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Effect of Genetic Polymorphism (rs2619363) on *SNCA* Gene among Iraqi Patients with Parkinson's and some Gastrointestinal Disorders

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Abstract: Parkinson's disease (PD) consider as a progressive ageing neurodegenerative disease, Parkinson's consider as a heterogenous disease, with mainly initiate through correlation between genetic and epigenetic by inducing of different factors on some related genes, these factors like (environmental, toxicants, nutrition, heavy metals, pesticides, some drugs) and also(trauma on head ,strokes) in addition to unknown reasons which cause an idiopathic PD .Current study aims to focusing on specific related PD gene called *SNCA* by single nucleotides polymorphism (rs2619363) as a risk factor for PD initiation disease in PD patients in addition to study the effect of polymorphisms on random Iraqi patients with different gastrointestinal tract disorders to proof the previous hypophysis that suggested about PD initiation which may started from gastrointestinal tract disorders . The chosen samples belong to participants suffering with PD in addition to others suffering with different gastrointestinal tract disorders (GITD) in addition to healthy people. In current study; number of participants were 133 collected in period (March-2022 to November- 2022) from participants whom attended to Al-yarmouk teaching hospital and Baghdad hospital in medical city), and mainly divided to (48 patient with PD, 49 patient with GITD and 36 healthy participants), the blood samples were kept in EDTA tubes for molecular tests, DNA was extracted from the blood samples and then used real-time polymerase chain reaction (PCR) technique with complementary primer, then used singer sequencing to analysis the data. The results revealed the genotypes of all participants, a wild type in PD was (CC) more OR with (1.40) than (CA) with (0.76) and (AA) as mutant with (0.73), respectively, while in GITD (CC) also more genotype appeared with OR (1.00), but (CA) more than (AA) with OR (1.10 and 0.72, respectively). In PD and GITD (C allele) frequency more in all patients, while A allele more frequency in healthy. In conclusion polymorphism of study target SNP on *SNCA* gene not revealed significance on both PD and GITD because of, the nature of Iraqi population samples in addition to small samples not give the real reflect or influence of this alternation on *SNCA* gene as a risk factor on Iraqi population than other communities and populations.

Keywords: Parkinsons disease, Gastrointestinal tract disorders, *SNCA* gene, rs2619363.

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Introduction

Parkinson disease or (PD), mainly related with progressive age disease or also called decline disease. Most cases have cellular decline progression with most significant non motor signs like anxiety, depression, difficulties in sleep, anosmia, amnesia then started the most visual symptoms like speech stutter shuffle steps,

bradykinesia, rigidity, hands or legs tremor, dystonia and constipation (1, 2). Some previous studies mentioned that causes PD mainly caused by external factors like environmental pollution, toxicants heavy nanoparticles and pesticides and some kinds of drugs (3,4,5). Studies also referred about some kinds of gastrointestinal tract disorders or GITD may contributed with idiopathic initiation PD by accumulation the neurotoxins on enteric nervous system (ENS) and reach to brain in specific region in mid brain substantia nigra (SN) which the region having the dopaminergic neurons which has responsible to dopamine production mainly work as neurotransmitters connection among to neurons which responsible for muscles rhythm and controlling (especially skeletal muscle) (6, 7). All these neurotoxins transferring to brain from gut by vagus nerve (VN) or as known as Brain-Gut-Axis (BGA). theses neurotoxins came from interaction of microbiota action and immune activity through repeated inflammations in gut canal (8,9,10) ,Genetic polymorphism mean that any changing happening at the level of DNA sequence leads to changing phenotyping features (11).There are many genes which may related with PD like *PTEN* and induced kinase 1 (*PINK1*), Parkinson disease gene 7,(*PARK7*), deglycase *DJ-1* gene, Leucine-rich repeat kinase 2 (*LRRK2*).While *SNCA* which mainly responsible for alpha-synuclein protein. *SNCA* consider as a first gene was discovered which related with PD initiation through expression of α-syn protein with misfolding forms and work as neurotoxic on synapses instead to functional work as neurotransmitter protein among neurons (12).

Current study depends on gene polymorphism related with PD gene at the rs2619363 level like many articles that focusing on how this specific polymorphism appear the significant expression as a risk factor on PD and on GITD patients and how the alleles frequency distribution on specific population under study (13, 14, 15). The polymorphism of *SNCA* gene under study for many articles in different populations like east Europe and middle Asia e.g. Russia (16) or in middle east like Iranian population (17).

The current study aimed to find the effect of expression *SNCA* gene at rs2619363 in random Iraqi population with PD and different types of GITD under the study.

Materials and methods

The total number of the study participant was 133, fresh blood samples were aspirated through venipunctures from the participants during their attendance to AL-Yarmouk teaching hospital and Baghdad hospital /Medical city. The collecting samples done in the period from (March-2022 until November-2022), and the age range of participants was (20-70 years) .The samples were divided according to the nature of case and detection ways in to (48 with PD and 49 with GITD and 36 healthy persons). By used a protocol guideline in addition to PD detection criteria, neuroscientist detected and examined the patients with PD with depending on medical evidences, while the patient with GITD (different types) detected through the endoscopic instrument for upper portion of digestive system by used oesophago-

gastro-duodenoscopy called (OGD) and colonoscope for detection the lower portion of digestive system. while control group also passed through same conditions of examination.

Genomic DNA was isolated from thawed blood for 133 samples by using specific DNA extraction Kit (Norgen/ Canada), then the DNA yield samples purified by the specific assay solution belong Qubit™ dsDNA HS Assay Kit (Thermofisher/USA).

The Qubit dsDNA HS (Highly Sensitivity) assay kit is designed specifically for use with the (Qubit Fluorometer). The assay is highly selective for double-stranded DNA (dsDNA) over RNA. The assay is performed at room temperature, and the signal is stable for 3 hours. The common contaminants such as salts, free nucleotides, solvents, detergents, or protein are well tolerated in the assay. The assay is highly selective for double-stranded DNA (dsDNA) over RNA and is accurate for initial sample concentrations from 10 pg/µL to 100 ng/µL. The DNA extraction samples stored at –20C° until used.

Polymerase chain reaction was done by using real time thermocycler instrument (Eppendorf Germany) by applying a specific primer which illustrated in (Table1), the primer alignment was designs according to instructions of National Center for Biotechnology Information (NCBI).

All PCR reaction steps were illustrated in (Table 2). The steps of reaction parameters, initial denaturation at 94°C for 4 min, followed by 35 cycles of PCR amplification including denaturation at 94°C, annealing at 51°C, extension at 72°C (each comprising 30 s), and the final extension at 72°C for 5 min. The PCR product was checked by electrophoresis on 1.5% agarose gels with red safe stain (10 ng/100 mL of agarose solution in Tri's borate EDTA buffer) with red safe stain for bands identification.

Table (1): Specific primers for *SNCA* with single nucleotide polymorphism

Name of Primer	Sequence	Product size (bp)	Annealing Temp(C°)	Reference
rs2619363	F-5`- GTCCCATTTCTAGAGCGAAAA -3` R-5`- GCTAACCTGTCGTCGAATG -3`	1041	51C	Current study

Table (2): PCR optimization additions components

Step	Temperature (ºC)	Time	Cycle
Initial-denaturation	94	4 min.	one
Denaturation	94	30 sec.	35x
Annealing	51	30 sec.	
Extension	72	30 min.	
Final extension	72	5 sec.	one

Statistical analysis

Analysis of data was carried out using the available statistical package of SPSS-26 (Statistical Packages for Social Sciences- version-26) and also by used WinPiP software. Data were presented in simple mathematically forms with measures including frequency, percentage, odd ratio (OR), confidence interval (CI). Probability value that mainly refers to statistically significant at (P<0.05), statistically very significant

at (P <0.01) and statistically highly substantial at (P < 0.001) (18).

Results and discussion

The product size of PCR (1041bp), while the ladder size was (1614 bp), all the genotypes were independently verified by Sanger sequencing using the previously prepared PCR amplicon (Macrogen, Seoul, South Korea).

Gel electrophoresis of PCR yield product rection represented in Figure1, while (Figure 2) represented the alternation of single nucleotides at *SNCA* gene (rs2619363).

In (Table3), data revealed the genotype of the (rs2619363) in patients with Parkinsons compared with control, the mainly genotypes were (CC, CA, AA), the participants in the table mainly divided into (48 in PD and 36 in control). The higher genotypic ratio were found in PD patients represented by wild type (CC) with 68.8% while in control was 61.1% with odd ratio (1.40), while the lowest value was in mutant (AA) with 6.2 % in PD and 8.3% in control with odd ratio (0.73), while (CA) ratio were (25% in PD and 30.6 % in control respectively with odd ratio (0.76). The frequency of C allele more in PD and control than A allele with (78 time in PD and 81.2%) vs 55 time in control and 76.4%) with odd ratio (1.34) while A allele reading was (18 time in PD with 18.8% vs 17 time in control with 23.6%). In general sight on (Table 3), *p-value* for the all data which related with rs2619363 not appeared a significance, even the p-*value* that related with alleles frequency, *all* p-values were higher than (0.05). In spite of the OR values ranging from (< 1 and >1) with disappeared of significance, that mean not correlation between the PD inducing gene *SNCA* at (rs2619363) with PD in Iraqi samples (under study). In spite of the effect of the *SNCA* gene and give significance in different populations (19, 20).

The current study disagrees with most studies about the polymorphism and effect on population with highly risk of PD, current study suggested many reasons about these results like small population samples (under-study samples) and even the difference of family history among the Iraqi patients and healthy in addition to random marriage and variety of the genetics among the Iraqi population study samples. In (Table 4) represented the genotypes of the patient's group with different GIT disorders compared with healthy, genotypes were (CC, CA and AA), the participants number with GITD were (49 while the control were 36), the highest genotype ratio was the wild type (CC) with 61.2% while in control was 61.1% and OR (1.00). While (CA) ratio was 32.7% and in control 30.6% OR was (1.10). The lowest genotype was (AA) with 6.2% in GITD and 8.3% in control and odds ratio was (0.72).

About allele frequency the C allele was more in two groups than A with (76 times in GITD with 77.6% than 55 times in control with 76.4%; 22 times in GITD with 22.4% and 17 times with 23.6% in control, respectively) with odds ratio (1.07 and 0.94, respectively), rs2619363 not showed significancy about the effect of polymorphism on the Iraqi population (under-study random society samples), in spite of the variation of allele between the GITD and control, but the

polymorphism not appeared the risky effect among the Iraqi population under the study at *P-values* were (>0.05).

There were not previous studies and reviews that may give the clear linking between the (rs2619363) polymorphism and it effect on patients with GITD, and give the clear correlation between PD and GITD at the genetic levels, most previous studies correlated between GITD with different types of genes, some studies comparing the Ulcerative colitis as a type of GITD with control in serological tests only (21) and others more related with the risk factors test in disorders like gastritis (22, 23, 24).While other studies working on other GITD disorder with other type of gene called Cytotoxin-associated gene A(25).

Current study suggested about less findings of other comparisons data with current findings ;There are two types of comparison studies related with these kinds of scientific paper, most of similar work studies in different societies like middle east or in other regions of the world more focusing on the samples counting because of increasing population and increasing rates of PD and different GITD patients in those societies, so the risk factor or protective factor or even the effect of specific SNP alternation of related disease gene on specific society appears clearly. And the other types of studies in Iraqi population samples related with same disease but with different genes, or study the same gene but different SNPs or in different diseases, so the comparison related with these types of projects need more similar way projects and more financial capabilities or fundings to including more counts of study samples participants to give more clearance evidences about the direct effect reflection of these types of genes in Iraqi population.

Figure (1): Gel electrophoresis of the *SNCA* with specific primer.

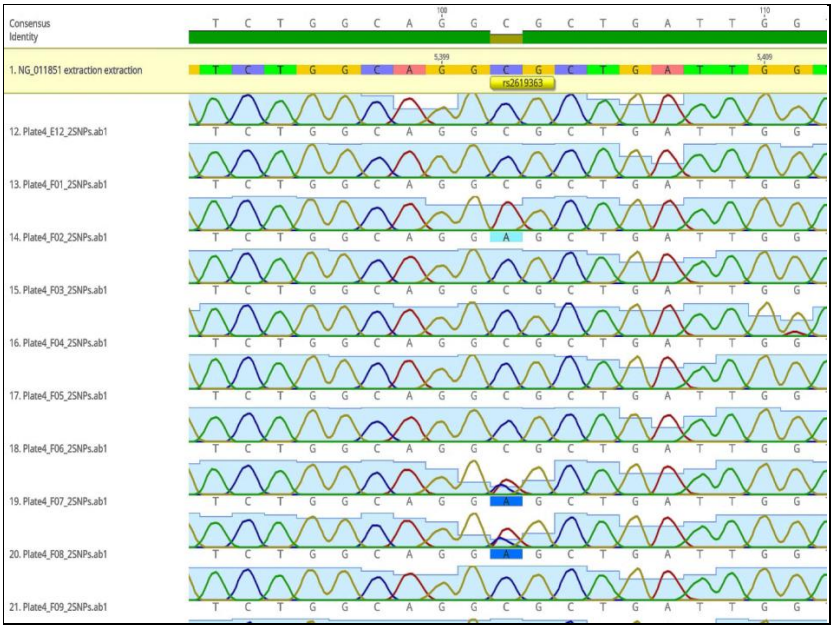

Figure (2): The single nucleotides Polymorphism alternations in sequence.

Table (3): The single nucleotide polymorphisms rs2619363 in parkinson disorder compared with Healthy Control.

SNP (rs2619363)		Parkinson patients (n=48)	Healthy Control (n=36)	OR	95%C1	p-value
Genotype	Wild CC	33(0.688)	22(0.611)	1.40	0.57-3.43	0.49
	Hetero CA	12(0.250)	11(0.306)	0.76	0.29-1.96	0.62
	Mutant AA	3 (0.062)	3 (0.083)	0.73	0.14-3.79	1.0
Allele frequency	C	78(0.812)	55(0.764)	1.34	0.64-2.80	0.45
	A	18(0.188)	17(0.236)	0.75	0.36-1.57	0.45
HWE	P-value	0.21	0.35			
	Chi^2	1.54	0.84			

N=Frequency, Single nucleotide polymorphism (SNP), confidence interval (CI), odds ratio (OR), p-value= Probability value, Chi^2= chi square, HWE: A simple calculator to determine whether observed genotype frequencies are consistent with Hardy-Weinberg equilibrium.

Table (4): The single nucleotide polymorphisms rs2619363 in gastritis disorder compared with Healthy Control.

SNP (rs2619363)		GITD patients (N=49)	Healthy Control (N=36)	OR	95%C1	p-value
Genotype	Wild CC	30(0.612)	22(0.611)	1.00	0.42-2.40	1.00
	Hetero CA	16(0.327)	11(0.306)	1.10	0.44-2.75	1.00
	Mutant AA	3(0.061)	3(0.083)	0.72	0.14-3.70	0.69
Allele frequency	C	76(0.776)	55(0.764)	1.07	0.52-2.19	0.85
	A	22(0.224)	17(0.236)	0.94	0.46-1.92	0.85
HWE	P-value	0.66	0.35			
	Chi^2	0.189	0.842			

N=Frequency, Single nucleotide polymorphism (SNP), confidence interval (CI), odds ratio (OR), p-value= Probability value, Chi^2= chi square, HWE: A simple calculator to determine whether observed genotype frequencies are consistent with Hardy-Weinberg equilibrium.

Conclusion

According to the current study data of polymorphism at (rs2619363) on *SNCA* gene in the Iraqi population (under study) samples not revealed the significance and indicated the risk of parkinson on Iraqi samples and also not give the direct connection between PD and GITD in distinct way , that depended on many other factors like the history of Iraqi population (Chosen samples) ,sample size in addition to randomized marriage which mainly effect on activation or expression of PD responsible different genes like *SNCA* gene and increasing genetic variations which minimizing the risk of PD.

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