Association between Adora2A rs2298383 polymorphism and Parkinson's disease in a Greek population

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ABSTRACT: Parkinson's Disease (PD) is a serious neurodegenerative syndrome that affects mostly the movement control in PD patients. L-dopa is the main drug that is given as a treatment in PD patients, but its chronic use is linked with the occurrence of additional motor problems, such as motor fluctuations and dyskinesia. Recent data support that Adora2A rs2298393 polymorphism may be a potential risk factor not only for PD, but for Levodopa-Induced Dyskinesia (LID), as well. In this study, we examined 127 Greek patients with PD and 102 healthy controls with Polymerase Chain Reaction-Restriction Fragment Length Polymorphism (PCR-RFLP) method, in order to evaluate the association between Adora2A rs2298383 polymorphism and PD in a Greek population. In addition, we explored the association between this particular polymorphism and LID. Our results do not seem to find significant correlation between rs2298393 polymorphism of Adora2A gene with PD or LID.

KEYWORDS: *Parkinson's Disease, Adora2A gene, Levodopa-Induced Dyskinesia, rs2298383 polymorphism*

INTRODUCTION

Parkinson Disease (PD) is the second most common neurodegenerative disease, after Alzheimer's Disease named by James Parkinson, who was the first one who described it with medical terms in his report "An Essay on the Shaking Palsy".[1] Although the disease was first described in medical terminology in 1817 by Parkinson, a description of the disease already existed in literature from ancient times. Specifically in the Old Testament there are at least two descriptions of "tremor" which is the characteristic symptom of Parkinson's disease. James Parkinson himself confessed that he relied on other previous studies of other scientists that described tremor as a symptom of the disease. Among these scientists that James Parkinson acknowledged, is one of the greatest Greek doctors of ancient times, Galen (129-126 AD) who first noticed and described various types of terror that can appear as symptoms of the disease.[2]

The main pathologoanatomical characteristic in PD is the loss of dopaminergic neurons in substantia nigra that result in discoloration of the solid portion in substantia nigra. This is normal with aging but in Parkinson's disease it is greatly accelerated.[3] The physiological function of these neurons, which contain melanin, is the production of dopamine, a main neurotransmitter, which is necessary for the communication between nerve cells.[3,4] The degeneration of these neurons greatly reduces the levels of dopamine in the brain and this decrease affects mostly the control of the movement in PD patients, who face a number of motor symptoms[5] like dyskinesia, resting tremor, rigidity and instability. Besides kinetic problems, there is also a number of non motor symptoms that PD pa-

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tients may experience. These include bowel dysfunction, hyposmia, depression and apathy, sleep disorders and impaired mental functioning which can even lead to dementia.[5] The disease progression is gradual and may take up to twenty years.[6] For the evaluation of the progress but also the severity of the disease, scientists use various rating scales. Most frequently used are Hoehn & Yahr scale and UPDRS(Unified Parkinson's disease Rating Scale).[5]

Unfortunately, up to now there is no treatment that can stop or even slow down the degeneration of the brain neurons and as a result the disease evolves slowly and gradually exhibits its symptomatology, which is more pronounced as the neurodegeneration progresses.[1,7] The primary pathological feature, as mentioned above, is the lack of dopamine, however its exogenous administration is not effective due to its low bioavailability since it cannot penetrate the blood-brain barrier.[8] Today there are several classes of drugs that are administered to patients with Parkinson's disease, either in combination or as monotherapy. When medication is not effective then neurosurgery is judged necessary.[9]

The main treatment that is given to PD patients to alleviate mostly their motor symptoms is L-dopa, a dopamine precursor. Normally, L-dopa is detected in increased concentration in the tissues of the plant Mucuna pruriens, which were used in ancient times from the Indians for treating a neural disease, which displays motor problems similar to those of Parkinson's disease.[2] The first time that it was used to treat Parkinson's disease was by Hornykiewicz in 1960, but the results were not very encouraging. 7 years later, the Greek, Georgios Kotzias, was the one who presented the important results of the administration of synthetic L-dopa to patients with Parkinson's disease, helping to establish L-dopa as a medication in Parkinsonian patients.[10]

In the first years of its administration, L-dopa dramatically improves the day-to-day life of patients by virtually eliminating the mobility problems they present, but unfortunately its chronic use is connected with additional motor symptoms like dyskinesia.[11]

For more than two decades and despite the developments and research in the field of Neuroscience, the causes that lead to the development of Parkinson's disease remain unknown. It is argued that degeneration of substantia neurons, which is the main pathological feature in Parkinson's disease, is due to genetic as well as environmental factors. The environmental factors that affect the onset of the disease include rural life, bacterial and viral infections, drinking water and pesticides while coffee and smoking seem to act as protective factors against neurodegeneration caused by the disease. [12] As regards to genetic factors, a set of genes have been associated with the onset of the disease while several other genes are under investigation (Olzweska McCarthy et al). Although only a 10-20% of PD cases have a family history, it is generally accepted that PD has a genetic etiology.[13] Recent studies found that Adenosine A2A receptor (Adora2A) is highly expressed in the putamen-one of the basal ganglia of the brain- of PD patients, as well as in peripheral blood cells.[14] Furthermore, other studies show that Adenosine A2A receptors are overexpressed in the stratium of PD patients who exhibited Levodopa Induced Dyskinesia (LID).[15] That reveals a possible connection between the gene that encodes Adora2A and PD, as well as LID. The Adora2A gene encodes the A2A adenosine receptor and is located in the long arm of the 22th chromosome (22q11.23). Adora2A gene includes a number of single nucleotide polymorphisms. Among them rs2298383 polymorphism (located in the intron 1) attracts the interest, because of its recent association with LID.[11]

The purpose of this study was to examine the role of rs2298383 polymorphism of Adora2A gene in PD, as well as the association between this particular polymorphism and LID, in a Greek cohort.

METHODOLOGY

Our study group consisted of 127 individuals diagnosed for sporadic PD [average age 63.39 ± 0.910 years (range 38 – 83 years); average age at disease onset: 51.53 ± 0.950 years (range: 30 – 72 years)] according to UK Parkinson's Disease Society Brain Bank Clinical Diagnostic Criteria (Hughes et al), in Papanikolaou Hospital, Thessaloniki, Greece and 102 control individuals [average age: 74.42 ± 0.858 years (range: 61 – 94 years)]. Of the 127 patients with Parkinson's disease studied, the 94 patients received medication as L-dopa with average dosage the 629.39 ± 67.2 mg. 32 of them were free of dyskinesias, 24 showed dyskinesia in 1-3 years after the initiation of medication with L-dopa while 38 showed dyskinesia after 3 years from the start of medication with L-dopa. In our associations, we count as positive, patients who experienced dyskinesia in 1-3 years after the initiation of medication with L-dopa. As negative we count patients that did not experience dyskinesia at all and those who experienced dyskinesia after 3 years from the start of medication with L-dopa. The presence of dyskinesia in PD patients was evaluated with Unified Dyskinesia Rating Scale. All the individuals of our control group were neurologically examined and were found healthy and had also a negative family history of neurological

diseases. All PD and control individuals were ethnic Greek residents of northern Greece, and gave informed consent for this study.

Genomic DNA was extracted in all subjects from peripheral blood leukocytes according to standard procedures. The rs2298383 genotypes were determined using the PCR-RFLP method. Amplification of the relative region encompassing this variant was accomplished using the following set of primers: forward primer: 5'-CTG ACC GCG TGG AAT CCT AT-3', reverse primer: 5'-CCT CAC CAA GAG CAC TGG AA-3'

PCR products were digested overnight, at 37 ^{o}C with the BsoBI restriction enzyme (*New England Biolabs*). Mutated homozygotes T/T were represented by an undigested PCR 390 bp product. Mutated heterozygotes C/T were represented by the fragments 390+ 255 + 135 bp and the wild-type homozygotes C/C were represented by the fragments 255+ 135 bp. All fragments were were resolved on 2% agarose gel, stained in ethidium bromide solution and visualized with an ultraviolet light. Genotype and allele frequencies were estimated by direct counting and were compared between patients and controls by means of $\chi 2$ analysis. $P < 0.05$ was considered significant.

RESULTS

The genotype and allele distributions for the studied rs2298383 polymorphism of Adora2A gene are shown in tables 1 and 2. Statistically significant differences were not found, either in genotype or allele frequencies regarding the Adora2A rs2298393 polymorphism in our total PD Greek cohort. When examining the subgroup of PD patients with LID compared to PD patients without LID, no statistically significant differences were not found, either in genotype or in allele frequencies, as well.

DISCUSSION

Parkinson's disease is a chronic disease, characterized by degeneration of the brain's nigral neurons. These neurons have as a normal function the production of a neurotransmitter, dopamine, which is necessary for the transmission of messages between nerve cells. Neurodegeneration therefore, leads to the appearance of mainly motor problems in patients, which intensify as the disease progresses.

Although the disease is inherited as a simple Mendelian character in only 10-20% of all Parkinson's disease cases, a set of genes have been associated with the onset of the disease while several other genes are under investigation.[16,17,18] The main drug that is given to Parkinson's disease patients is L-dopa, a dopamine precursor that is primarily used to treat mostly the kinetic symptoms of the disease. However, chronic use of L-dopa from Parkinson's patients results in additional motor problems, namely dyskinesia. This makes it even more difficult to treat the motor problems of the disease. An attempt to understand the pathogenetic mechanisms that cause both the disease and the dyskinesias that occur due to L-dopa, have turned the interest of the researchers into the genes involved in neurotransmission.[19,20] Results of recent investigations conducted by Casetta, Vincenzi *et al.* show that A2A receptors of adenosine are elevated in the shell, one of the basal ganglia of the brain, of patients with Parkinson's disease, indicating that there may be correlation between the gene coding for these receptors (Adora2A) and Parkinson's disease.[14] Additional studies show elevated levels of adenosine A2A receptors in the striatum of the brain of parkinsonian patients who have developed dyskinesia, reinforcing the previous view and therefore suggesting that there might be some correlation between the gene coding for these receptors (Adora2A) and the disease.[15] Apart from the studies that associate adenosine A2A receptors with the disease, there are also several studies, such as Cie lak, Komoszy ski *et al*., claiming that A2A receptor antagonists of adenosine have neuroprotective activity.[21] Tsuboi indirectly supports the same view, concluding that caffeine protects against neurodegeneration by blocking A2A receptors of adenosine thus demonstrating their important role in neuroprotection.[22] Cie lak, Komoszy ski *et al.* suggest that adenosine A2A receptor antagonists when given in combination with L-dopa may have a very significant improvement in motor symptoms and also may achieve neuroprotection that could delay or even stop the progression of the disease.[23] Thus, it is understood that A2A receptors of adenosine could be new therapeutic targets for Parkinson's disease. Morelli, Blantini *et al.* underline that, while many studies have shown that adenosine A2A receptor antagonists have positive effects on L-dopa dyskinesia, there are not enough clinical tests to prove these findings.[24] All of this data suggest that the Adora2A gene should be studied extensively in terms of its potential to be a marker of predisposition for both Parkinson's disease and dyskinesias caused by L-dopa.

The Adora2A gene encodes the A2A receptor of adenosine, a receptor that binds to G proteins and is highly expressed in the striatum of the brain. There, it competes with D2 dopaminergic receptors indirectly, regulating the transmission of messages through the neurons. It includes several polymorphisms of which rs2298383 is of particular interest because of its positive

correlation with the occurrence of L-dopa induced dyskinesia in Parkinson's disease patients, as studied by Rieck, Schumacher-Shuh *et al.* in 2015. Since 2012 there was already a study by Greenbaum, Cohen *et al.* which revealed the association of rs2298383 polymorphism of the Adora2A gene with L-dopa induced dyskinesia.[11] Generally, this polymorphism has been associated with the appearance of many diseases. In particular, Childs, Hohoff *et. al* in their study claim that rs2298383 polymorphism of the Adora2A gene causes increased anxiety due to caffeine consumption (caffeine acts competitively against A2 adenosine receptors)[25] while Freitag, Agelopoulos *et al*., after studying this polymorphism in relation to autism, found that there was a correlation between them.[26] Additionally, this polymorphism was associated with anxiety disorder[27] and schizophrenia.[28] One study associated Parkinson's disease with two polymorphisms of the Adora2A gene, namely rs2298383 and rs2298383.[29] The data thus, suggest that there may be a correlation between the Adora2A gene and Parkinson's disease or even between the rs2298383 gene polymorphism with the occurrence of dyskinesia caused by L-dopa medication in patients with Parkinson's disease.

The present study aims to investigate the correlation between the rs2298383 polymorphism of the Adora2A gene with Parkinson's disease and the occurrence of dyskinesia caused by L-dopa medication in Greek patients with Parkinson's disease. The results do not reveal a statistically significant correlation of this polymorphism with either Parkinson's disease or the occurrence of dyskinesia due to treatment with L-dopa. It should be noted that there were some limitations regarding the number of patients with Parkinson's disease studied. In particular, of the 127 patients with Parkinson's disease, only 24 had dyskinesia, so only in these patients there was a possibility of studying the distribution of alleles and genotypes of polymorphism. It is therefore necessary to conduct further studies in the Greek population, including a larger number of patients and other polymorphisms of the Adora2A gene.

CONCLUSION

Adora2A has been acknowledged recently as a possible candidate gene not only for Parkinson's disease predisposition, but also for the prediction of specific adverse effects of L-dopa medication, such as dyskinesia. In our PD Greek cohort, no statistically significant differences were found, either in genotype or allele frequencies regarding the Adora2A rs2298383 polymorphism. Moreover when examining the subgroup of PD patients with LID compared to PD patients without LID, no statistically significant differences were found. However, additional studies including a larger number of patients, and examining different polymorphisms of Adora2A gene, will delineate the role of Adora2A gene in the pathogenesis of PD, and of Levodopa Induced Dyskinesia.

CONFLICTS OF INTEREST: NO

Συσχέτιση μεταξύ του rs2298383 πολυμορφισμού του γονιδίου Adora2A και της νόσου του Parkinson σε ΄Ελληνες ασθενείς

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Περίληψη: Σκοπός της συγκεκριμένης μελέτης ήταν η αναζήτηση συσχέτισης μεταξύ του rs2298383 πολυμορφισμού του γονιδίου Adora2A με την νόσο του Parkinson, καθώς και με την εμφάνιση δυσκινησίας που προκαλείται από την φαρμακευτική αγωγή με L-dopa σε ΄Ελληνες ασθενείς με νόσο του Parkinson. Στην έρευνα πήραν μέρος συνολικά 127 ΄Ελληνες ασθενείς με την νόσο του Parkinson, που αποτέλεσαν την ομάδα μελέτης και 102 υγιείς, που αποτέλεσαν την ομάδα ελέγχου και οι οποίοι ελέγχθηκαν ως προς την ύπαρξη του πολυμορφισμού. Από τους 127 ασθενείς με Parkinson που μελετήθηκαν οι 94 ασθενείς έλαβαν ως φαρμακευτική αγωγή L-dopa και από αυτούς οι 24 εμφάνισαν δυσκινησία ως παρενέργεια της φαρμακευτικής αγωγής με L-dopa. Τα αποτελέσματά μας δεν φανερώνουν σημαντική συσχέτιση του rs2298383 πολυμορφισμού του γονιδίου Adora2A με την νόσο του Parkinson, αλλά ούτε και με την εμφάνιση δυσκινησίας που προκαλείται από την φαρμακευτική αγωγή με λεβοντόπα σε ΄Ελληνες ασθενείς με νόσο του Parkinson. Αξίζει να σημειωθεί ότι είναι αναγκαίο να διεξαχθούν ανάλογες έρευνες στον ελληνικό πληθυσμό, όπου οι ομάδες μελέτης θα περιλαμβάνουν μεγαλύτερο αριθμό ασθενών. Επιπλέον μελέτες και σε άλλους πολυμορφισμούς του γονιδίου Adora2A θα συμβάλλουν σημαντικά τόσο στην κατανόηση της παθογένεσης της νόσου του Parkinson αλλά και των φαρμακοεπαγώμενων δυσκινησιών.

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Table 1. Genotypes and Alleles' Frequencies of the rs2298383 polymorphism of Adora2A gene in PD patients and in the controls in a Greek population.

	Genotypes			**Alleles**	
	T/T	**C/T**	**C/C**	**T**	**C**
PD patients (n=127)	37.8%	47.2%	15%	61.4%	38.6%
Controls (n=102)	34.4%	52.9%	12.7%	60.8%	39.2%
X^2		P=0.659		P=0.807	

Table 2. Genotypes and Alleles' Frequencies of the rs2298383 polymorphism of Adora2A gene in PD patients with LID and in PD patients without LID.

	Genotypes			**Alleles**	
	T/T	**C/T**	**C/C**	**T**	**C**
PD patients with LID	42%	42%	16%	62.5%	37.5%
PD patients without LID	38.5%	44.4%	17.1%	61%	39%
X^2		P=0.964		P=0.827	