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 which was reported to cosegregate with Parkinson's disease (PD) has been screened in Egyptian patients with PD. It is a starting step that may modulate drug therapy of Parkinson's disease among Egyptians based on the genetic findings of that mutation.

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 and onset of the disease 6 months-20 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 mutations G2019S & I2020T were targeted for analysis and the exon 41 was amplified and sequenced for the 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 is of a low incidence among the Egyptian Parkinson’s patients sample studied. However, further studies are required with larger number of subjects to reach a precise information about the penetrance of this mutation and its correlation to clinical practice in Egyptian patients with PD.

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], and these two domains are known to harbor most of PD-linked mutations (figure 1) [4]. Importantly, it has been shown that LRRK2 protein plays a significant role in regulating neural functions [5]. One of the significant mutations determined in LRRK2 is G2019S and when being over-expressed in LRRK2 is which displayed neurite impairment and loss of dopaminergic neurons with more severe loss of dopamine neurons [6]

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 [7,8].

There are some indications that the course of LRRK2 G2019S-associated parkinsonism is more benign with slower progression, less cognitive impairment compared with idiopathic PD [9].
Although the clinical response to dopamine-replacement regimens was not different between LRRK2 G2019S-associated PD and idiopathic PD, but patients with idiopathic PD needed treatment earlier than patients with G2019S mutation in LRRK2. Furthermore, the time of onset of drug-induced dyskinesia was longer in patients with mutations in LRRK2 than idiopathic PD [10]. The current study was planned to look for the presence or not of LRRK2 G2019S mutation in Egyptian patients with Parkinson’s disease. It is a starting step that may modulate drug therapy of Parkinson’s disease among Egyptians based on the genetic findings of that mutation.

Methods
Sixty nine patients with PD and 96 adult healthy controls were recruited for the study. The patients 16 women and 53 men with mean age ± SD of 60.7± 16 years with onset of the disease between 6 months and 20 years. Clinical assessment of the patients as followed in Assiut University Hospital was performed according to the Unified PD Rating Scale (UPDRS) to determine the severity of the disease [11]. All subjects included in the study (patients & controls) were of Egyptian origin. Genomic DNA was isolated from the peripheral blood for each subject using QIA amp DNA Blood Mini kits. The large size of the LRRK2 makes it impractical to provide comprehensive screening of the whole gene. Therefore, the point mutations G2019S & I2020T were targeted for analysis and the exon 41 of LRRK2 was amplified from genomic DNA using PCR and directly sequenced for the possible mutations.

Results
Sequencing of the coding region in LRRK2 revealed that one patient only was carrying the G2019S mutation in the gene and he was a heterozygous carrier. The rest of all other subjects
(controls and patients) did not have that mutation. Therefore, a very low incidence of the mutation (1.45% prevalence) was considered among the Egyptian patients studied. The demographic criteria of that 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 low prevalence (1/64; 1.45% incidence) of LRRK2 G2019S mutation reported in the study may be attributed to the small sample size. In literature, only one Egyptian study has been published where screening of the LRRK2 G2019S mutation in 113 Egyptian patients with PD and 87 healthy controls have detected 11 patients (9.7%) with PD, all of them were heterozygous, but the mutation was not observed in any of the controls [12]. The meeting point between the two Egyptian studies is the heterozygosity among Egyptians with PD who were carrying this kind of mutation. However, the difference in the incidence of LRRK2 G2019S mutation between the two studies for the same population may be ascribed to many factors like the sample size included in each study, selection bias, age difference, study design, and statistical methods of calculation [2,13]. Therefore, further multicenter studies are required among Egyptians but on larger number of patients and control subjects to determine a possible real figure of the prevalence of that mutation in Egyptians with PD.

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 life style 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].

Our data provide a provisional (but non-confirmatory) finding of low prevalence of LRRK2 G2019S mutation in Egyptians with Parkinson’s disease. Further studies on larger number of subjects (patients and controls) are needed to identify precise information about the penetrance of G2019S mutation in LRRK2 protein among Egyptians with PD. It is recommended also to widen the scope of screening for other mutations related to PD in LRRK2 protein among Egyptians with PD.

References:


[PubMed: 18539534].
Legends of figures:

Figure 1. Domain structures of LRRK2 protein. ANK (ankyrin repeats), LRR (Leucine-rich repeats), ROC (Ras of Complex proteins), COR (C-terminal of ROC).
Protein-protein interactions  GTPase  Kinase