**Short Research Article**

Title page

Genetic analysis of Leucin-rich repeat kinase 2 (LRRK2) G2019S mutation in a sample of Egyptian patients with Parkinson's disease. A pilot study

Competing Interests: Authors declare that no competing interests exist.

AUTHORS' CONTRIBUTIONS
Authors A: suggested the idea of the study, shared in the study design, participated in the practical genomic part and wrote the paper.
Author B: managed the clinical part of the study, interpreted the clinical data
Author C: managed DNA extraction from patients and interpretation of the results
Author D: participated in the practical genomic part, interpretation of the results
All authors read and approved the final manuscript.

CONSENT (WHERE EVER APPLICABLE)
We here certify that we have got a patient consent (it is in Arabic language format) for every patient before start of the study on each patient. This consent is a must according to the rules in research followed in the Faculty of Medicine Assiut University which never give approval to start the study before seeing this consent.

ETHICAL APPROVAL
The study has been approved first by pharmacology department council followed by approval of the Ethical Committee of Faculty of Medicine, Assiut University.
Abstract

Aim: Many causative genes and susceptibility loci have been identified to be associated with Parkinson's disease (PD) in different ethnic populations. One of these genes is the Leucin-rich repeat kinase 2 (LRRK2) gene. The most common mutation identified in the LRRK2 protein that leads to an amino acid substitution and cosegregates with PD is the G2019S substitution. We investigated the presence and frequency of that mutation in 69 patients with PD compared with 96 controls and all subjects are Egyptians and inhabitants in Upper Egypt.

Place and Duration of Study: Departments of pharmacology, neurology, and clinical pathology, Assiut University (Egypt) and Department of Neurodegenerative Diseases, Hertie-Institute for Clinical Brain Research, University of Tübingen, Tübingen, Germany between June 2010 and September 2011.

Methodology: Sixty nine patients with PD of sporadic type and ninety six controls were included in the study. All subjects were assessed by direct sequencing of their DNA for the point mutation G2019S in the exon 41 of LRRK2.

Results

Genotyping analysis and sequencing of DNA showed heterozygous mutation of LRRK2 G2019S gene in only one of the patients with PD (1/69; incidence: 1.45%). All other patients and control subjects were negative for that mutation. The heterozygous patient was female, 56 years old, married, with the onset of the disease at her age of 54.5 years and her disease condition was mild and presented as resting tremors, depressed mood and slight salivation.

Conclusion: LRRK2 G2019S mutation of the heterozygous genotype seems to be of very low incidence in Egyptians who are inhabitants in Upper Egypt. We suggest testing of this mutation on a larger number of subjects with Parkinson’s disease who are inhabitants of this geographical
area of Egypt to reach a real incidence and possible influence of this mutation on the clinical course of the disease.

**Keywords**: Leucin-rich repeat kinase 2 G2019S; mutation; Parkinson's; Egyptians

**Introduction:**

Parkinson’s disease (PD) is the second most common neurodegenerative disorder after Alzheimer disease [1] and many causative genes and susceptibility loci have been linked to both familial and sporadic forms of that disease [2]. One of the effective genes identified in relation to PD is the leucin-rich repeat kinase 2 gene (LRRK2) which is a large, multi-domain GTPase/kinase protein that harbors most of the mutations linked to PD [3,4]. One of these significant mutations is G2019S which is associated with neuronal impairment and loss of dopaminergic neurons [5]. A previous Egyptian study revealed an incidence of 9.7% of heterozygous mutation in LRRK2 G2019S among the Egyptians with sporadic PD who were the inhabitants in Alexandria Governorate and nearby region in the North part of Egypt.

In the present study, we have investigated LRRK2 gene for the presence or not of G2019S mutation in a group of Egyptian patients with PD who are inhabitants of Assiut Governorate and nearby regions in Upper Egypt.

**Methods**

**Patients & control subjects**

Sixty nine patients with sporadic PD (16 women and 53 men) with mean age ± SE of 60.7± 2.3 years and the onset of the disease between 6 months and 12 years and 96 control subjects (30 women and 66 men) with mean age ± SE of 61.5 ± 1.28 years were included in the study. All
were inhabitants in Assiut Governorate and surroundings in Upper Egypt. In the outpatient clinic of the neurology department in Assiut University hospital, the patients were diagnosed based on the presence of at least one or more of the published criteria for Parkinson’s disease: resting tremors, rigidity, bradykinesia and/or postural instability [6] and severity of the disease was rated according to the Unified Parkinson’s Disease Rating Scale (UPDRS) and Hoehn and Yahr staging [7]. The patients had a negative history of previous head trauma, brain tumor or medication with dopamine depleting agents within the last year before selecting. The control subjects were free of any neurological disorder, and reside in the same ethnic background as the patients with PD. The clinical criteria of the patients are demonstrated in table 1.

**Genetic screening**

Genomic DNA was isolated from the peripheral blood for each subject using QIA amp DNA Blood Mini kits. Polymerase chain reaction (PCR) was carried out to amplify exon 41 of LRRK2 from genomic DNA. The total volume of the genomic mix was 16 µl including 0.4 µl of each primer (10 pm/µl), 0.4 µl of each dNTP, 2.4 µl of MgCl₂, 0.1 µl of Taq polymerase, 4.0 µl of buffer plus 8.3 µl H₂O. 4 µl of genomic DNA (10 ng/µl) or H₂O (as a negative control) were added to the mix and annealing temperature was at 60°C. The forward and reverse primers used to amplify the LRRK2 exon 41 were as follows: GCACAGAATTTTTGATGCTTG/

GAGGTCAGTGGTTATCCATCC) as previously reported [8]. PCR-amplified DNA fragments were analyzed on 2% agarose gel and visualized by ethidium bromide staining. The PCR products were then sequenced in forward and reverse direction and the computer program TREV [9] was used to evaluate the resulting chromatogram for fluorescence peaks and calls the nucleotides in the order they passed through the viewer to determine any change in nucleotide
sequences compared to the normal LRRK2 gene sequence. The chromatogram of the sequencing analysis showed heterozygosis as two overlapping peaks of low height in comparison with other peaks and also with control chromatogram at the same location.

The Ethics Committee in Faculty of Medicine & University Hospital approved the study and written informed consent was obtained from each subject.

Results

Sequencing of the coding region in LRRK2 revealed only one patient who was carrying the G2019S mutation in the gene (incidence 1.45%) and it was of heterozygous style as defined by the two overlapping peaks of corresponding sequencing analysis chromatogram compared with the other peaks as well as the control chromatogram at the same location. All other subjects (controls and patients) did not show the same mutation. The demographic criteria of that heterozygous patient reflected a mild form of the disease. The patient was 56 years old, with age at onset of the disease of 54.5 years. She was complaining of resting tremors, rigidity, bradykinesia, postural instability, slight salivation and mood depression. No information was available for positive family history of the disease.

Discussion

The prevalence of LRRK2 G2019S mutation shows wide variability and is ethnic dependent. The worldwide frequency of LRRK2 G2019S was 1% of patients with sporadic PD and 4% of patients with hereditary PD. The highest frequency of LRRK2 G2019S was seen in north African Arabs (hereditary 36%, sporadic 39%) and Ashkenazi Jews (hereditary 28%, sporadic 10%). The frequency was higher in southern European countries than in northern European countries. LRRK2 G2019S was rarely seen in Asians [10-13].
In the present study, heterozygous mutation in LRRK2 G2019S gene was observed in only one of the patients but not in the other patients or the control group. This very low incidence of that mutation (1.45%) is different from the incidence reported before (9.7%) for the same mutation in a previous study performed on the Egyptians with PD though heterozygosity was a common factor in the two Egyptian studies [14]. The previous Egyptian study [14] was carried out on Egyptians who were inhabitants of Alexandria Governorate and its surroundings. This area lies in the North of Egypt and it was exposed throughout its existence to multiple genetic influences due to migration from neighboring ethnic groups especially Greeks, Arabs and Turks. Furthermore, strong trade connections had also led many Tunisian and Moroccan merchants to settle in Alexandria hundreds of years ago. [15]. High incidence of LRRK2 mutation in LRRK2G2019S was reported in Tunisians with PD [10,12,15]. In our study, all patients investigated were inhabitants in Upper Egypt where the circumstances of immigration and mixing with other populations of ethnic differences were not available. Other possible factors included the sample size of patients in each study, selection bias, study design, and techniques used in genetic analysis for determination of mutation (PCR-RFLP in the older study versus the fluorescence based sequencer in our study) [2,11,15].

The low prevalence of LRRK2 G2019S mutation in Egyptian Parkinson’s patients according to our study (1.45%) and the other Egyptian study (9.7%) in comparison with the high incidence among Tunisians (45%) though Egypt and Tunis are Arab countries located in the Middle East area is of interest. The ethnic difference, environmental factors and different life style between Tunisian and Egyptian populations may explain the prevalence difference [2].

Conclusion: LRRK2 G2019S mutation of heterozygous genotype has been observed in only one of 69 patients of Egyptian origin with Parkinson’s disease who are inhabitants in Upper Egypt.
This finding reflects the possibility of low incidence of that mutation among Egyptians living in this geographical area of the country. This pilot study encourages the extension of research on a larger number of subjects, inhabitants in this area of the country to reach a real representative incidence of that mutation and to correlate it with the clinical course of the disease. It is also recommended to check for other points of mutation in LRRK2 gene in relation to the disease in Egyptians with Parkinson’s disease.

References:


Table 1. Clinical Criteria of Egyptian patients (n=69) with idiopathic Parkinson’s disease.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Incidence</th>
<th>Score</th>
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<tbody>
<tr>
<td>Age</td>
<td>60.7 ± 2.3 years</td>
<td></td>
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<tr>
<td>Disease duration</td>
<td>(range: 6 months-12 years)</td>
<td></td>
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<tr>
<td>Clinical symptoms:</td>
<td></td>
<td></td>
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<tr>
<td>Tremors at rest</td>
<td>95%</td>
<td>1.8 ± 0.14</td>
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<tr>
<td>Bradykinesia</td>
<td>85%</td>
<td>1.5 ± 0.15</td>
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<tr>
<td>Rigidity</td>
<td>83%</td>
<td>1.1 ± 0.16</td>
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<tr>
<td>Postural instability</td>
<td>65%</td>
<td>1.08 ± 0.11</td>
</tr>
<tr>
<td>UPDRS overall score</td>
<td></td>
<td>22.39 ± 2.0</td>
</tr>
</tbody>
</table>

Values are means ± standard errors

UPDRS: Unified Parkinson’s disease Rating Scale