



Genetic Analysis of Leucin-Rich Repeat Kinase 2 (*LRRK2*) G2019S Mutation in a Sample of Egyptian Patients with Parkinson's Disease, a Pilot Study

**Ehab. S. El Desoky^{1*}, Eman. M. Khedr², Mohamed. S. Khalil³
and Thomas Gasser⁴**

¹Department of Pharmacology, Faculty of Medicine and Assiut University Hospital, Assiut, Egypt.

²Department Neurology, Faculty of Medicine and Assiut University Hospital, Assiut, Egypt.

³Department Clinical Pathology, Faculty of Medicine and Assiut University Hospital, Assiut, Egypt.

⁴Department of Neurodegenerative Diseases, Hertie-Institute for Clinical Brain Research, University of Tübingen, Tübingen, Germany.

Authors' contributions

This work was carried out in collaboration between all authors. Authors ESED suggested the idea of the study, shared in the study design, participated in the practical genomic part and wrote the paper. Author EMK managed the clinical part of the study, interpreted the clinical data. Author MSK managed DNA extraction from patients and interpretation of the results. Author TG participated in the practical genomic part, interpretation of the results. All authors read and approved the final manuscript.

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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 G2019S substitution in that gene is the most common mutation identified to co-segregates with PD. In the North part of Egypt (Alexandria and nearby region), an

*Corresponding author: Email: ehegypt@yahoo.com;

incidence of 9.7% of heterozygous mutation in *LRRK2 G2019S* was reported in a sample of Egyptians with sporadic PD. We investigated the same mutation in 69 Egyptian patients with sporadic PD and 96 ethnically matched controls who all were inhabitants of Upper Egypt to find out if it could be a susceptibility gene for PD among Egyptians.

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 and all were inhabitants of Assiut Governorate and nearby region in Upper Egypt. PCR-genotyping analysis for the point mutation *G2019S* in the exon 41 was performed and presence or absence of mutation was confirmed by direct sequencing of the probands identified of the DNA.

Results: Genotyping analysis and sequencing of DNA showed only one patient who was carrier to the mutation *G2019S* (1/69; incidence: 1.45%) and it was of heterozygous style. The rest of subjects (patients and control) were not carrying the mutation. This rarity of this kind of mutation among the Egyptian sample studied suggests that it may be a rare cause of PD in Upper Egypt region. However, if it is observed, it may have a trend of heterozygosity genotyping style as previously defined in the Egyptians living in the North region of Egypt

Conclusion: The very low incidence of *G2019S* mutation in Egyptians living in Upper Egypt compared to Egyptians inhabitants in North Egypt suggests a multicenter study on a large number of Egyptians with Parkinson's disease to reach a real incidence of that mutation and if it has (or not) a correlation to causation and course of Parkinson's disease among Egyptians.

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

1. 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 candidate and susceptibility genes in PD pathogenesis is the leucin-rich repeat kinase 2 gene (*LRRK2*) which is a large, multi-domain GTPase/ kinase protein [3,4]. One of the significant mutations in *LRRK2* linked to PD is the *G2019S* which has been found associated with neuronal impairment and loss of dopaminergic neurons [5]. It accounts for 6.6% of familial and 1.6% of sporadic PD cases in Caucasians [6,7]. Its frequency is high in the North African Arabs (40%) [8] and Ashkenazi Jews (30%) [9], moderate in the Portuguese (8–9%) and very low (< 1%) in countries like Austria, Belgium, Denmark, Germany, Greece, Japan, India, Iran and Poland [10]. In Egypt, only one study to check the incidence of *G2019S* mutation among Egyptians with PD has been published [11]. The study reported an incidence of 9.7% of heterozygous mutation in *LRRK2 G2019S* and the patients studied were of sporadic type of PD and inhabitants of the North part of Egypt (Alexandria Governorate and nearby region). These findings encouraged us to check for the same mutation in another group of Egyptians

diagnosed as PD of sporadic type but inhabitants of Upper Egypt (Assiut Governorate and nearby region). Because of the large size of the *LRRK2* (51 exons spanning 145 kb region on chromosome 12p11.2-q13.1) [12], and the high cost of this type of genetic studies, the point mutation *G2019S* was targeted in the present study where the exon 41 of *LRRK2* was amplified from genomic DNA using PCR and directly sequenced for the possible mutation. However, this approach of genetic screening from our side does not rule out the presence of other mutations or risk variants that might be associated with the disease pathogenesis.

2. METHODS

2.1 Patients and Control Subjects

Sixty nine patients with sporadic PD (16 women and 53 men) and a mean age \pm SE of 60.7 \pm 2.3 years who attended the outpatient clinic of the neurology department in Assiut University hospital were included in the study. The onset of the disease was between 6 months and 12 years. The patients were diagnosed clinically as PD based on the presence of at least one or more of the following criteria: resting tremors, rigidity, bradykinesia and/or postural instability [13]. The severity of the disease was rated

according to the Unified Parkinson's Disease Rating Scale (UPDRS) and Hoehn and Yahr staging [14]. The patients had a negative history of previous head trauma, brain tumor or medication with dopamine depleting agents within the last year before selecting. Ninety six ethnically matched controls (30 women and 66 men) with mean age \pm SE of 61.5 ± 1.28 years were also genotyped for carrying of the same mutation or not to explore if the mutation G2019S in LRRK2 gene could have a potential susceptibility in causation of PD among Egyptians. All subjects (patients and controls) were inhabitants of Assiut Governorate and nearby area in Upper Egypt. The clinical criteria of the patients are demonstrated in Table 1.

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

| Parameter | | |
|----------------------|----------------------------|-----------------|
| Age | 60.7 \pm 2.3 years | |
| Disease duration | (Range: 6 months-12 years) | |
| Clinical symptoms: | Incidence | Score |
| Tremors at rest | 95% | 1.8 \pm 0.14 |
| Bradykinesia | 85% | 1.5 \pm 0.15 |
| Rigidity | 83% | 1.1 \pm 0.16 |
| Postural instability | 65% | 1.08 \pm 0.11 |
| UPDRS overall score | | 22.39 \pm 2.0 |

Values are means \pm standard errors, UPDRS: Unified Parkinson's disease Rating Scale

2.2 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 μ l including 0.4 μ l of each primer (10 pm/ μ l), 0.4 μ l of each dNTP, 2.4 μ l of MgCl₂, 0.1 μ l of Taq polymerase, 4.0 μ l of buffer plus 8.3 μ l H₂O. 4 μ l of genomic DNA (10 ng/ μ l) 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 [15]. 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 [16] 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.

3. RESULTS

PCR genotyping analysis of DNA for control subjects and patients followed by sequencing of the coding region in LRRK2 revealed that one patient only was carrier to the targeted mutation G2019S in the gene (incidence 1.45%) and it was of heterozygous style compared with the rest of patients and control subjects who were described as non-carrier to that mutation. The patient with that heterozygous mutation was 56 years old female, with onset of Parkinson's disease when she was 54.5 years. The clinical symptoms detected in the patient included resting tremors, rigidity, bradykinesia, postural instability, slight salivation and mood depression. No information was available for positive family history of the disease in that patient.

In Table 2, summation of our findings and that of the previous Egyptian study [11], as regards the incidence and genotyping style of LRRK2 G2019S mutation showed a total incidence of 7% for the mutation with heterozygosity.

Table 2. The incidence of LRRK2 G2019S mutation in Egyptians with Parkinson's disease (Present study vs. a previous study)*

| Egyptian study | LRRK2 G2019S mutation | | | |
|-------------------|-----------------------|------------------|--------------|----------|
| | Carriers | | Non-carriers | |
| | Control | Patients | Control | Patients |
| Hashad | - | 11 [#] | 87 | 102 |
| DI* | | (9.7%) | (100%) | (90.3%) |
| EL Desoky | - | 1 [#] | 96 | 68 |
| et al** | | (1.45%) | (100%) | (98.55%) |
| Total (Egyptians) | - | 12 ^{\$} | 183 | 170 |
| | | (7.1%) | | (92.9%) |

* Reference 14 (published 2011), ** (The present study), # Number of carriers compared to the corresponding patients who were non-carriers, Values in brackets represent an incidence, \$ The total number of Egyptians in the two studies who were diagnosed as Parkinson's disease and carriers to the mutation

4. DISCUSSION

In the present study, we did not observe the targeted mutation *G2019S* in *LRRK2* gene in the study sample (patients and control) except in one patient with the disease. She was a female with 1.5 years onset of the disease and the mutation was heterozygous. This very low incidence of mutation (1/64; 1.45%) among the Egyptian patients studied suggests that it may be a rare cause of PD among Egyptians who are inhabitants of Upper Egypt. The incidence is also lower than that previously reported in Egyptians with sporadic PD (9.7%) who were inhabitants of the North of Egypt (Alexandria Governorate and its surroundings) [11]. Historically, the North area of Egypt 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 [17] and Tunisians with PD are known with the high incidence of *LRRK2* mutation in *LRRK2G2019S* [8,10,18]. In Upper Egypt, these circumstances of immigration and mixing with other populations of ethnic differences were not available. Other possible factors that may explain the different incidence of mutation in the two Egyptian studies are 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) [2,8,9]. However, heterozygosity of the mutation *G2019S* was a common finding in Egyptians based on the two Egyptian studies. The issue of finding only one subject with this kind of mutation among the sample studied has been reported before in an Indian study where 1012 individuals (PD, 800; controls, 212) were screened for the same mutation [19].

The low prevalence of *LRRK2 G2019S* mutation in Egyptians with sporadic PD (7%; Table 2) compared with Tunisians (45%) though Egypt and Tunis are Arab countries located in the Middle East is of interest. The ethnic difference, environmental factors and different life style between Tunisian and Egyptian populations may explain the prevalence difference [2].

5. CONCLUSION

LRRK2 G2019S mutation is of low incidence among Egyptians but it could be of heterozygous

style. This suggests that this mutation in *LRRK2* may not be a susceptible gene for causation of Parkinson's disease. Also, the incidence of *G2019S* mutation may be different among Egyptians living in Upper Egypt compared with those living in the North region of the country. This encourages for a multicenter study on a larger number of subjects in both regions of the country to reach a real representative incidence of that mutation which helps to determine its relation to causation of the disease. Also, mutations other than *G2019S* in *LRRK2* which are expected to be correlated with Parkinson's disease should be considered in genetic screening of Egyptians with the disease.

CONSENT

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.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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