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: The G2019S substitution in Leucin-rich repeat kinase 2 (LRRK2) gene has been linked with occurrence of Parkinson's disease (PD). The current pilot study has been carried out to screen the presence and frequency of this kind of mutation in a group of patients with PD in comparison with controls. All subjects included in the study are of Egyptian origin and inhabitants in Upper Egypt.

Place and Duration of Study: Departments of Neurology, pharmacology 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 (53 men, 16 women; age range 52-76 years and onset of the disease 6 months-12 years) were selected for the study. Ninety six control healthy adult subjects were also included for genetic comparison. All participants were of Egyptian origin. Clinical assessment of the patients was performed to determine the severity of the disease. Genomic DNA was isolated and because of the large size of the LRRK2 gene, the point mutation G2019S was targeted for analysis in the exon 41, amplified and sequenced for that possible mutations.

Results

Genotyping analysis revealed only one patient who was carrier for the mutation (1/69; 1.45% incidence) and he was also heterozygous. All other PD patients and the controls were negative for the mutation. The heterozygous patient was female, 56 years old, married, with her age at onset of the disease of 54 years. Parkinson’s disease was presented as resting tremors, depressed mood slight salivation in that particular patient included
Conclusion: LRRK2 G2019S mutation of heterozygous genotype has been determined in only one of 69 patients of Egyptian origin with Parkinson’s disease who are living in Upper Egypt. This finding reflects the low incidence of that mutation among Egyptians living in this geographical area of the country. Multicenter studies are required on larger number of subjects to reach a real incidence of that mutation among Egyptians and to correlate the incidence to the clinical course of Parkinson’s 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 at present, many causative genes and susceptibility loci have been linked with both familial and sporadic forms of the 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 [3], known to harbor most of PD-linked mutations [4]. One of the significant mutations in LRRK2 is G2019S and its over-expression is associated with neuronal impairment and loss of dopaminergic neurons [5] [6]. A previous study was carried out on Egyptian patients with PD living who were inhabitants in Alexandria Governorate and nearby region in the North part of Egypt. The incidence of G2019S mutation in LRRK2 gene in that study was 9.7% and it was of heterozygous style.

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

Patients & control subjects

Sixty nine patients with sporadic PD who attended the outpatient clinic in Assiut University Hospital were selected to the study. Clinical diagnosis was based on published criteria (at least 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 should have negative history of previous head trauma, brain tumor or medication with dopamine depleting agents within the last year before selection. The control subjects (n=96) were all healthy, free of any neurological disorder, age, sex matched and reside in the same ethnic background as the patients with PD.

Genetic screening

Genomic DNA was isolated from the peripheral blood for each subject using QIAamp 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 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: GCACAGAATTGTGATGCTTG//GAGGTCAGGTGTATCATCC 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 normal LRRK2 gene sequence.

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

Results

The patients involved in the study were 16 women and 53 men with mean age ± SE of 60.7 ± 2.3 years with onset of the disease between 6 months and 12 years. The clinical criteria of the patients are demonstrated in table 1. The control subjects were 96 subjects (30 women and 66 men) with mean age ± SE of 61.5 ± 1.28 years.

Sequencing of the coding region in LRRK2 revealed only one patient who was carrying the G2019S mutation in the gene and it was of heterozygous style. The chromatogram of sequencing analysis showed heterozygosity as two overlapping peaks of low height in comparison with other peaks and also with control chromatogram at the same location (figure 1). The rest of all other subjects (controls and patients) did not have that mutation as observed in the chromatograms.

The demographic criteria of the heterozygous patient were as follows: female sex, 56 years old, married, with age at onset of the disease of 54.5 years. The clinical features of PD in that particular patient included: depressed mood, slight salivation, resting tremors. No information was available for positive family history of the disease.

Discussion

The prevalence of LRRK2 G2019S mutation shows a great variability and is ethnic dependent. While G2019S prevalence is very rare in Asia, South Africa and in some European countries
such as Poland and Greece, it accounts for 13% of sporadic and 30% of familial PD among Askenazi Jews and 41% of sporadic and 37% of familial PD in North African Arabs [10,11].

In the present Egyptian study, a low incidence of mutation G2019S (1.45%) in LRRK2 has been reported in only one of the patients with PD (1/69) while the remainders and the control subjects did not have this type of mutation. The mutation was also of heterozygous genetic style. A previous Egyptian study showed an incidence of 9.7% for the same mutation in 113 patients with PD but all mutations were also of heterozygous genetic style. [12]. The two studies confirm heterozygosity in G2019S as the genotype of mutation in LRRK2 among Egyptians but different incidence of that mutation between the two studies for Egyptians may be ascribed to many factors. In the older study [12], the Egyptian subjects investigated 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. [13].

High incidence of LRRK2 mutation in LRRK2G2019S was reported in Tunisians with PD [10,11,14] [7-9]. In our study, all patients were living in Upper Egypt where the circumstances for immigration and mixing with other populations of ethnic differences were not available. The methodology for genetic screening was also different between the two studies. In our study, the fluorescence based sequencer was employed for direct sequencing of PCR product to investigate the base sequence of each single strand of DNA and to confirm any present point of mutation at base level (SNP). In the older study [12], LRRK2 G2019S mutation was carried out by restriction fragment length polymorphism (RFLP) technique and the genotyping mutation was
confirmed by allelic discrimination using the 5’ nuclease assay. PCR-RFLP is allows very rapid, simple, and inexpensive detection of point mutations within the sequences of PCR products. The mutation is discriminated by the specific restriction endonuclease and is identified by gel electrophoresis followed by staining with ethidium bromide. However, failure to cut the genomic sample at the expected restriction sites may result in an identifiably larger than expected fragment implying that there is a possible mutation at the point of the restriction site. Unfortunately, the combined factors of the high complexity of most eukaryotic genomes, and the requirement for specific endonucleases, may let the exact mutation cannot necessarily be resolved in a single experiment, and the slow nature of gel assays make RFLP a poor choice for high throughput analysis [15]. Other possible factors that may explain the incidence difference between the two Egyptian studies are the sample size in each study), selection bias, study design, and statistical methods of calculation [2,16].

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 lifestyle differences between Tunisian and Egyptian populations [2] in addition to the above factors mentioned as regards variability in prevalence of that mutation in the Egyptian studies may explain the prevalence difference [2].

Conclusion: LRRK2 G2019S mutation of heterozygous genotype has been determined in only one of 69 patients of Egyptian origin with Parkinson’s disease who are living in Upper Egypt. This finding reflects the low incidence of that mutation among Egyptians living in this geographical area of the country. Multicenter studies are required on larger number of subjects to reach a real incidence of that mutation among Egyptians and to correlate the incidence to the
clinical course of Parkinson’s disease. It is also recommended to check for other points of mutation in LRRK2 gene in correlation with Parkinson’s disease among Egyptians.

References:


Table 1. Clinical Criteria of Egyptians patients (n=65) with idiopathic Parkinson’s disease.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Incidence %</th>
<th>Score ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>60.7 ± 2.3 years</td>
<td></td>
</tr>
<tr>
<td>Disease duration</td>
<td>(range: 6 months-12 years)</td>
<td></td>
</tr>
<tr>
<td>Clinical symptoms:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tremors at rest</td>
<td>95%</td>
<td>1.8 ± 0.14</td>
</tr>
<tr>
<td>Bradykinesia</td>
<td>85%</td>
<td>1.5 ± 0.15</td>
</tr>
<tr>
<td>Rigidity</td>
<td>83%</td>
<td>1.1 ± 0.16</td>
</tr>
<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
Legends of figures:

Figure 1. Chromatogram of LRRK2G2019S sequencing. A&B panels represent control & patients with no mutation. Panel C shows mutation of heterozygous style as defined by the two overlapping peaks which are of low height in comparison with peaks at the same location in A&B panels.
Control

Patients with no mutation

Patient with heterozygous mutation (the two overlapping peaks as defined by the arrow). A