FA value in the substantia nigra on DTI was lower in PD compared with healthy controls without a strong discriminative power 2- Inverse correlation of clinical severity (H&Y score) with the FA values
G. Gattellaro,et al. (2009)	White Matter Involvement in Idiopathic Parkinson Disease: A Diffusion Tensor Imaging Study	10	10	1 ; 2	clinical criteria of Gelb.	63.8 (15.7)	yes	(MD)mean diffusibility and (FA) fractional anisotropy maps	ROI SLF CI Genu SN	1.5T	Widespread microstructural damage to frontal and parietal white matter occurs already in the early stages of PD.
C. Tessa, et al. (2008)	A Whole-Brain Analysis in De Novo Parkinson Disease	27	16	1 ; 2	UKBB (UPDRS)	60.9 (9.7)	n	T1-weighted images and MD and (FA) maps calculated from diffusion tensor imaging (DTI)	GM WM	1.5T	increase of FA values in novo PD, more pronounced in patients with the akinetic-rigid type, probably reflecting diffuse subtle GM loss
D.E. Vaillancourt,et al. (2013)	Thalamic Projection Fiber Integrity in de novo Parkinson Disease	20	20	(UPDRS) part III	UKBB	57.9 (8.9)	NA	FA analysis and fiber tracking in the thalamus, we used a high-resolution DTI protocol	AN VA DM VL VPL/Vpm PU	3.0T	The present study provides preliminary in vivo evidence of thalamic projection fiber degeneration in de novo PD and sheds light on the extent of disrupted thalamic circuitry as a result of the disease itself.
Koji Kamagata ,et al. (2013)	Diffusional kurtosis imaging of cingulate fibers in Parkinson disease: Comparison with	15	15	1; 2; 3 (2.7)	UKBB	65.0 (9.3)	yes	(DKI)diffusional kurtosis imaging; mean kurtosis (MK) and (FA)	anterior cingulum	3.0T	(FA) DKI can detect alterations of the anterior cingulum in PD patients more sensitively than can conventional diffusion tensor imaging
Ricarda A. Menke, et al. (2010)	Connectivity-based segmentation of the substantia nigra in human and its	10	10	1; 2; 3; (2.7)	clin diagosed	63.7 (6.7)	yes	single pulse observation of T1 (DESPOT1)-HIFI quantitative imaging method	SNr SNc	3.0T	study demonstrated that tractography-based parcellation might be a useful tool for the segregation of SNc and SNr
D.E. Vaillancourt, et.al (2009)	High-resolution diffusion tensor imaging in the substantia nigra of de novo Parkinson disease	14	14	1; 2 UPDRS III	UKBB	57.2	No	high-resolution DTI protocol at 3 Tesla to study specific segments of degeneration in the SN (FA)	rSN mSN cSN	3.0T	High resolution diffusion tensor imaging in the substantia nigra distinguishes early stage, de novo patients with Parkinson disease (PD) from healthy individuals
K Yoshikawa, et al. (2004)	Early pathological changes in the parkinsonian brain demonstrated by diffusion tensor MRI	12	8	1; 2	UKBB	71.3 (7.7)		The mean FA values of all the ROIs	SN	1.5T	1- PD had decreased FA values in two striatal circuits in which dopaminergic neurones 2 -Significant change in subcortical white matter among the cases of advanced PD
Wang Zhan et al (2011)	Regional alterations of brain microstructure in Parkinson's disease using diffusion tensor imaging.	12	20	NA	UPDRS	67.4 (8)	Off	Measures of Fractional Anisotropy and Mean Diffusivity	SN	4.0T	FA reductions in PD compared with controls superior PRG, postcentral gyrus, white matter areas close to supplementary motor areas, substantial nigra, inferior PRG, external and internal capsules, right thalamus, and left putamen
Guangwei Du et al. (2011)	Combined R2* and diffusion tensor imaging changes in the substantia nigra in Parkinson disease	16	16	(UPDRS-III)	clin diagosed	59.2 (6.9)	n	High resolution MRI (T2-weighted, T2*, and DTI)	SN	3.0T	In this study, the combination of R2* and FA measures improved the overall discrimination between PD and Controls
Menke, R. A., et al. (2009)	MRI characteristics of the substantia nigra in Parkinson's disease: A combined quantitative T1 and DTI study	10	10	1; 2; 3; (2.7)	clin diagosed	63.7 (6.7)	yes	Combination of DESPOT and DT imaging	SN	3.0T	
		244	**242**								

Table 7 - Selected Studies of DTI in Parkinson's disease

Quality assessment of the DTI studies:

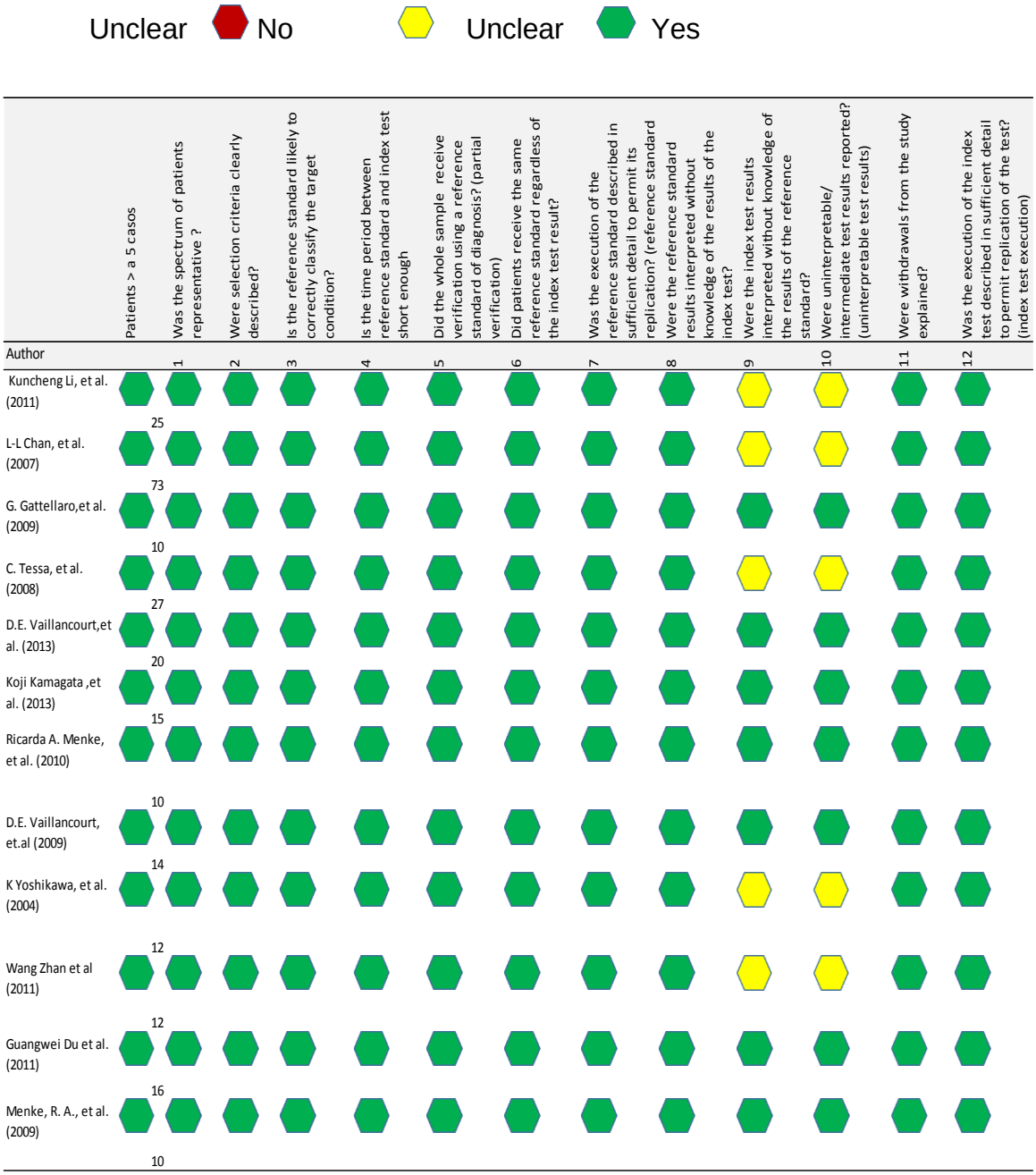

Table 8 - Quality assessment of DTI studies

In the second group (DTI) we analyzed the differences in FA of the SN.

In this group, from 12 selected studies, 4 were not included because the ROI of determination of FA was different, the right rectus gyrus and anterior cingulum and not SN, table 9.

Author (Year)	Title	PD	HC	Studied Region	M.F. Tesla	+info	t-test p-value
DTI							
Kuncheng Li, et al. (2011)	Voxel-based analysis of diffusion tensor indices in the brain in patients with Parkinson's disease	25	25	WB	3.0 T	FA MD	0,001
C. Tessa, et al. (2008)	A Whole-Brain Analysis in De Novo Parkinson Disease	27	16	GM WM	1.5T	FA	0,009
D.E. Vaillancourt,et al. (2013)	Thalamic Projection Fiber Integrity in de novo Parkinson Disease	20	20	AN VA DM VL VPL/Vpm PU	3.0T	FA	0,016 0,009 0,006
Koji Kamagata ,et al. (2013)	Diffusional kurtosis imaging of cingulate fibers in Parkinson disease: Comparison with conventional diffusion tensor imaging	15	15	anterior cingulum	3.0T	FA	0,017

Table 9 - Studies not included in the DTI Meta-Analysis

In the other 8 studies, table10, there was a significant decrease of FA in SN:

Author (Year)	Title	PD	HC	Studied Region	M.F. Tesla	+info	t-test p-value
DTI							
L-L Chan, et al. (2007)	Case control study of diffusion tensor imaging in Parkinson's disease	73	78	SN	1.5T	FA	0,001
G. Gattellaro,et al. (2009)	White Matter Involvement in Idiopathic Parkinson Disease: A Diffusion Tensor Imaging Study	10	10	ROI SLF CI Genu SN	1.5T	MD	 0,01 0,005 0,002 0,05
Ricarda A. Menke, et al. (2010)	Connectivity-based segmentation of the substantia nigra in human and its	10	10	SNr SNc	3.0T	Vol	0,005 0,010
D.E. Vaillancourt, et.al (2009)	High-resolution diffusion tensor imaging in the substantia nigra of de novo Parkinson disease	14	14	rSN mSN cSN	3.0T	FA	0,001
K Yoshikawa, et al. (2004)	Early pathological changes in the parkinsonian brain demonstrated by diffusion tensor MRI	12	8	SN	1.5T	FA	0,05
Wang Zhan et al (2011)	Regional alterations of brain microstructure in Parkinson's disease using diffusion tensor imaging.	12	20	SN	4.0T	FA	0.0001
Guangwei Du et al. (2011)	Combined R2* and diffusion tensor imaging changes in the substantia nigra in Parkinson disease	16	16	SN	3.0T	FA R2*	0,05 0,001
Menke, R. A., et al. (2009)	MRI characteristics of the substantia nigra in Parkinson's disease: A combined quantitative T1 and DTI study	10	10	SN	3.0T	l. SN r. SN	0,02 0,01

Table 10 - Studies included in the DTI Meta-Analysis of FA in SN

Meta-Analysis of DTI studies.

The effect size for the reduction was pooled from all (8) with a total of 157 PD and 166 HC (using Hedges's g) showing a large mean effect size (-0,811, 95% confidence interval -1,036 to -0,586, p < 0,0001). Studies have low level of heterogeneity (Q [6] =7,327, p =0,396 I^2 = 4,465). (Table 11)(*)

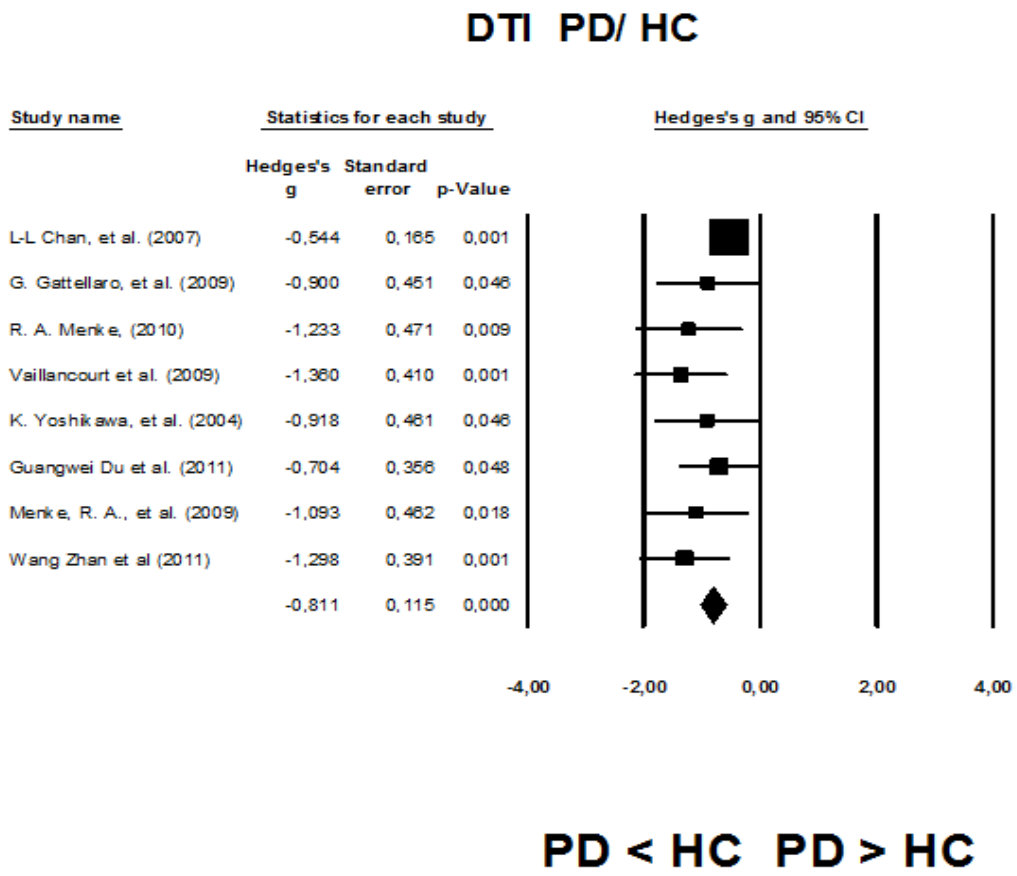

Table 11 - Meta-Analysis of DTI (FA) in SN

(*) For the statistic calculations was used the software "Comprehensive Meta-Analysis" of Biostat,Englewood, USA version 2.2.064 of July 27, 2011 licensed to Jose Gominho

3rd - Single-voxel Spectroscopy

In the early studies of the application of MRS in DP, Holshouser BA, et al., 1995, in a multicenter pilot study with 151 PD and 97 age-matched HC, show that there was not a significant reduction in the proportion of NAA/creatine in the Striatum In group IPD when compared with the controls. The authors observed a decrease in the ratio of NAA/choline in older patients with IPD (Idiopathic Parkinson's Disease) and a significant decrease in the mean NAA/Cho ratio was observed in patients not being treated with L-dopa. This study suggests that the ratio NAA / Cho can be affected by treatment with L-dopa, which can provide a reversible marker of the neuronal dysfunction in striatum [20].

In a 1H-MRS study, the absolute quantification of metabolites in millimolar concentration, did not show significant differences, of NAA, choline and creatine, in addition, there was no significant changes in concentration of glutamine or glutamate in the basal ganglia [9]. Ellis, Lemmens et al. [15] did not demonstrate any significant difference in the proportion of NAA/creatine in a group of patients with DPI. However, there was a significant reduction of the proportions of NAA/choline in drug naive patients with DPI, compared to a treated group and a control group.

Gulin Oz, et al., 2006, in a study using a single-voxel spectroscopy in small volumes in a 4Tesla scanner, with 10 PD patients with mean age of 59 years old and 11 HC, detected that the neurochemical profile of the SN is unique and characterized by a fourfold higher GABA/Glu ratio compared to the cortex, in excellent agreement with established neurochemistry [70].

Bing Zhou et al., 2013, recently published a study, where used equipment of 3.0 Tesla, stating that there was an improvement of signal-to-noise ratio and resonance magnetic resolution and found significant differences in the reasons NAA/ Cr, NAA/ Cho /NAA (Cho + Cr) observed in SN between PD patients and healthy controls ($P < 0.05$), as well as the affected extremity between the ipsilateral and contralateral in PD patients ($P <0.05$) were also observed significant differences in the reasons NAA/ Cr, NAA/ Cho, NAA/ (Cho + Cr) among patients with slight and severe PD. It was verified that 1H-RMS can be used to detect metabolites of substantia nigra in PD patients, which can be useful in the early diagnosis and in assessment of DP [66].

E. Hattingen, et al., 2009, using 3Tesla scanner a combined 1H/31P MR spectroscopy in 29 PD patients with mean age of 62 and 19 age matched HC, focus on the substantia nigra and on the putamen since these are anatomical key areas in the neurodegenerative process of the dopaminergic system in Parkinson's disease found a bilateral reduction of high-energy phosphates such as adenosine triphosphate and phosphocreatine as final acceptors of energy from mitochondrial oxidative phosphorylation. In contrast, low-energy metabolites such as adenosine diphosphate and inorganic phosphate were within normal ranges. These results provide strong in vivo evidence that mitochondrial dysfunction of mesostriatal neurons is a central and persistent phenomenon in the pathogenesis cascade of Parkinson's disease which occurs early in the course of the disease [71].

The selected studies and quality assessment are in the table 12 and table 13 respectively.

Author (Year)	Title	PD	HC	Hoehn & Yahr Stage	Diag. Criteria	Mean Age	Medication	Modality	Studied Region	M.F. Tesla	Conclusions
Spectroscopy											
B. Zhou, et al. (2013)	Application of proton magnetic resonance spectroscopy on substantia nigra metabolites in Parkinson's disease	30	20	1; 2	clin diagosed	58.9 (10)		spectroscopy (1H- MRS).of 3.0 T technology, improvements in the signal-to-noise ratio and resolution	SN	3.0 T	Significant differences in NAA/Cr, NAA/Cho, NAA/ (Cho + Cr) were observed between PD patients and healthy controls (P<0.05) as well as between the ipsilateral and contralateral. It can be used to detect substantia nigrametabolites in PD patients, which may be useful for early diagnosis and evaluation of PD
D. Axelson, et al. (2002)	Applications of Neural Network Analyses to In Vivo 1H Magnetic Resonance Spectroscopy of Parkinson Disease Patients	31	14	NA	diagnostic criteria of Gelb	63.7	yes	spectroscopy (1H- MRS).		1.5T	In conclusion, ANNs could successfully be trained to distinguish control spectra from spectra recorded from PD patients. Furthermore, networks could be trained to distinguish the four groups of probable PD, possible PD, atypical PD, and controls.
R. M. Camicioli, et al. (2007)	Magnetic Resonance Spectroscopic Evidence for Presupplementary Motor Area Neuronal Dysfunction in Parkinson's Disease	44	38	UPDRS)17 Hoehn & Yahr mean 2.25	UKBB	71.2 (4.4)	yes	Spectroscopy with voxel 2x2x2cm at Anterior Cingulum(AC) Posterior Cingulum(CP) e pré MAS		1.5T	AC and PC NAA/Cr and Cho/Cr in any region did not differ (P 0.05). In conclusion, pre-SMA NAA/Cr was selectively decreased in PD, consistent with neuronal (P= 0.045) disfunction. This should be further examined as a biomarker of disease in PD.
B. C. Bowen, et al. (1995)	Proton MR Spectroscopy of the Brain in 14 Patients with Parkinson Disease	14	13	NA	clinical diagnosis	64 (15)	yes	single-voxel (27-cm3 volume) proton MR spectroscopy of the occipital lobe		1,5T	Increase in cerebral lactate in patients with Parkinson disease support the hypothesis that Parkinson disease is a stemic disorder characterized by an impairment of oxidative energy metabolism
E. Hattingen, et al. (2009)	Phosphorus and proton magnetic resonance spectroscopy demonstrates mitochondrial dysfunction in early and advanced Parkinson's disease	29	19	1;2 and 3; 4	UKBB	62.4 (9.2) 66.7 (7.6)	yes	combined 1H/31P MR spectroscopy	MSR	3T	Combined 1H/31P MR spectroscopy revealed a significant decrease of high energy phosphates in the mesostriatal region (MSR)of patients with Parkinson's disease in vivo. The data strongly support the hypothesis that mitochondrial dysfunction is involved early in the pathogenesis of Parkinson's disease and a major constituent of the pathogenetic cascade.
Gulin Oz, et al. (2006)	Proton MRS of the Unilateral Substantia Nigra in the Human Brain at 4 Tesla: Detection of High GABA Concentrations	10	11	UPDRS (27)	clinical diagnosis	59 (10)	No (>12h)	single-voxel spectroscopy in small volumes	SN	4T	Single-voxel spectroscopy of the unilateral human SN is feasible in both healthy volunteers and patients with PD. It provides an in vivo neurochemical profile that includes neurotransmitter (Glu and GABA) and antioxidant (GSH) levels that are in excellent agreement with neurochemistry literature.
Barbara A Holshouser, et.al. (1995)	Localized Proton NMR Spectroscopy in the Striatum of Patients with Idiopatic Parkinson's Disease: A Multicenter Pilot	151	97	1 to 4	UKBB	27 to 83	yes	single-voxel spectroscopy	Striatum	1.5T	A Trend in NAA/Cho redution, being statistically significant in a subgroup of older patients might be indicative of neuronal cell death in the striatum in the progression of PD
Groger, A., et al. (2011)	Three-dimensional magnetic resonance spectroscopic imaging in the substantia nigra of healthy controls and patients with Parkinson's	17	8	2.5 to 3	clinical diagnosis	69	yes	3D-MRSI	SN	3T	These findings from this study indicate that regional variations of the SN may diferentiate patients with Parkinson´s disease and healthy controls.
	Total	326	220								

Table 12 - Selected Spectroscopy Studies

Quality assessment of the Spectroscopy studies:

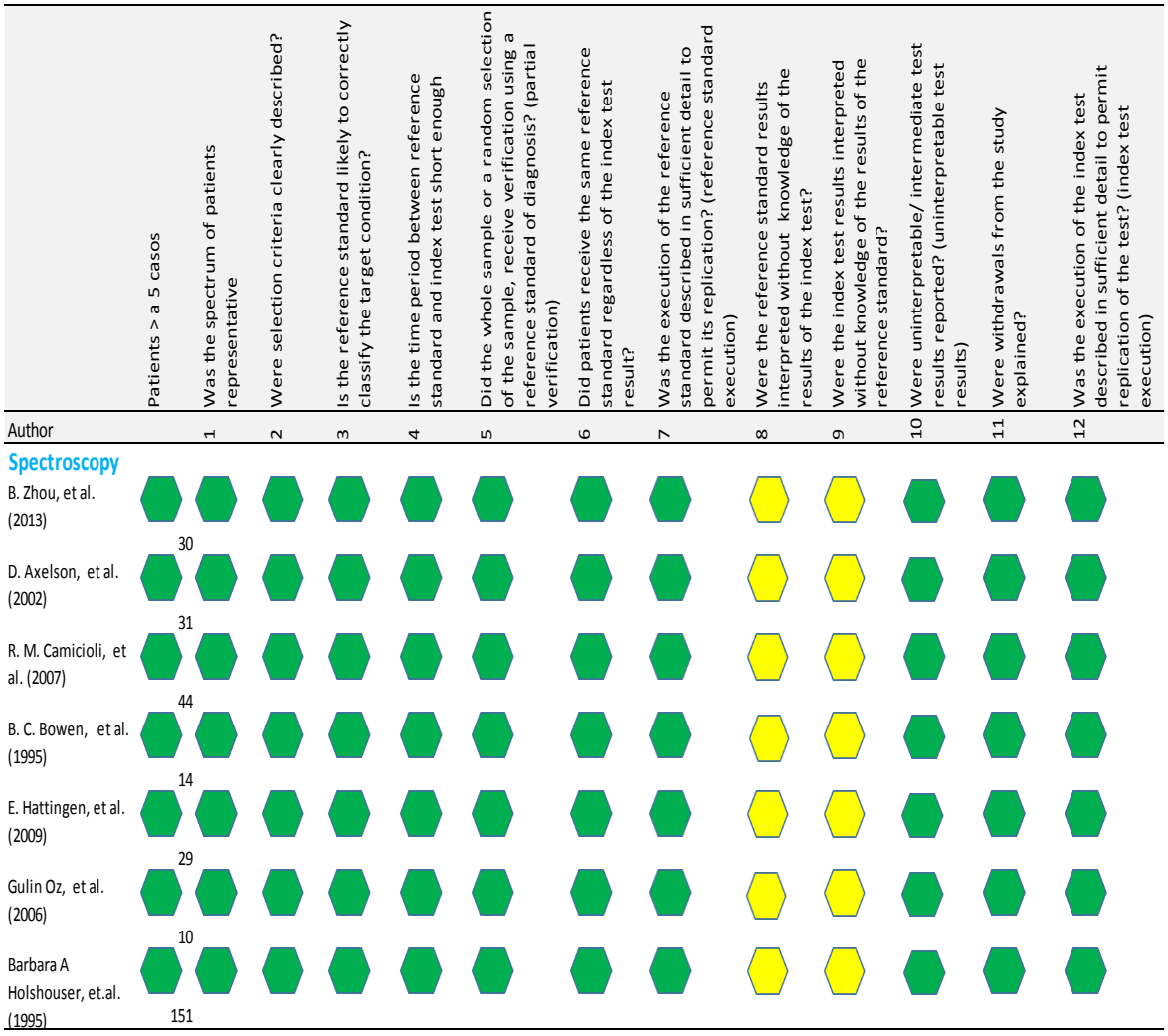

Table 13 - Quality assessment of the Spectroscopy studies

The quality of the studies was good. Due to the technique execution and interpretation they do not refer about the knowledge, of the standard and the patient's results, of the tests.

Although a quantitative meta-analysis of the data was planned, the significant heterogeneity in both the methodology of the studies, particularly echo time

(TE), and the results suggested a qualitative approach was more advisable.

4th - fMRI that includes RS-fMRI (resting state fMRI).

Review of fMRI studies

Cortex

Reviewing the studies published up to date, involving the comparison of Parkinson's patients with healthy controls, we found:

Tessa et al., 2010, in the study "Decreased and increased cortical activation coexist in New Parkinson's disease" with 20 PD patients, mean age 71, and 11 age matched HC, show a complex pattern of cortical activation with hypo and hyperactive areas present simultaneously. Hypo activation in left SM1 may indicate that deafferentation effects are predominant in this cortical region in an early stage of the disease. The increased activation in the left temporal–parietal cortex adjacent to SM1 and in right SM1 and adjacent cortex could either reflect compensatory mechanisms put in place by the diseased brain to compensate the striatal–cortical network failure or simply represent an extension of the disease-related dysfunction. The correlation of the cortical activity in both left SM1 and

SMA/cingulum area with the clinical severity of the disease supports the theory that both deafferentation and hyperactivity phenomena are closely related to disease progression [53].

Maria F-Seara et al., 2012, claim, using another technique previously described, the ASL, in 25 PD patients with a mean age of 63.2 and 25 age matched HC, with their results support the hypothesis that cortical perfusion and metabolism are reduced in PD, even at the early disease stages, earlier than most other previous studies have suggested. In addition, this work demonstrates that the ASL technique has enough sensitivity to detect this perfusion decreases by means of absolute CBF comparisons, without the need for normalization techniques. They say that the cortical perfusion deficits is likely related to gray matter atrophy in some areas, as demonstrated by the overlap of atrophy and decreased perfusion found in our group of patients [17].

D. Vaillancourt, et al., 2013, in a study with 20 PD patients with mean age of 53.7 in NTD (Non-Tremor Dominant) and 57.3 in TD (Tremor Dominant) and 20 age matched HC, concluded that they have a robust findings across both voxel-wise and ROI analyses showed that compared to TD patients, NTD patients had reduced activation in ipsilateral dorsolateral prefrontal cortex globus pallidus of NTD PD patients compared to both TD PD patients and controls. Previous studies have shown that the TD variant has a slower rate of progression and less deterioration of health related quality of life [59].

Basal ganglia

David Vaillancourt, et al.,2010, using a 3.0 Tesla equipment compared the activation by (BOLD) in all basal ganglia of 20 Parkinson's patients (mean age 57.9) with healthy controls using a paradigm, could reach 3 conclusions: First there is a significant negative correlation between the sign BOLD and the UPDRS bilaterally in the caudate nucleus, putamen and sub thalamic nucleus; contralateral in substantia nigra, thalamus and external segment of the globus pallidus; second, that the bradykinesia is the symptom that most consistently predicted the BOLD activation in the basal ganglia and thalamus. In addition, the BOLD activation in the internal globus pallidus contralateral was related to tremor; third, that reduced cortical activity in the primary motor cortex and supplementary motor area in recent PD does not relate to the motor symptoms [58].

Tau Wu et al. using fMRI in a study, concludes that Parkinson's patients can achieve automations after proper training but with greater difficulty and using more brain activity to offset the dysfunction of the basal ganglia. These patients also has an increased activity of the cerebellum, the pre motor area, precuneus cortex and prefrontal [51].

Hu Liu et al., 2005, in an article published recently states that using fMRI (BOLD contrast), in a Resting State, he found in the selected sample of 18 (mean age 61.4) PD patients a decreased connectivity between the cerebellum and inferior parietal lobe. The inferior parietal lobe (BA40) is part of the Executive network, which is required for the selection and maintenance of working memory and information necessary for the preparation of the action 21.

Tao Wu et al., 2012, demonstrates in 16 PD patients (age mean 58.1) that the combination of fMRI and network analysis is likely a useful tool to establish the BG (basal ganglia) pathways model in vivo in human subjects. The causal connectivity from the substantia nigra to different brain networks suggests that the dopaminergic system exerts influences on an extensive brain functions, both motor and cognitive behaviors. The pattern of this connectivity is abnormal in PD, secondary to dopamine depletion and becomes more significant as the disorder progresses. The disrupted BG networks may contribute to the motor and some non-motor impairments in PD, the fMRI has been a useful method to demonstrate "in vivo" these pathways [62].

D. Vaillancourt, et al. (2013) confirmed that fMRI differences in the basal ganglia and cortex between patients with PD and control subjects are primarily due to patients with the (NTD) non-tremor dominant subtype rather than the (TD) tremor dominant subtype. These findings suggest that objective measures of brain function may be useful in future, genotype-phenotype analyses and targeted therapeutic trials focused on PD subtypes [59].

Resting State fMRI.

Since its discovery by Biswal et al. (1995) resting State fMRI (RS-fMRI) has been applied successfully to identify a variety of networks cortico-intrinsic, and cortico sub cortical with a homogeneous resolution in range mm.

Simon Baudrexel et al., 2011, studied the functional connectivity of the sub thalamic nuclei (STN) with the cortical areas of Brodmann 4 and 6, in a group of 31 PD patients (mean age 58.4) and 44 age matched HC and confirmed, as previously demonstrated by electrophysiological studies, that there is an increase

in functional connectivity (FC) among them and also the increased FC primarily among the basal ganglia. These results are in agreement with the recent experimental data, which suggest that the increased synchronicity mediated STN-motor cortex, the call the hyper-direct sub thalamic motor cortex via and which can play a key role in the pathophysiology of DP. Comparing the PD patients with healthy controls, patients with tremor showed increased FC STN specifically in the hand area of the primary motor area and primary sensory cortex. In patients without tremor, were also found an increased values FC between STN and median motor cortical areas, including the supplementary motor area (SMA) 1.

A. Tessitore et al., 2012, Using RS-fMRI and studying 29 PD patients with akinesia or "Freezing of gait", and comparing with 15 HC, concluded that in these patients in resting state, functional connectivity reveals an interruption of "Executive attention" (right Middle frontal gyrus and in angular gyrus) and neurological vision network (right occipital-temporal gyrus), that can be associated with these phenomenon and that the severity is correlated with the decrease of this connectivity [54].

The selected studies of fMRI and RS-fMRI are represented in the table 14 and the quality assessment in table 15.

Author (Year)	Title	PD	HC	Hoehn & Yahr Stage	Diag. Criteria	Mean Age	Medication	Modality	Studied Region	M.F. Tesla	Conclusions
fMRI											
D. Vaillancourt, et al. (2010)	Blood Oxygenation Level Dependent Activation in Basal Ganglia Nuclei Relates to Specific Symptoms in De Novo Parkinson's Disease	20	20	UPDRS	UKBB	57.9	No	blood oxygenation level dependent (BOLD) activation in the ROI	BG	3.0T	1-Compared with control subjects, patients with PD had reduced PSC in all contralateral and ipsilateral nuclei of the BG. 2- Patients with PD also had reduced PSC in the medial and lateral thalamus, M1, and SMA
Tao Wu, et al. (2005)	A functional MRI study of automatic movements in patients with Parkinson's disease	12	14	1; 2	Clinical Diagnostic	61.2	Yes (12h)	functional magnetic resonance imaging (fMRI)	BG Cortex	1.5 T	Patients had greater activity in the cerebellum, premotor area, parietal cortex, precuneus and prefrontal cortex compared with normal subjects while performing automatic movements
Hu Liu , et al. (2013)	Altered resting-state functional connectivity of the dentate nucleus in Parkinson's disease	18	18	H&Y (1.4) and UPDRS (16)	Clinical Diagnostic	61.4 (8.5)	?	The RS-fMRI scanning with (BOLD)contrast		3.0T	Found a decreased connectivity between the cerebellum and the inferior parietal lobule in the PD patients sample. The inferior parietal lobule (BA40) is part of the executive network, which is required for the selection
Tao Wu, et al. (2012)	Basal ganglia circuits changes in Parkinson's disease patients	16	16	H&Y (1.4) and UPDRS (21.5)	Clinical Diagnostic	58.1 (4.8)	No	fMRIs were performed: Blood-oxygen-level dependent (BOLD) data were acquired with gradient-echo echoplanar sequences.	ROI	3.0T	Demonstrate that the combination of fMRI and network analysis is likely a useful tool to establish the BG pathways model in vivo in human subjects. The causal connectivity from the substantia nigra to many brain networks suggests that the dopaminergic system exerts influences on extensive brain functions, both motor and cognitive behaviors.
D. Vaillancourt, et al. (2013)	Brain activation differences in tremor and non-tremor dominant Parkinson's disease (Author Manuscript)	20	20	1; 2	UKBB	NTD= 53.7 (8.7) TD 0 57.3(8.9)	No	Blood oxygenation level dependent activation and percent signal change.	ROI	3.0T	Robust findings across both voxel-wise and ROI analyses showed that compared to TD patients, NTD patients had reduced activation in ipsilateral dorsolateral prefrontal cortex globus pallidus of NTD PD patients compared to both TD PD patients and controls
Il-H. Choea,et al. (2013)	Decreased and increased cerebral regional homogeneity in early Parkinson's disease	22	25	H&Y (1.6) and UPDRS (10.4)	UKBB	58.3 (2.4)	Y and N	Regional homogeneity (ReHo) data which records the blood oxygen level-dependent (BOLD)	ROI	3.00T	ReHo was increased in the left inferior parietal lobule, the angular gyrus, the supramarginal gyrus, the middle occipital gyrus and the parahippocampal gyrus.
Tao Wu, et al. (2009)	Changes of functional connectivity of the motor network in the resting state in Parkinson's disease	22	22	H&Y (off medication) 1.7±0.5	Clinical Diagnostic	59.5 (10.1)	Y and N	fMRI (BOLD)	ROI	1.5T	Functional connectivity of the motor network in the resting state is disrupted in PD. This change is secondary to dopamine deficiency, and related to the severity of the disease
M. A. F.-Seara, et al. (2012)	Cortical hypoperfusion in Parkinson's disease assessed using arterial spin labeled perfusion MRI	25	25	1; 2	UKBB	63.2 (6.6)	Y and N	Arterial spin labeled (ASL) perfusionMRI The ASL technique utilizes electromagnetically labeled arterial blood water as an endogenous tracer, yielding quantitative CBF	whole brain VWC and ROI	3.0T	Assessment of absolute cerebral perfusion in PD using the ASL technique yielded a pattern of cortical perfusion deficit, affecting frontal, parietal and occipital areas. Our results largely confirm prior studies of perfusion and metabolism measuring absolute CBF ou CMRglc
C. Tessa , et al. (2010)	Decreased and increased cortical activation coexist in de novo Parkinson's disease	20	11	1; 2 (1.6)	UKBB	71 (3.1)	Y and N	high resolution contiguous 3D T1 weighted images, with a MPRAGE sequence . ,(FLAIR) sequence _fMRI and T2*-weighted echo-planar imaging (EPI) sequence	WB voxel-wise comparison and ROI	1.5T	Lower activation in the left (SM1) cortex and cerebellum and higher activation in the left temporal– parietal cortex adjacent to the SM1 and in right SM1.PD patients the disease severity correlated significantly with activation of left SM1 and supplementary motor area and cingulum, bilaterally.
D. E. Vaillancourt, et al. (2007)	Role of hyperactive cerebellum and motor cortex in Parkinson's disease	8	8	UPDRS (31,3)	Clinical Diagnostic	59.4 (8.4)	Yes	Using a BOLD contrast fMRI paradigm PD patients and healthy controls	WB VWC and ROI	3.0T	Evidence supporting the hypothesis that hyperactivation in the ipsilateral cerebellum is a compensatory mechanism for the defective basal ganglia.
S. Baudrexel, et al. (2011)	Resting state fMRI reveals increased subthalamic nucleus–motor cortex connectivity in Parkinson's disease	31	44	1; 2	UKBB	59.4 (10.7)	N (12)	High resolution quantitative T1 maps were calculated from two RF-spoiled 3D gradient echo data sets acquired with different flip angles as described recentlv	WB VWC and ROI	3.0T	Increased resting state FC between the STN and cortical motor areas. PD rigor and tremor symptoms might be linked to an abnormal coupling of these areas
A. Tessitore , et.al (2012)	Resting-state brain connectivity in patients with Parkinson's disease and freezing of gait	29	15	1; 2	UKBB	FOG+ 66.94 (6.0) FOG- 66.31 (6.3)	Yes	Resting-state functional magnetic resonance imaging	WB voxel-wise comparison and ROI	3.0T	Patients with freezing of gait exhibit significantly reduced functional onnectivity within both "executive-attention" (in the right middle frontal gyrus and in the angular gyrus) and visual networks (in the right occipito-temporal gyrus) [p < 0.05 corrected for multiple comparisons].
T. R. Melzer, et al. (2011)	Arterial spin labelling reveals an abnormal cerebral perfusion pattern in Parkinson's disease	61	29	UPDRS part III	UKBB	64.7 (8.6); MCI 69.8 (9.1);D 75.1 (6.7)	N and Y	ASL a MRI perfusion method	ROI	3.0T	This network was characterized by decreased cortical and preserved subcortical and sensorimotor cortical perfusion
	Total	304	267								

Table 14 - Selected studies of fMRI and RS-fMRI

Quality assessment of the fMRI and RS-fMRI studies:

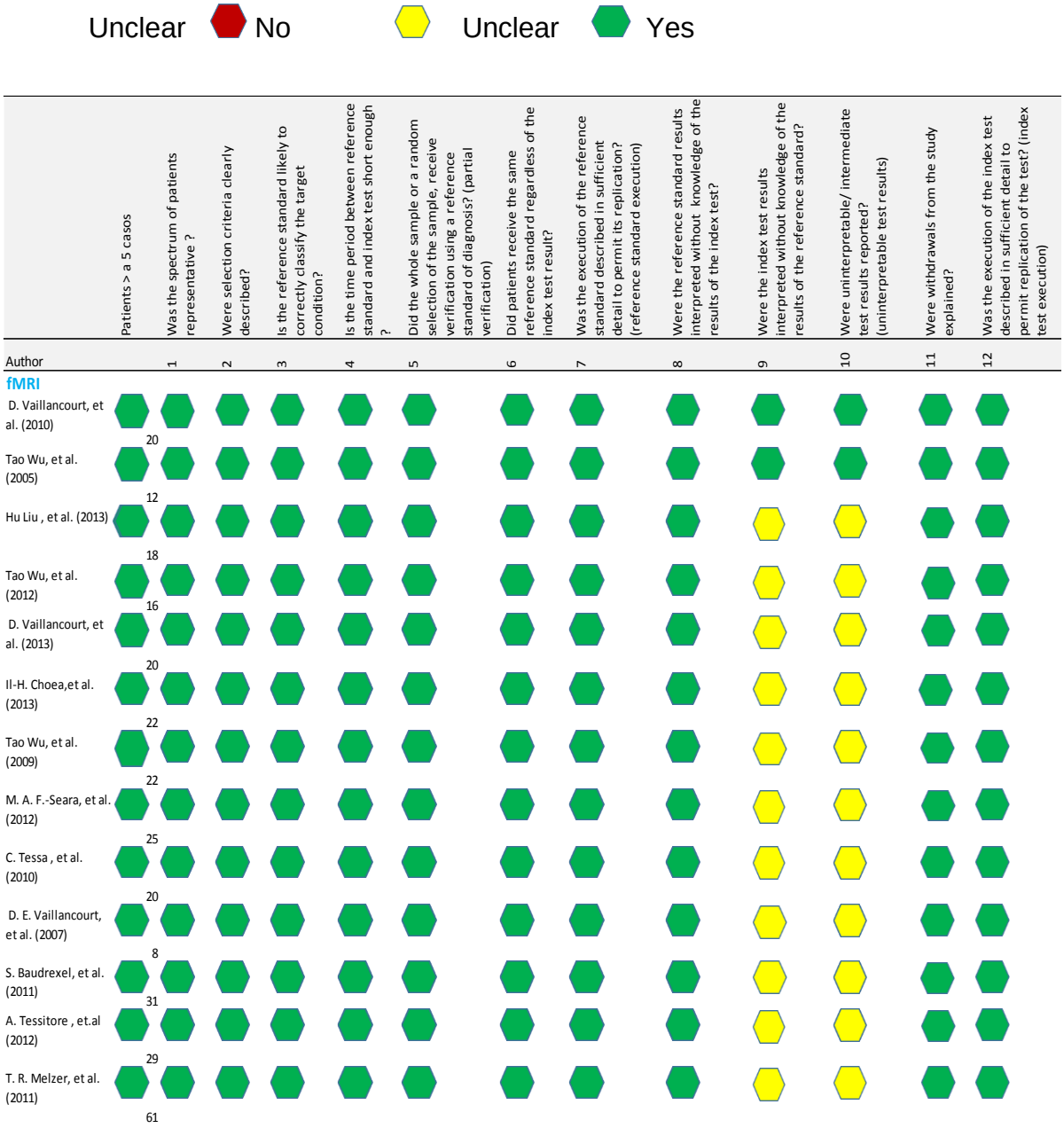

Table 15 - Quality assessment of the fMRI and RS-fMRI studies

The quality of the studies was in average good.

Although a quantitative meta-analysis of the data was planned, the significant heterogeneity in both the methodology of the studies, and the results suggested a qualitative approach was more advisable.

Discussion

After reviewing several studies of various imaging modalities of MRI I will divide the discussion as previously, into the four groups, as presented in the results:

1st- Structural and Iron and Neuromelanin MRI; 2nd - DTI (diffusion tensor imaging) with FA and MD; 3rd-Spectroscopy (MRS); 4th- fMRI that includes RS-fMRI (resting state fMRI)

Structural and Iron and Neuromelanin MRI

In this group of studies we can conclude (probably due to the number of 3T systems) that using T1 Inversion recovery imaging (the area) [24]; T2W in (the volume) [33]; T1p (neuronal loss) [46]; MRI neuromelanin-sensitive (volume measurement) [23] we found a significant decrease in SN of the PD patients when compared with age-matched HC; And an significant increase of R2* (=1/T2*) and T2p, (sensitive to iron deposition) of values in the PD patients [41;27] when compared with HC. Even mixing systems with different magnetic fields.

DTI (diffusion tensor imaging) with FA and MD

With this modality we can find in PD patients, changes in FA and MD in whole brain [74] but more pronounced in the frontal and parietal white matter reflecting a widespread microstructural damage that occurs already in the early stages of PD[75], in thalamic projection fibers [11], MK and FA in the anterior cingulum were significantly lower [22], in the motor, pre-motor and supplementary motor cortex [64], white matter areas close to supplementary motor areas external and internal capsules, right thalamus, and left putamen [65] and as we have shown in the meta-analysis a significant reduction in FA in the SN [31; 14; 65; 64; 60; 32; 7].

Spectroscopy (MRS)

In the Spectroscopy on substantia nigra SN metabolites in Parkinson's disease were observed between PD patients and healthy controls significant differences in NAA/Cr, NAA/Cho, NAA/ (Cho + Cr) [66; 76] It provides an in vivo neurochemical profile that includes neurotransmitter (Glu and GABA) and antioxidant (GSH) levels that are in excellent agreement with neurochemistry literature [70] . In the pre-SMA, NAA/Cr was selectively decreased in PD, consistent with neuronal (P= 0.045) dysfunction [73]. In the putamen and midbrain was found a bilateral reduction of high-energy phosphates such as adenosine triphosphate and phosphocreatine as final acceptors of energy from mitochondrial oxidative phosphorylation [71].

Functional magnetic resonance imaging, including RS-fMRI (resting state fMRI).

Using different paradigms and comparing PD patients with HC we found in the different studies: reduced PSC (Percent Signal Change) in all contralateral and ipsilateral nuclei of the Basal Ganglia, medial and lateral thalamus M1 and SMA. There were significant negative correlations between total motor Unified Parkinson's Disease Rating Scale (UPDRS) and BOLD activation in bilateral caudate, bilateral putamen, contralateral external segment of the globus pallidus, bilateral sub thalamic nucleus, contralateral substantia nigra, and thalamus. Bradykinesia was the symptom that most consistently predicted BOLD activation in the basal ganglia and thalamus. Also, BOLD activation in the contralateral internal globus pallidus was related to tremor.

The reduced cortical activity in primary motor cortex and supplementary motor area in de novo PD did not relate to motor symptoms [58].

Parkinson's disease patients require more brain activity in the cerebellum, premotor area, parietal cortex, precuneus and prefrontal cortex compared with normal subjects to compensate for basal ganglia dysfunction while performing automatic movements [51]. Using the combination of fMRI and network analysis they found that the causal connectivity from the substantia nigra with many brain networks, suggests that the dopaminergic system exerts influences on extensive brain functions, both motor and cognitive behaviors [62].

Using RS-fMRI to study functional connectivity (FC) it was found that PD patients has a disrupted motor network (Tao Wu et al. 2009). Increased resting state FC between the STN and cortical motor areas and PD rigor and tremor symptoms might be linked to an abnormal coupling of these areas. Finally that the PD patients with freezing of gait exhibit significantly reduced functional connectivity within both "executive-attention" (in the right middle frontal gyrus and in the angular gyrus) and visual networks (in the right occipito-temporal gyrus).

These recent studies, from 2012 and 2013, included in this review with PD patients with 3-T, 4-T and 7-T scanners confirms what was previously written in EFNS/MDS-ES Guidelines (2012) "newer quantitative imaging techniques implemented on 3-T have shown promising results in detecting abnormalities in the SN and nigrostriatal pathways with high diagnostic accuracy in separating PD patients from healthy controls (class II evidence)" [72]. But these findings warrant further a large prospective confirmatory studies with all stages and including postmortem histopathology.

Limitations.

There are some methodology and data limitations to be considered for this review. The number of studies and the size of studies are modest, limiting the generalizability of the results. No publication bias was detected for the Meta-Analysis but this cannot be excluded. Although no significant statistical heterogeneity was detected in the Meta-Analysis of SN in PD, there were many differences among the total studies in modalities of MRI, data acquisition, data analysis, protocols, magnetic field strength and subject details, with associated limitations. Clinical diagnostic criteria were used for patient selection but without neuropathology verification in the studies, thus misdiagnosis cannot be excluded.

The stage of a patient's disease, either early or advanced, may influence MRI findings. Patients with early disease were underrepresented in the studies, most likely because clinical diagnostic accuracy is higher in the later stages. Patient groups also often included those who were already initiated on therapy, and antiparkinsonian medications are another factor that modulate in some cases the MRI findings as we have seen in some studies. A Lack of prospective studies with all stages including postmortem histopathology for confirmation is not present.

Conclusion

Magnetic Resonance Imaging may prove valuable in supporting the diagnosis in Parkinson's disease because it has a good accuracy in separate PD patients from Healthy Controls, and could have a role in detecting pre manifest disease, monitoring progression and drug therapeutic impact. Prospective and longitudinal studies using DTI, Spectroscopy, fMRI, RS-fMRI and other modalities of MRI on larger cohorts of patients with Parkinson´s disease, particularly including at-risk and early-disease populations, are needed to investigate some of the actual encouraging preliminary findings. Future research will be facilitated by the increasing availability of higher magnetic field (4; 7 and 14 Tesla) and multimodal neuroimaging systems that are actually available, but was not the objective of this study, will benefit the "*in vivo"* diagnostics. We think that now with a better communication between the investigation groups, the standardization of protocols will be a reality, in the future and that will help us to get a better and comparable results.

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Attachment – 1 (All data collected from structural studies)

Author (year)	Title	PD	HC	Hoehn & Yahr Stage	Diag. Criteria	Mean Age	Medication	Modality	Studied Region	Tesla	Conclusions	Sem	Spec	PPV	NPV	LR	ROC	Info.	t-test p-value
Structural Changes																			
M. Hutchinson, et al. (1999)	Parkinson's disease: a novel MRI method for determining structural changes in the substantia nigra	6	6	1; 2 ; 3	clinical diag	58	yes	T2 weighted images	SN	1.5T	Substantia nigra degenerates from lateral to medial and in a rostral to caudal direction	NA	NA	NA	NA	NA	NA	NA	0.001
C. Haegelen, et al. (2013)	Automated segmentation of basal ganglia and deep brain structures in Parkinsons's disease	57	10	NA	clinical diag	58.9 (8)	NA	T1-weighted and T2-weighted MRI	BG	3.0T	The construction of an MR image template specific to PD, and also evaluated a method of achieving the accurate segmentation of the basal ganglia and deep brain structures	NA	NA	2,3	NA	NA	NA	NA	0.05
Jan Linder,et al. (2009)	Degenerative changes were common in brain magnetic resonance imaging in patients with newly diagnosed Parkinson's disease	66	30	UPDRS-III	UKBB	68 (9.6)	NA	T1 W and T2 W covering the whole brain.	All brain	1.5T	Therefore, they are of limited value in distinguishing among subgroups at clinical presentation and do not change daily practice	NA	NA	NA	NA	NA	NA	NA	NA
L. Minati,et al. (2007)	Imaging Degeneration of the Substantia Nigra in Parkinson Disease with Inversion-Recovery MR Imaging	8	8	All - 2	clinical diag	66.5 (5.0)	yes	T 1 contrate (Inversion-recovery imaging)	SN	1.5T	A way to visualize nigral degeneration. SN	NA	NA	NA	NA	NA	NA	NA	0.04
M. Hutchinson, et al. (2006)	Inversion Recovery MRI in Idiopathic Parkinson Disease Is a Very Sensitive Tool to Assess Neurodegeneration in the Substantia Nigra	12	12	UPDRS	clinical diag		NA	Segmented inversion recovery (IR) ratio imaging (SIRRIM). ie, WMS/GMS.	SN	1.5T	Is sensitive enough to identify patients with early-stage IPD. All patients with IPD were identified correctly, and full dichotomization between healthy volunteers and patients was obtained with our database	NA	NA	NA	NA	NA	NA	NA	
Yonca Anik, et al. (2007)	Magnetization Transfer Ratio in Early Period of Parkinson Disease	33	30	1st year early	clinical diag	66.9 (8.49)	yes	Magnetization transfer ratio (MTR) imaging	SNpc SNpr RN Pons	1.5T	MTR analysis is a useful technique for initial PD assessment. MTR decreases in all PD	NA	NA	NA	NA	NA	NA	SNpc SNpr RN Pons	0.001 0.006 0.037 0.046
N. D. Forkert,et al. (2011)	Image-based Classification of Parkinsonian Syndromes Using T2'-Atlases	33	24	?	clin diagosed	61.5 (10.4)	NA	1/T2' = 1/qT2*-1/qT2 a Selectively measured using T2' MR imaging		1.5T	Automatic classification using the generated atlases.	0.94	0.91	NA	NA	NA	NA	NA	NA
R. A. Menke , et al. (2009)	MRI characteristics of the substantia nigra in Parkinson's disease: A combined quantitative T1 and DTI study	10	10	1; 2; 3	clinical diag	63,7 (6,7)		Driven equilibrium single pulse observation of T1 (DESPOT1) and (DTI)	SN-Put SN-Tal VCDR	3.0T	Combining SN volumetry and its connectivity with the thalamus improved the classification sensitivity to 100% and specificity to 80% for PD	1.0 1.0	0.80 0.90	NA	NA	NA	0.80 0.90	NA	0.05
I. Nestrasil , et al. (2010)	T1q and T2q MRI in the evaluation of Parkinson's disease	9	9	UPDRS and HY	clinical diag	59.0 (7.1)		T1p and T2p	SN	4.0T	Was demonstrated the ability of MRI methods to separate PD from controls. T1q may be a useful marker of asymmetry in mild-moderate PD	1.0	0.95	NA	NA	95	0.98 9 0.95 6	Tiq T2q both	0.001
O. Monchi , et al. (2011)	Patterns of cortical thickness and surface area in early Parkinson's disease	49	33	UPDRS and HY 1 to 2,5	UK PDSBB	63.3 (7.3)	yes	MRI volumes / Voxel based morphometry (VBM)	Cortex	3.0T	Mean cortical surface area was significantly larger in the PD than in the HC group (2.30±0.18 mm2 vs. 2.19±0.16 mm2; p<0.005)	NA	NA	NA	NA	NA	NA	NA	0.005
H. Oikawa,et al. (2002)	The Substantia Nigra in Parkinson Disease: Proton Density–Weighted Spin-Echo and Fast Short Inversion Time Inversion-Recovery MR Findings	22	22	1; 2; 3	clinically established diagnosis	59.8 (9.2)	NA	T2-weighted -- STIR sequence	SN	1.5T	Was demonstrated the ability of MRI methods to separate PD from controls. T1q may be a useful marker of asymmetry in mild-moderate PD	NA	NA	NA	NA	NA	NA	NA	>0,05
S. Michaeli,et al. (2007)	Assessment of Brain Iron and Neuronal Integrity in Patients with Parkinson's Disease Using Novel MRI Contrasts	8	8	2	(UPDRS)	61 (16)	yes (7)	Rotating frame transverse (T2p) and longitudinal (T1p) relaxation MRI methods	SN	4.0T	novel adiabatic T2 and T1 MRI relaxation methods used here for the measurement of the load and distribution of iron and neuronal loss may provide unique information on the pathogenesis of PD.	NA	NA	NA	NA	NA	NA	T2p T1p	0.01 0.036
Z.-H. Cho, et al. (2011)	Direct Visualization of Parkinson's Disease by In Vivo Human Brain Imaging Using 7.0T Magnetic Resonance Imaging	10	9	1 and 3	(UPDRS)	58.3 (8.5) & 59 (16)	?	7.0T T2*-weighted MR images of the SN	SN	7.0T	1- This study has demonstrated that by using 7.0T MRI, one can visualize the pathologic features of PD within the SN. 2- Clear image of SNc and surroundings such as CC	NA	NA	NA	NA	NA	NA	NA	0.0002
M. Rossi,et al. (2013)	Clinical MRI for iron detection in Parkinson's disease	36	21	na	(UPDRS)	71	yes (4)	Maplt, SWI, and T2-weighted sequences T2* (R2*=1/T2*)	BG SNpc	3.0T	In conclusion, both R2* and SWI detected differences between patients and controls	NA	NA	NA	NA	NA	NA	NA	0.001
M.M. Lewis et al. (2013)	Higher iron in the red nucleus marks Parkinson's dyskinesia	38	23	1; 2; 3	(UPDRS) (21)	60.6	yes	Iron content was estimated from bilateral RN and SN transverse relaxation rates (R2*)	RN SN	3.0T	RN R2* values correlated with off-drug Unified Parkinson's Disease Rating Scale-motor scores RN R2* values were significantly higher in PDpDYS compared with control and PD DYS	NA	NA	NA	NA	NA	NA	NA	0.019 0.002
	Total	397	255																
Neuromelanin																			
Kenichi Kashihara, et al. (2011)	Neuromelanin magnetic resonance imaging of nigral volume loss in patients with Parkinson's disease	80	54	HY	UKBB	70.9 (8.2)	yes	Axial T1-weighted MRI (volume)	SNc	3.0T	The mean volumes for the left and right SNc were significantly reduced in patients with PD compared to the controls. Volume loss became marked in parallel with disease severity and duration. Neuromelanin MRI may be considered as a biomarker of nigral degeneration in patients with PD.	NA	NA	NA	NA	NA	NA	NA	0.001 0.05
Kohsuke Kudo, et al. (2013)	3D neuromelanin-sensitive magnetic resonance imaging with semi-automated volume measurement of the substantia nigra pars compacta for diagnosis	18	27	1; 2; 3; 4	UKBB	68.8 (6.4)	NA	3D turbo field echo (TFE) sequence for neuromelaninsensitive MRI	SNc	3.0T	method can distinguish the PD group from the control group with high sensitivity and specificity, especially for early stage of PD	0.83	0.85	NA	NA	NA	0.88	NA	0.01
Penny A. Gowland, et al. (2013)	Visualization of nigrosome 1 and its loss in PD Pathoanatomical correlation and in vivo 7 T MRI	10	8		UKBB	64 (5)	NA	with high-resolution T2*-weighted MRI scans	SNpc	7.0T	high-resolution 7 T MRI can directly visualize nigrosome 1. The absence of nigrosome 1 in the SNpc onMRI scans might prove useful in developing a neuroimaging diagnostic test for PD	NA	NA	NA	NA	NA	NA	NA	NA
Chigumi Ohtsuka, et al. (2013)	Changes in substantia nigra and locus coeruleus in patients with early-stage Parkinson's disease using neuromelanin-sensitive MR imaging	61	22	1–2 and 3-5	UKBB	50–78 (66.5)	nou	we obtained oblique-axial fast spin-echo T1- weighted images	SN LC	3.0T	Neuromelanin-sensitive MR imaging is able to detect significant signal attenuation in the lateral part of the SNc and in the LC, even in patients with early PD	72,7 81,8	86,7 90.0	NA	NA	NA	0.86 0.88	NA	0.0001
	Total	169	111																

Attachment – 2 (All data collected from DTI studies)

Author (Year)	Title	PD	HC	Hoehn & Yahr Stage	Diag. Criteria	Mean Age	Medication	Modality	Studied Region	M.F. Tesla	Conclusions	Sem	Spec	PPV	NPV	LR	ROC	+info	t-test p-value
DTI																			
Kuncheng Li, et al. (2011)	Voxel-based analysis of diffusion tensor indices in the brain in patients with Parkinson's disease	25	25	1; 2; 3	UKBB	58.4 (9.8)	yes	Voxel-based analysis (VBA) is a technique that can identify the changes of diffusion indices in any part of the whole brain (FA) and (MD)	WB	3.0 T	Abnormal diffusivity in the white matter of bilateral cerebellar hemispheres The positive correlation of the FA values with the TOI and negative correlation of the MD values with the TOI in cerebellum indicated that the cerebellar hemispheres may play an	NA	NA	NA	NA	NA	NA	FA MD	0,001
L-L Chan, et al. (2007)	Case control study of diffusion tensor imaging in Parkinson's disease	73	78	1; 2; 3; 4	UKBB	63.6 (9.8)	yes	DTI = Difusion Tensor imaging (AF), (ADC)	SN	1.5T	1-FA value in the substantia nigra on DTI was lower in PD compared with healthy controls without a strong discriminative power 2- Inverse correlation of clinical severity (H&Y score) with the FA values	49	72	61	60		0.653	FA	0,001
G. Gattellaro,et al. (2009)	White Matter Involvement in Idiopathic Parkinson Disease: A Diffusion Tensor Imaging Study	10	10	1 ; 2	clinical criteria of Gelb.	63.8 (15.7)	yes	(MD)mean diffusibility and (FA) fractional anisotropy maps	ROI SLF CI Genu SN	1.5T	Widespread microstructural damage to frontal and parietal white matter occurs already in the early stages of PD.	NA	NA	NA	NA	NA	NA	MD	0,01 0,005 0,002 0,05
C. Tessa, et al. (2008)	A Whole-Brain Analysis in De Novo Parkinson Disease	27	16	1 ; 2	UKBB (UPDRS)	60.9 (9.7)	n	T1-weighted images and MD and (FA) maps calculated from diffusion tensor imaging (DTI)	GM WM	1.5T	increase of FA values in novo PD, more pronounced in patients with the akinetic-rigid type, probably reflecting diffuse subtle GM loss	NA	NA	NA	NA	NA	NA	FA	0,009
D.E. Vaillancourt,et al. (2013)	Thalamic Projection Fiber Integrity in de novo Parkinson Disease	20	20	(UPDRS) part III	UKBB	57.9 (8.9)	NA	FA analysis and fiber tracking in the thalamus, we used a high-resolution DTI protocol	AN VA DM VL VPL/Vpm PU	3.0T	The present study provides preliminary in vivo evidence of thalamic projection fiber degeneration in de novo PD and sheds light on the extent of disrupted thalamic circuitry as a result of the disease itself.	NA	NA	NA	NA	NA	NA	FA	0,016 0,009 0,006
Koji Kamagata ,et al. (2013)	Diffusional kurtosis imaging of cingulate fibers in Parkinson disease: Comparison with conventional diffusion tensor imaging	15	15	1; 2; 3 (2.7)	UKBB	65.0 (9.3)	yes	(DKI)diffusional kurtosis imaging; mean kurtosis (MK) and (FA)	anterior cingulum	3.0T	(FA) DKI can detect alterations of the anterior cingulum in PD patients more sensitively than can conventional diffusion tensor imaging	0.87 0.80	0.94 0.64	NA	NA	NA	0.912	FA	0,017
Ricarda A. Menke, et al. (2010)	Connectivity-based segmentation of the substantia nigra in human and its	10	10	1; 2; 3; (2.7)	clin diagosed	63.7 (6.7)	yes	single pulse observation of T1 (DESPOT1)-HIFI quantitative imaging method	SNr SNc	3.0T	study demonstrated that tractography-based parcellation might be a useful tool for the segregation of SNc and SNr	NA	NA	NA	NA	NA	NA	Vol	0,005 0,010
D.E. Vaillancourt, et.al (2009)	High-resolution diffusion tensor imaging in the substantia nigra of de novo Parkinson disease	14	14	1; 2 UPDRS III	UKBB	57.2	No	high-resolution DTI protocol at 3 Tesla to study specific segments of degeneration in the SN (FA)	rSN mSN cSN	3.0T	High resolution diffusion tensor imaging in the substantia nigra distinguishes early stage, de novo patients with Parkinson disease (PD) from healthy individuals	100 100 100	7.10 35 100	NA	NA	NA	0.651 0.834 1.00	FA	0,001
K Yoshikawa, et al. (2004)	Early pathological changes in the parkinsonian brain demonstrated by diffusion tensor MRI	12	8	1; 2	UKBB	71.3 (7.7)		The mean FA values of all the ROIs	SN	1.5T	1- PD had decreased FA values in two striatal circuits in which dopaminergic neurones 2 -Significant change in subcortical white matter among the cases of advanced PD	NA	NA	NA	NA	NA	NA	FA	0,05
Wang Zhan et al (2011)	Regional alterations of brain microstructure in Parkinson's disease using diffusion tensor imaging.	12	20	NA	UPDRS	67.4 (8)	Off	Measures of Fractional Anisotropy and Mean Diffusivity	SN	4.0T	FA reductions in PD compared with controls superior PRG, postcentral gyrus, white matter areas close to supplementary motor areas, substantial nigra, inferior PRG, external and internal capsules, right thalamus, and left putamen	NA	NA	NA	NA	NA	NA	FA	0.0001
Guangwei Du et al. (2011)	Combined R2* and diffusion tensor imaging changes in the substantia nigra in Parkinson disease	16	16	(UPDRS-III)	clin diagosed	59.2 (6.9)	n	High resolution MRI (T2-weighted, T2*, and DTI)	SN	3.0T	In this study, the combination of R2* and FA measures improved the overall discrimination between PD and Controls	NA	NA	NA	NA	NA	NA	FA R2*	0,05 0,001
Menke, R. A., et al. (2009)	MRI characteristics of the substantia nigra in Parkinson's disease: A combined quantitative T1 and DTI study	10	10	1; 2; 3; (2.7)	clin diagosed	63.7 (6.7)	yes	Combination of DESPOT and DT imaging	SN	3.0T		100	80					l. SN r. SN	0,02 0,01
		244	**242**																

Attachment – 3 (All data collected from Spectroscopy studies)

Author (Year)	Title	PD	HC	Hoehn & Yahr Stage	Diag. Criteria	Mean Age	Medication	Modality	Studied Region	M.F. Tesla	Conclusions	Sem	Spec	PPV	NPV	LR	ROC	+info	t-test p-value
		244	242																
Spectroscopy																			
B. Zhou, et al. (2013)	Application of proton magnetic resonance spectroscopy on substantia nigra metabolites in Parkinson's disease	30	20	1; 2	clin diagosed	58.9 (10)		spectroscopy (1H- MRS).of 3.0 T technology, improvements in the signal-to-noise ratio and resolution	SN	3.0 T	Significant differences in NAA/Cr, NAA/Cho, NAA/ (Cho + Cr) were observed between PD patients and healthy controls (P<0.05) as well as between the ipsilateral and contralateral. It can be used to detect substantia nigrametabolites in PD patients, which may be useful for early diagnosis and evaluation of PD	NA	NA	NA	NA	NA	NA		
D. Axelson, et al. (2002)	Applications of Neural Network Analyses to In Vivo 1H Magnetic Resonance Spectroscopy of Parkinson Disease Patients	31	14	NA	diagnostic criteria of Gelb	63.7	yes	spectroscopy (1H- MRS).		1.5T	In conclusion, ANNs could successfully be trained to distinguish control spectra from spectra recorded from PD patients. Furthermore, networks could be trained to distinguish the four groups of probable PD, possible PD, atypical PD, and controls.	0.97	0.93	NA	NA	NA	NA		
R. M. Camicioli, et al. (2007)	Magnetic Resonance Spectroscopic Evidence for Presupplementary Motor Area Neuronal Dysfunction in Parkinson's Disease	44	38	UPDRS)17 Hoehn & Yahr mean 2.25	UKBB	71.2 (4.4)	yes	Spectroscopy with voxel 2x2x2cm at Anterior Cingulum(AC) Posterior Cingulum(CP) e pré MAS		1.5T	AC and PC NAA/Cr and Cho/Cr in any region did not differ (P 0.05). In conclusion, pre-SMA NAA/Cr was selectively decreased in PD, consistent with neuronal (P= 0.045) disfunction. This should be further examined as a biomarker of disease in PD.	NA	NA	NA	NA	NA	NA		
B. C. Bowen, et al. (1995)	Proton MR Spectroscopy of the Brain in 14 Patients with Parkinson Disease	14	13	NA	clinical diagnosis	64 (15)	yes	single-voxel (27-cm3 volume) proton MR spectroscopy of the occipital lobe		1,5T	Increase in cerebral lactate in patients with Parkinson disease support the hypothesis that Parkinson disease is a stemic disorder characterized by an impairment of oxidative energy metabolism	NA	NA	NA	NA	NA	NA		
E. Hattingen, et al. (2009)	Phosphorus and proton magnetic resonance spectroscopy demonstrates mitochondrial dysfunction in early and advanced Parkinson's disease	29	19	1;2 and 3; 4	UKBB	62.4 (9.2) 66.7 (7.6)	yes	combined 1H/31P MR spectroscopy	MSR	3T	Combined 1H/31P MR spectroscopy revealed a significant decrease of high energy phosphates in the mesostriatal region (MSR)of patients with Parkinson's disease in vivo. The data strongly support the hypothesis that mitochondrial dysfunction is involved early in the pathogenesis of Parkinson's disease and a major constituent of the pathogenetic cascade.	NA	NA	NA	NA	NA	NA		
Gulin Oz, et al. (2006)	Proton MRS of the Unilateral Substantia Nigra in the Human Brain at 4 Tesla: Detection of High GABA Concentrations	10	11	UPDRS (27)	clinical diagnosis	59 (10)	No (>12h)	single-voxel spectroscopy in small volumes	SN	4T	Single-voxel spectroscopy of the unilateral human SN is feasible in both healthy volunteers and patients with PD. It provides an in vivo neurochemical profile that includes neurotransmitter (Glu and GABA) and antioxidant (GSH) levels that are in excellent agreement with neurochemistry literature.	NA	NA	NA	NA	NA	NA		
Barbara A Holshouser, et.al. (1995)	Localized Proton NMR Spectroscopy in the Striatum of Patients with Idiopatic Parkinson's Disease: A Multicenter Pilot	151	97	1 to 4	UKBB	27 to 83	yes	single-voxel spectroscopy	Striatum	1.5T	A Trend in NAA/Cho redution, being statistically significant in a subgroup of older patients might be indicative of neuronal cell death in the striatum in the progression of PD	NA	NA	NA	NA	NA	NA		0,03
Groger, A., et al. (2011)	Three-dimensional magnetic resonance spectroscopic imaging in the substantia nigra of healthy controls and patients with Parkinson's	17	8	2.5 to 3	clinical diagnosis	69	yes	3D-MRSI	SN	3T	These findings from this study indicate that regional variations of the SN may diferentiate patients with Parkinson´s disease and healthy controls.								
	Total	326	220																

Attachment – 4 (All data collected from fMRI and RS-fMRI studies)

Author (Year)	Title	PD	HC	Hoehn & Yahr Stage	Diag. Criteria	Mean Age	Medication	Modality	Studied Region	M.F. Tesla	Conclusions	Sem	Spec	PPV	NPV	LR	ROC	+info	t-test p-value
		244	242																
fMRI																			
D. Vaillancourt, et al. (2010)	Blood Oxygenation Level Dependent Activation in Basal Ganglia Nuclei Relates to Specific Symptoms in De Novo Parkinson's Disease	20	20	UPDRS	UKBB	57.9	No	blood oxygenation level dependent (BOLD) activation in the ROI	BG	3.0T	1-Compared with control subjects, patients with PD had reduced PSC in all contralateral and ipsilateral nuclei of the BG. 2- Patients with PD also had reduced PSC in the medial and lateral thalamus, M1, and SMA	NA	NA	NA	NA	NA	NA	Bold	<0,05
Tao Wu, et al. (2005)	A functional MRI study of automatic movements in patients with Parkinson's disease	12	14	1; 2	Clinical Diagnostic	61.2	Yes (12h)	functional magnetic resonance imaging (fMRI)	BG Cortex	1.5 T	Patients had greater activity in the cerebellum, premotor area, parietal cortex, precuneus and prefrontal cortex compared with normal subjects while performing automatic movements	NA	NA	NA	NA	NA	NA	Bold	0,001
Simon J.G. Lewis , Roger A. Barker	A pathophysiological model of freezing of gait in Parkinson's disease	0	0		clinic				BG Cortex			NA							
Hu Liu , et al. (2013)	Altered resting-state functional connectivity of the dentate nucleus in Parkinson's disease	18	18	H&Y (1.4) and UPDRS (16)	Clinical Diagnostic	61.4 (8.5)	?	The RS-fMRI scanning with (BOLD)contrast		3.0T	Found a decreased connectivity between the cerebellum and the inferior parietal lobule in the PD patients sample. The inferior parietal lobule (BA40) is part of the executive network, which is required for the selection	NA	NA	NA	NA	NA	NA	Bold	0,05
Tao Wu, et al. (2012)	Basal ganglia circuits changes in Parkinson's disease patients	16	16	H&Y (1.4) and UPDRS (21.5)	Clinical Diagnostic	58.1 (4.8)	No	fMRIs were performed: Blood-oxygen-level dependent (BOLD) data were acquired with gradient-echo echoplanar sequences.	ROI	3.0T	Demonstrate that the combination of fMRI and network analysis is likely a useful tool to establish the BG pathways model in vivo in human subjects. The causal connectivity from the substantia nigra to many brain networks suggests that the dopaminergic system exerts influences on extensive brain functions, both motor and cognitive behaviors.	NA	NA	NA	NA	NA	NA	NA	NA
D. Vaillancourt, et al. (2013)	Brain activation differences in tremor and non-tremor dominant Parkinson's disease (Author Manuscript)	20	20	1; 2	UKBB	NTD= 53.7 (8.7) TD 0 57.3(8.9)	No	Blood oxygenation level dependent activation and percent signal change.	ROI	3.0T	Robust findings across both voxel-wise and ROI analyses showed that compared to TD patients, NTD patients had reduced activation in ipsilateral dorsolateral prefrontal cortex globus pallidus of NTD PD patients compared to both TD PD patients and controls	NA	NA	NA	NA	NA	NA	NA	NA
Il-H. Choea,et al. (2013)	Decreased and increased cerebral regional homogeneity in early Parkinson's disease	22	25	H&Y (1.6) and UPDRS (10.4)	UKBB	58.3 (2.4)	Y and N	Regional homogeneity (ReHo) data which records the blood oxygen level-dependent (BOLD)	ROI	3.00T	ReHo was increased in the left inferior parietal lobule, the angular gyrus, the supramarginal gyrus, the middle occipital gyrus and the parahippocampal gyrus.	NA	NA	NA	NA	NA	NA	NA	0,01
Tao Wu, et al. (2009)	Changes of functional connectivity of the motor network in the resting state in Parkinson's disease	22	22	H&Y (off medication) 1.7±0.5	Clinical Diagnostic	59.5 (10.1)	Y and N	fMRI (BOLD)	ROI	1.5T	Functional connectivity of the motor network in the resting state is disrupted in PD. This change is secondary to dopamine deficiency, and related to the severity of the disease	NA	NA	NA	NA	NA	NA	NA	0,03
M. A. F.-Seara, et al. (2012)	Cortical hypoperfusion in Parkinson's disease assessed using arterial spin labeled perfusion MRI	25	25	1; 2	UKBB	63.2 (6.6)	Y and N	Arterial spin labeled (ASL) perfusionMRI The ASL technique utilizes electromagnetically labeled arterial blood water as an endogenous tracer, yielding quantitative CBF	whole brain VWC and ROI	3.0T	Assessment of absolute cerebral perfusion in PD using the ASL technique yielded a pattern of cortical perfusion deficit, affecting frontal, parietal and occipital areas. Our results largely confirm prior studies of perfusion and metabolism measuring absolute CBF ou CMRglc	NA	NA	NA	NA	NA	NA	NA	NA
C. Tessa , et al. (2010)	Decreased and increased cortical activation coexist in de novo Parkinson's disease	20	11	1; 2 (1.6)	UKBB	71 (3.1)	Y and N	high resolution contiguous 3D T1 weighted images, with a MPRAGE sequence . ,(FLAIR) sequence _fMRI and T2*-weighted echo-planar imaging (EPI) sequence	WB voxel-wise comparison and ROI	1.5T	Lower activation in the left (SM1) cortex and cerebellum and higher activation in the left temporal– parietal cortex adjacent to the SM1 and in right SM1.PD patients the disease severity correlated significantly with activation of left SM1 and supplementary motor area and cingulum, bilaterally.	NA	NA	NA	NA	NA	NA	NA	NA
D. E. Vaillancourt, et al. (2007)	Role of hyperactive cerebellum and motor cortex in Parkinson's disease	8	8	UPDRS (31,3)	Clinical Diagnostic	59.4 (8.4)	Yes	Using a BOLD contrast fMRI paradigm PD patients and healthy controls	WB VWC and ROI	3.0T	Evidence supporting the hypothesis that hyperactivation in the ipsilateral cerebellum is a compensatory mechanism for the defective basal ganglia.	NA	NA	NA	NA	NA	NA	NA	NA
S. Baudrexel, et al. (2011)	Resting state fMRI reveals increased subthalamic nucleus–motor cortex connectivity in Parkinson's disease	31	44	1; 2	UKBB	59.4 (10.7)	N (12)	High resolution quantitative T1 maps were calculated from two RF-spoiled 3D gradient echo data sets acquired with different flip angles as described recently	WB VWC and ROI	3.0T	Increased resting state FC between the STN and cortical motor areas. PD rigor and tremor symptoms might be linked to an abnormal coupling of these areas	NA	NA	NA	NA	NA	NA	NA	NA
A. Tessitore , et.al (2012)	Resting-state brain connectivity in patients with Parkinson's disease and freezing of gait	29	15	1; 2	UKBB	FOG+ 66.94 (6.0) FOG- 66.31 (6.3)	Yes	Resting-state functional magnetic resonance imaging	WB voxel-wise comparison and ROI	3.0T	Patients with freezing of gait exhibit significantly reduced functional connectivity within both "executive-attention" (in the right middle frontal gyrus and in the angular gyrus) and visual networks (in the right occipito-temporal gyrus) [p < 0.05 corrected for multiple comparisons].	NA	NA	NA	NA	NA	NA	NA	NA
T. R. Melzer, et al. (2011)	Arterial spin labelling reveals an abnormal cerebral perfusion pattern in Parkinson's disease	61	29	UPDRS part III	UKBB	64.7 (8.6); MCI 69.8 (9.1);D 75.1 (6.7)	N and Y	ASL a MRI perfusion method	ROI	3.0T	This network was characterized by decreased cortical and preserved subcortical and sensorimotor cortical perfusion	NA	NA	NA	NA	NA	0.66		
	Total	304	267																