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Naturally-Occurring Genetic Variation Scores for Dopamine Function among Patients with Parkinson's Disease in Palestine

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Thesis Approval

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Declaration

I certify that this thesis submitted for the degree of master is the result of my own research, except where otherwise acknowledged. This thesis has not submitted for a higher degree to any other university or institution.

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Dedication

I would like to dedicate this work and effort with all my love to the soul of my grandma (Hind Shurafa)- God bless her.

Niveen Betouni

Acknowledgment

Conducting my thesis has been a truly life-changing experience for me. Achieving this goal is a grace and blessing from Almighty Allah. I would like to express my deep thanks to my academic supervisor, Dr. Mohammad Herzallah who has made this achievement possible through his endless support. I am also, thankful to my partner, soul mate, and husband Rajwan Budeir for believing in me and for his lifelong support. Moreover, special thanks to my family, my mother, my sons Qusai and Bassel, and daughters Toujan, Layan, and Miral, my uncle's wife Mai Mani and to my friend Maram Mani for their emotional support and inspiration. In addition, I am grateful to all the staff members of the Palestinian Neuroscience Initiative, especially Leen Jayousi, for her constant support in each step of my study. Gratitude and appreciation are extended to all my instructors at the Arab American University, especially Dr. Nouar Quttob, Dr. Siba Shanak, Prof. Hisham Darwish, Dr. Zaidoun Salah and to the Committee members, Dr. Reham Nazal and Dr. Fawaz Awad. Many thanks to my colleagues, Osayed Zohoud and Sondos Odeh for their support. Finally, I would like to thank everyone who has assisted me in my study and everyone who has supported me at this stage of my life to let my dream come true.

Abstract

Objective: The aim of this study is to create a dopaminergic genetic risk score for Parkinson's disease based on naturally occurring polymorphisms in key dopamine genes.

Materials and methods: This case-control study of Parkinson's disease recruited 106 Palestinian subjects: 55 patients with PD and 51 healthy control subjects. The age of participants ranged from 55-75 years. Subject DNA was genotyped for a total of seven SNPs in the *DRD1* (rs686, rs4532), *DRD2* (rs1076560, rs6277), *SLC6A3* (rs3836790, rs28363170), and *COMT* (rs4680) genes.

Results: There was a significant difference between patients with PD and healthy individuals in the genotype and allelic frequency in *DRD1* rs4532, *SLC6A3* rs3836790, and *COMT* rs4680. Genotype combinations permutation analysis and Hardy-Weinberg equilibrium calculations revealed significant effects of the same SNPs. Dopamine genetic risk scores based on all SNPs did not reveal any difference between patients with PD and healthy individuals. A machine learning classifier based on *DRD1* rs4532, *SLC6A3* rs3836790, and *COMT* rs4680 identified patients with PD in 70% of cases.

Conclusion: Key SNPs in dopamine genes could serve as risk predictors and diagnostic markers for PD. Our findings could be developed into future clinical assessment tools.

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Abbreviations

PD: Parkinson Disease

WHO: World Health Organization

L-DOPA: L-3,4-Dihydroxyphenylalanine

SNP: Single Nucleotide Polymorphism

DAT: Dopamine Active Transporter

COMT: Catechol-O-Methyltransferase

DRD1: Dopamine Receptor D1

DRD2: Dopamine Receptor D2

SLC6A3: Solute Carrier Family 6 Member 3

3' UTR: 3 Prime Untranslated Region

VNTR: Variable Number Tandem Repeat

Int8 VNTR: Intron8 VNTR

MAO-B: Monoamine Oxidase B

cAMP: Cyclic Adenosine Monophosphate

mRNA: Messenger Ribonucleic Acid

bp: Base Pair

ADHD: Attention Deficit Hyperactivity Disorder

ASD: Autism Spectrum Disorders

D2S: Dopamine D2 Short Isoform

D2L: Dopamine D2 Long Isoform

DNA: Deoxyribonucleic Acid

EDTA: Ethylenediaminetetraacetic Acid

TE: Tris-EDTA

PCR: Polymerase Chain Reaction

RFLP: Restriction Fragments Length Polymorphism

TAE: Tris-Acetate-EDTA

TBE: Tris-Borate-EDTA

SPSS: Statistical Package for the Social Sciences

MAOA: Monoamine Oxidase A

Chapter1

Introduction

1.1 Parkinson Disease

Parkinson’s disease (PD) is a chronic neurodegenerative disorder and among the quickest growing neurological diseases contributing to disability. The prevalence and death due to PD from 1990 to 2015 have been increased by more than two folds (Feigin et al., 2017). According to the world health organization (WHO), the worldwide incidence of PD over the past 25 years has doubled (WHO, 2022). The number of patients suffering from PD worldwide exceeds 10 million. In the USA, it was estimated that a million individuals will develop PD per year by 2020. It was also estimated that this number will rise by 2030 to 1,238,000 patients per year (Marras et al., 2018). Different prevalence of PD was reported according to ethnic groups and geographic areas. For example, the prevalence of PD in the Arab Muslim population in Wadi Ara, Haifa is considered to be minimal (Masalha et al., 2010) compared to the high prevalence in the Jewish population (Herishanu et al., 1989; Anca et al., 2002). In turn, in northern California, PD prevalence was the highest mainly among Hispanic than non-Hispanic whites, followed by Asians and Blacks (Van Den Eeden et al., 2003).

PD is characterized by both motor symptoms, including tremor, bradykinesia, postural instability, and muscular rigidity, and non-motor symptoms such as depression, pain, constipation, chronic fatigue, genitourinary problem, sleep disturbance, and dementia (Schrag et al. 2015). The more the disease progresses, the more vital the manifestation of symptoms becomes, resulting in social and economic limitations that have a substantial impact on Parkinson's patients' quality of life; improving these symptoms becomes a significant task in managing the condition (Schrag & Quinn,

2020). Although the etiology of PD is not well understood, loss of dopamine, a neurotransmitter that is produced in the substantia nigra pars compacta (Cragg et al., 1997) is implicated as the most significant pathology of PD, which leads to the aggregation of neuronal inclusions known as Lewy bodies and the occurrence of α-synthesis (Gibb and Lees 1988; Spillantini et al,1997). Postmortem studies of patients with PD revealed severe decay in the nigrostriatal dopaminergic neurons (Hartmann, 2004). Pharmacological dopamine remediation approaches are used to recover dopamine levels in patients with PD. After discovering L-3,4-dihydroxyphenylalanine (L-DOPA) (Funk,1911), a precursor to dopamine (Ehringer & Hornykiewicz, 1998), the treatment of PD patients with L-DOPA has significantly improved the life expectancy of PD patients (Barbeau, Sourkes and Murphy 1961).

Different components of the dopamine system have been linked to both motor and nonmotor symptoms of PD. Single nucleotide polymorphisms (SNPs) in the different dopamine genes and have been identified as important factors in understanding the pathophysiology of PD and its risk (Magistrelli et al., 2021). For instance, the pharmacological and biochemical activities of dopamine in the brain and body are mediated by two different dopamine receptor families, D1 and D2, encoded by five different genes (Kebabian et al., 1972). Widely researched SNPs affect the functionality of the different dopamine receptors (Porta et al., 2012) and were linked to the risk of developing PD (Mishra et al., 2018). Aside from dopamine receptors, various SNPs were identified in dopamine clearance and degradation systems, such as the dopamine transporter (*DAT1*) gene, and the catechol-O-methyltransferase *(COMT)* gene (Tunbridge et al., 2019). Although these SNPs were examined individually by past research studies (Wardle et al., 2013), very few efforts were made to identify their

collective impact on the risk to develop PD motor and nonmotor symptoms (Baetu et al., 2015).

Although PD is mainly sporadic, the resemblance in both familial and sporadic forms can help in the further understanding of the pathophysiology and the molecular mechanisms of the disease (Meiser et al., 2013). Genetic and family history is considered one of the risk factors for having PD. For example, mutations in the *PRKN*, *SNCA*, *GBA1*, *DJ-1*, *LRRK2* and *VPS35* genes were linked with PD (X. S. Zeng et al., 2018). The first mutation related to PD was recognized in 1990 in the *SNCA* gene, while the first mutation in *PRKN* gene was identified in 1998 (Oczkowska et al., 2013). Monogenic and familial forms only affect 10-15% of patients with PD who exhibit symptoms early in life. Hence, genetic forms of PD are categorized into juvenile-onset if age is less than 21, and early-onset if age is 20-40 (Massano & Bhatia, 2012). Otherwise, PD is considered sporadic.

Cellular and molecular pathways interact and influence the progression of PD. For instance, neuroinflammation in the form of activated microglia and astrocytes can injure dopaminergic neurons in the substantial nigra (Zaman et al., 2021). Several insults, including mitochondrial dysfunction and environmental toxins, may play a role in initiating oxidative stress leading to the production of reactive oxygen species in dopaminergic neurons and their subsequent death (Zaman et al., 2021). Since dysfunctional mitochondria are usually removed by mitophagy from substantial nigra neurons, ineffective autophagy can lead to the aggregation for toxic proteins and the demise of the cell (Zaman et al., 2021). Furthermore, dopaminergic neurons die as a result of endoplasmic reticulum stress, which is shown in postmortem studies of the PD (X. S. Zeng et al., 2018).

Aside from genetics and cellular mechanisms, other factors can influence the risk for developing PD. Advancement of age is considered the main risk factor for developing PD (Collier et al., 2017). Sex is another important risk factor, where males tend to have at least two folds higher risk to develop PD than woman (Baldreschi et al., 2000; Van Den Eeden et al., 2003). As such, the expressions of the PD-related genes in dopaminergic neurons and response to PD treatment is highly associated with sex (Dias, Vera Junn, Eunsung Mouradian, 2008). Cohort studies also shed the light on the association between traumatic head injury and PD (Crane et al., 2016). Occupational exposure to certain pesticides, insecticides, herbicides, rodenticides, fungicides and heavy metals increase the risk of PD (Narayan et al., 2017). Other chemicals such as the halogenated solvent trichloroethylene and polychlorinated biphenyls were also associated with increase the risk for PD (De Miranda & Greenamyre, 2020).

1.2 General Objectives

The general aim of this study is to create a dopaminergic genetic risk score for PD. We examined the diagnostic power of common functional and naturally-occurring SNPs in the key genes of the dopamine system: dopamine transporter, receptors, and degradation enzymes.

1.3 Specific Objectives

We evaluated the following SNPs in key dopamine genes in patients with PD and matched healthy controls:

1. Dopamine receptor D1 gene (*DRD1*):
    a. rs686: Regulates D1 receptor expression.

   b. rs4532: Regulates the translation of *DRD1* mRNA.

2. Dopamine receptor D2 gene (*DRD2*):
   a. rs6277 (C957T): Regulates stability of *DRD2* mRNA folding.
   b. rs1076560 (SNP19): Regulates DRD2 mRNA stability and splicing.
3. Dopamine active transporter gene (*SLC6A3* or *DAT1*):
   a. rs3836790 (*DAT1* Intron8 VNTR): Regulates expression of *DAT1* gene transcription.
   b. rs28363170 (*DAT1* 3'UTR VNTR): Regulates density of presynaptic dopamine transporter.
4. Catechol-O-Methyltransferase (*COMT*):
   a. rs4680 (Val158Met): Regulates COMT enzyme abundance, stability and activity.

1.4 Significance

Dopamine dysfunction has been implicated in the pathophysiology of PD. Naturally-occurring variations in the genes encoding key proteins in the dopamine system could play a central role in the development of PD. This project offers a genetic score and systematic quantification of seven functional SNPs in dopamine genes among patients with PD and healthy controls in Palestine. We also quantify the significance of the concocted dopamine SNPs as a potential diagnostic tool for PD.

Chapter 2

Literature Review

2.1 Dopamine

Dopamine, 3-hydroxytyramine, is the most abundant catecholamine in the brain (Vallone et al., 2000). It was first considered a precursor and intermediate of other catecholamines. Afterward, it was shown as an independent transmitter synthesized from L-DOPA in the brain (Blaschko, 1957; Carlsson et al., 1957). Dopamine is produced in neurons within the ventral tegmental area and the substantia nigra in the midbrain, hypothalamus, olfactory bulb, periaqueductal gray and the periphery (e.g., the adrenal medulla and retina (Ayano, 2016). In the human brain, dopaminergic neurons project to the basal ganglia, mainly the caudate nucleus, putamen, and pallidum (Sano et al., 1959). Dopamine at the cellular level binds to various receptor proteins and exhibits multiple roles such as stimulation of adenylyl cyclase (Kebabian et al., 1972), activation of k+ channels (Sasaki & Sato 1987), regulation of gene expression, and modulation of the firing rate of neurons (Gerfen et al., 1990). Dopamine is also involved in regulating numerous functions such as movement (Bernheimer et al., 1973), reward (Sharot et al., 2009), memory (Shohamy & Adcock, 2010), cognition and attention (Nieoullon, 2002), and addiction (Volkow et al., 2011). Moreover, disturbances in dopaminergic function have also been associated with several neurological disorders such as Parkinson's disease, Huntington's disease (Bernheimer et al., 1973), and schizophrenia (Laruelle et al., 1996). Dopaminergic pathways are affected by dopamine synthesis, which is influenced by the accessibility of tyrosine and enzymes such as dopa decarboxylase and tyrosine hydroxylase; these enzymes are responsible for converting tyrosine to dopa and then to dopamine (Lovenberg et al., 1962). Degradation of dopamine also

occurs by the action of monoamine oxidase B (MAO-B) (Rosengren, 1960) or COMT enzymes (Axelrod, n.d.) see figure 2.1. There are two types of dopamine receptor families, D-1 like and D-2 like (Kebanian and Calne, 1979). There are multiple subtypes of dopaminergic receptors. They can be either post-synaptic or pre-synaptic, and they can act as excitatory or inhibitory according to their subtype and to the G-coupled protein (Ayano, 2016). D-1 like and D-2 like subtypes are mainly found in the basal ganglia. The distribution of D-1 receptors in the medial caudate nucleus is very high, while D-2 receptors are dispersed evenly through the caudate. Similar densities were found in the caudate nucleus and putamen between both D-1 and D-2 receptors, while in some limbic and neocortical regions D-1 were more abundant than D-2 dopamine receptors (Hall et al.,1994). Only D1, D2, and D3 receptors exhibit considerable levels of dopamine receptor expression in the striatum. The caudate, putamen, and nucleus accumbens are evenly distributed with D1 and D2 receptor messenger ribonucleic acids (mRNAs). D3 receptor mRNA is moderately abundant in the ventral putamen and is particularly available in the nucleus accumbens. The mRNA D1 and D4 receptors is more prevalent in the prefrontal cortex than other receptors. The temporal neocortex exhibits a comparable pattern. While D2, D3, and D5 are found in the occipital cortex at low levels of expression, D1 receptor mRNA is the most prevalent and D3 is the least common. (Meador-Woodruff et al., 1996). Dopamine acts by binding to one of these receptors to produce numerous second messenger effects via cyclic adenosine monophosphate (cAMP) (Vallar and Meldolesi, 1989). The stimulation of D1 receptors increases the activity of adenylyl cyclase, while the stimulation of D2 inhibits adenylyl cyclase (Caron et al., 1978). Differences between the two types of dopamine receptors are illustrated in the table (2.1).

Table 2.1: Differences between D-1 and D-2 like dopamine receptors.

D1-Like Dopamine Receptors	**D2-Like Dopamine Receptors**
D1 and D5 subtypes	D2, D3, and D4 subtypes
Coupled with Gs regulatory proteins	Coupled with Gi regulatory proteins
Activate adenylyl cyclase	Inhibit adenylyl cyclase
Catalyze the production of cAMP	Inhibit the production of cAMP
Activate phosphatidylinositol	Inhibit phosphatidylinositol
Decrease K+ channel activity	Increase K+ channel activity
Stimulate Ca^{+2} mobilization	Inhibit Ca^{+2} mobilization

Dopamine released in the synapse binds to either excitatory or inhibitory receptors on the postsynaptic neurons. It can be reuptaken back by binding to the dopamine transporters on the presynaptic neurons (Kilty et al., 2016). When there is excess release of dopamine, it binds to inhibitory auto-receptors on the presynaptic neurons (Ford, 2014).

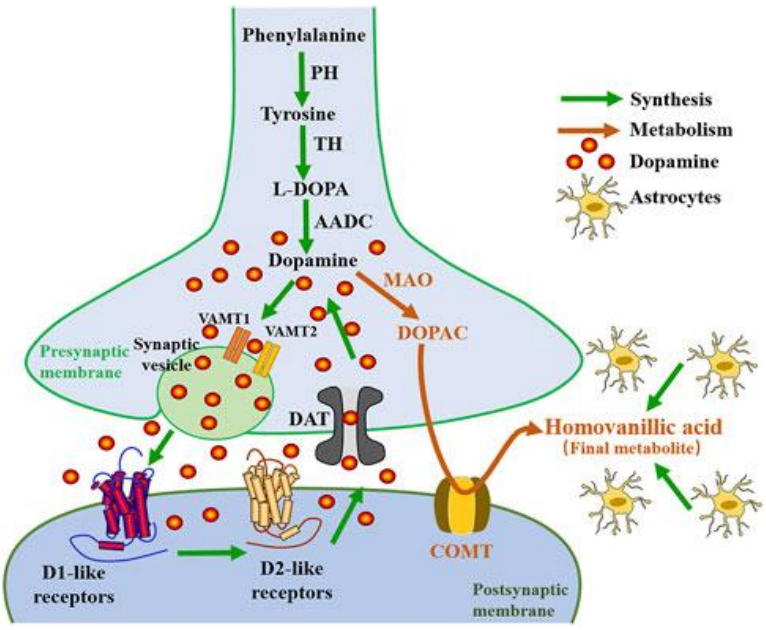


Figure 2.1: The synthesis and degradation of dopamine. Phenylalanine hydroxylase catalyzes the conversion of the amino acid phenylalanine into tyrosine, from which dopamine is produced either directly or indirectly. Tyrosine enters the neuron and is converted into L-3,4-dihydroxyphenyl-L-alanine (L-DOPA) by tyrosine hydroxylase in the cytoplasm. Large neutral amino-acid transporters absorb L-DOPA, which is then converted to dopamine by the aromatic L-amino acid decarboxylase (AADC) found in dopaminergic neuron. Dopamine is transported by vesicular monoamine transporter (VMAT) into synaptic vesicles where they are stored before being released extracellularly. Degradation of dopamine happens through two enzymatic reactions, one mediated by monoamine oxidase (MAO) which catalyzes dopamine to 3,4-dihydroxyphenylacetic acid (DOPAC), and the other through catecholamine-O-methyltransferase (COMT), where homovanillic acid (HVA), a major dopamine metabolite, is produced (from Zhao et al., 2022).

2.2 Parkinson's Disease and Dopamine

Patients with PD exhibit a reduction in dopamine concentrations in the basal ganglia (Bertler, 1961) due to the demise of neurons in the substantia nigra pars compacta. The degree of degeneration of dopaminergic neurons in the pars compacta is positively correlated with dysregulation of the dopamine metabolism in the basal ganglia. Furthermore, the disruption of nigrostriatal dopaminergic neurons lowers the enzymatic activity of the L-tyrosine hydroxylase and L-DOPA decarboxylase and thus inhibits the synthesis of dopamine (Bernheimer et al., 1973). The severely decreased level of striatal dopamine (putamen and caudate nucleus) is responsible for the exhibition of motor symptoms in PD patients (Schapira, 2009). The implication of dopamine in PD is also seen from improving symptoms such as rigidity and akinesia when patients are treated with L-DOPA (Barbeau, Sourkes, and Murphy 1962).

2.3 Candidate Genes and SNPs

Since the primary cause of PD is the decay of dopamine neurons, we selected the following dopamine genes (*DRD1*, *DRD2*, *SLC6A3*, *COMT*) that encode dopamine receptors, transporter and COMT enzyme to build the dopamine genetic risk scores for PD. Selected genes and SNPs are illustrated in table (2.2).

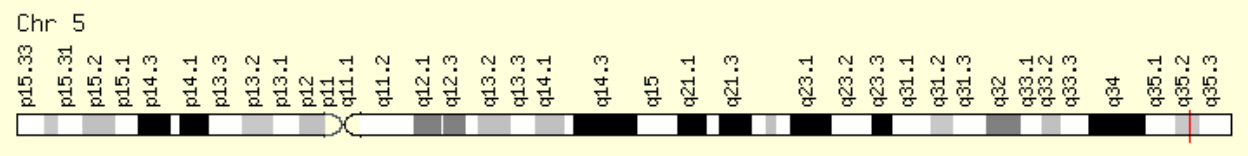
Table 2.2: Selected genes, molecular location of genes, selected SNPs, Alleles, and SNP location[**].

Gene	Molecular Location of gene: base pair (bp)	SNP	Alleles	SNP location	Examples of diseases and disorders associated with SNP
DRD1	Chromosome 5: from bp. 175,440,036 to 175,444,182 on reverse strand	rs686	G>A / G>C /G>T	chr5:175441697 Exon 2 (3'UTR)	Schizophrenia, (Zhu et al., 2011) nicotine dependence, (Huang et al., 2008)
DRD1	Chromosome 5: from bp. 175,440,036 to 175,444,182 on reverse strand	rs4532	C>T	chr5:175443147 Exon 1 (5'UTR)	Impulsive control disorder, (Zainal Abidin et al., 2015) schizophrenia, (Allen et al., 2008)
DRD2	From bp: 113,409,605 to 113,475,691 on chromosome 11	SNP19 rs1076560	C>A	chr11:113412966 Intron 6	Schizophrenia, (Zheng et al., 2012) Opioid addiction, (Clarke et al., 2014)
DRD2	From bp: 113,409,605 to 113,475,691 on chromosome 11	C957T rs6277	G>A	chr11:113412737 Exon 7	Schizophrenia, (Betcheva et al., 2009) Post-traumatic stress disorder, (Voisey et al., 2009)
SLC6A3	From bp: 1,392,794 to 1,445,440 on chromosome 5	Intron 8-VNTR rs3836790	5-repeats 6-repeats	chr5:1411740 Intron 8	Heroin addiction, (Koijam et al., 2020)
SLC6A3	From bp: 1,392,794 to 1,445,440 on chromosome 5	3'UTR-VNTR rs28363170	9-repeats 10-repeats	chr5:1393785 3'UTR	Parkinson disease,(Q. Zeng et al., 2021) Alzheimer's disease, (Fehér et al., 2014)
COMT	From bp: 19,941,371 to 19,969,975 on chromosome 22	Val/Met rs4680	G>A	Chr22:19963748 Exon 3	substance abuse, (Sonia et al., 2021) Risk of breast cancer, (Rai et al., 2017)

2.3.1 *DRD1* Gene

DRD1 is a protein-coding gene located on the long arm on chromosome 5 at position q.35.1(Dearry et al., 1990; Grandy et al., 1990; Sunahara et al., 1990). The location and structure of *DRD1* are illustrated in figure (2.2).

[**] Molecular Location of gene and SNP location obtained from NCBI database https://www.ncbi.nlm.nih.gov/.



(A)

(B)

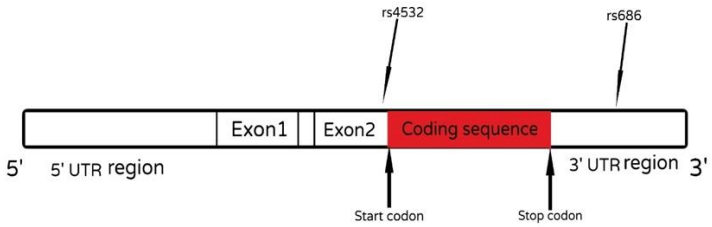


Figure 2.2: (A) The location of the DRD1 gene. (B) Schematic representation of the DRD1 gene.

The *DRD1* gene has an intronless coding region, it has two exons, and it encodes a dopamine receptor D1 subtype transmembrane protein consisting of 446 amino acids (Dearry et al., 1990; Sunahara et al., 1990). The dopamine receptor D1 is the most prevalent in the central nervous system. D1 receptor mRNA is highly distributed in caudate nucleus, olfactory tubercle, and nucleus accumbens. However, it is scarcely found in the substantia nigra, kidney, liver, or heart (Dearry et al., 1990). D1 receptor G-protein activates the cAMP-dependent protein kinase by triggering adenylyl cyclase (Hemmings et al., 1987). D1 receptors control neuronal growth differentiation, mediate various behavioral responses, and modify D2-mediated dopamine receptor activities (Lankford et al., 1988; Seeman et al., 1989). *The DRD1*'s two transcription variants result in alternative beginning locations for the transcription production. *DRD1* has been linked to several neurological disorders such as attention-deficit hyperactivity disorder (ADHD) (Bobb et al., 2005), autism (Adel E. El-Tarras, 2012), and bipolar disorder (Ni et al., 2002).

2.3.1.1 rs4532

The functional single nucleotide polymorphism rs4532 is located in the promoter area of the *DRD1* gene with a 30% frequency in the Caucasian population (Magistrelli et al., 2021). Previous studies has shown that the rs4532 A variant is associated with increased risk for autism spectrum disorders (ASD) alongside deficits in executive and social cognition; men seem to be more severely impacted than women (Hettinger et al., 2008). The G-allele of rs4532 exhibited a dose-response gradient with an increased risk for antipsychotic resistance in schizophrenia as a potential pharmacogenetic marker (Ota et al., 2012).

Furthermore, a meta-analysis of 725 schizophrenia patients have demonstrated an association with rs4532 (Allen et al., 2008), impulsive control disorder in Malaysian PD patients (Zainal Abidin et al., 2015), and the recovery from stroke-induced dysphagia, mainly in older people (Park et al., 2021).

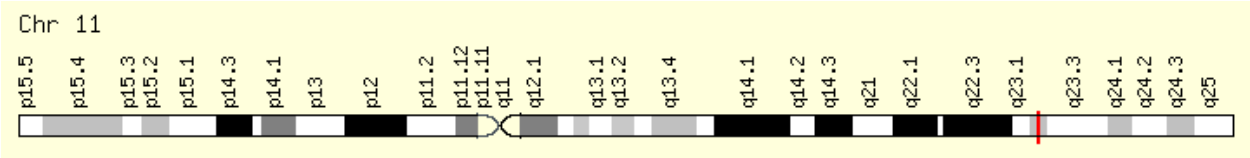
2.3.1.2 rs686

The rs686 (A/G) single nucleotide polymorphism is located in the 3’ end of the untranslated region of *DRD1* gene (Huang et al., 2008). It was associated with higher promoter activity and regulates differential *DRD1* gene expression in luciferase assays (Huang et al., 2008) as a consequence of its location in the binding region of microRNA-504 (Huang & Li, 2009). The A allele has a prevalence of approximately 60% in the Caucasian population. D1 with the A allele exhibits higher activity than the G allele (Magistrelli et al., 2021). Risk of visual hallucination in patients with PD was associated with rs686 A allele (Ferrari, et al., 2016). Research suggests that the G allele of rs686 was associated with decreased risk to schizophrenia relative to the A allele (Zhu et al., 2011). Another study showed that the minor alleles of rs686 (and rs4532) in

a Chinese sample were associated with decreased opioid dependence (Zhu et al., 2013). Further, depressive symptom severity was associated with rs686 variants in a Colombian sample (Jiménez et al., 2018). Finally, in 1000 African Americans and 600 European Americans argues that there are is a significant association between rs686 (and rs4532) and nicotine dependence (Huang et al., 2008).

2.3.*2 DRD2* Gene

The human D2 dopamine receptor gene (*DRD2*) is located on the long arm of chromosome 11 at position q22-q23. Investigation in the structure of *DRD2* showed that the coding region of D2 receptors consists of six introns (Grandy et al., 1989), although the *DRD2* gene consists of 8 exons. Exon 1 is about 250 kilobase upstream of exons 2 through 8. The location and structure of *DRD2* gene are illustrated in figure (2.3).



(A)

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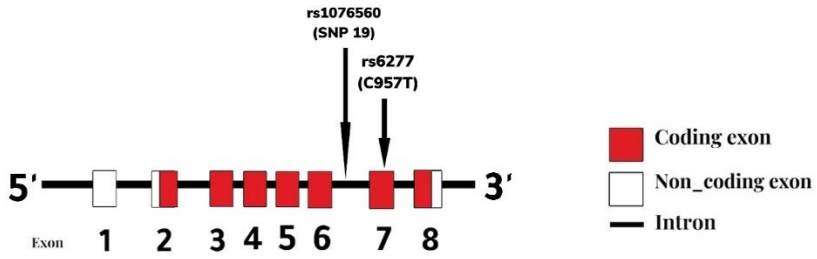


Figure 2.3: (A) The Location of DRD2 gene. (B) Schematic of the DRD2 gene with the location of the two SNPs of interest.

The *DRD2* gene spreads over 270 kilobases and consists of an intron of ~250 kilobase that isolates the initial exon from exons responsible for encoding the receptor protein (Eubanks et al., 1992). *DRD2* encodes for the postsynaptic D2 dopamine receptor protein with seven transmembrane domains (Giros et al., 1989). It is mainly expressed in the striatum and specific areas of the neocortex. The mRNA of the D2 receptor is highly dispersed in the putamen, caudate, and nucleus accumbens (Meador-Woodruff et al., 1996). Activation of this receptor inhibits the activity of adenyl cyclase, inhibits the transition of phosphatidylinositol, leads to higher activity of the K+ channel, and lowers the induction of Ca^{+2} (Monsma et al., 1989). Aside from its postsynaptic function, D2 also exerts its effect as an autoreceptors on dopaminergic neurons to regulate the firing rate and release of dopamine (Carlsson, 1975; Groves et al., 1975). The *DRD2* gene codes for two different alternative isoforms known as D2-short (D2S) and D2-long (D2L) due to the alternative splicing of the RNA messenger. D2S is 415 amino acids long while D2L is 444 amino acids in length (Giros et al., 1989). The main difference between the D2L and D2S isoforms comes from 29 amino acids in the third intracellular domain (Giros et al., 1989; Monsma et al., 1989). The two isoforms have different distributions (Khan et al., 1998; Giros et al., 1989). D2S is usually expressed as presynaptic autoreceptors while D2L mostly functions on the postsynaptic element (Usiello et al., 2000). In areas expressing the D2 receptor, excluding the brain stem, D2S is less abundant than D2L. Both variants act by inhibiting adenylyl cyclase. Nevertheless, D2S has higher efficiency in inhibiting adenylyl cyclase than D2L (Montmayeur & Borrelli, 1991).

Neurological and psychiatric disorders associated with *DRD2* include schizophrenia (J. P. Zhang et al., 2015), cocaine dependence (Volkow et al., 2011),

myoclonus dystonia (Klein et al., 1999), alcoholism (Blum et al., 1990), pathological gambling (De et al., 2001), and a variety of movement disorders (Koning et al., 2012).

2.3.2.1 C957T (rs6277)

C957T (rs6277, C/T) is a synonymous mutation located in exon 7 of the human *DRD2* gene (Seeman et al., 1993). It regulates the expression of *DRD2* by changing the folding of the predicted mRNA. C957T has been associated with decreasing the stability of mRNA and the production of the D2 dopamine receptor in vitro (Duan et al., 2003). The TT genotype of C957T was associated with a higher risk cognitive decline, impaired attention, and memory declines in patients with PD (Cools et al., 2002). Studies revealed that healthy subjects carrying the T allele noticeably had less availability in the D2 receptor and the effect of CC>CT>TT on the binding potential of the D2 receptor (Hirvonen et al., 2004). Another study found that the C957T genotypes altered the density and affinity of the D2 dopamine receptor in 45 healthy subjects studied using [11C]raclopride and position emission tomography (Hirvonen et al., 2009). It was shown that C957T C-homozygotes exhibited better performance in motor learning and slighter alteration in heart rate among both moderate/low-stress conditions than carriers of the T allele (Huertas et al., 2012). Conversely, the TT genotype of C957T was implicated in adaptive learning and shown to improve learning from punishments and reflective behaviors (Xie et al., 2015). The C957T C-allele was more prevalent among patients with schizophrenia (Hänninen et al., 2006). Furthermore, patients with schizophrenia who are homozygous for the C957T T-allele with higher performance on visual recognition tasks (Golimbet et al., 2017).

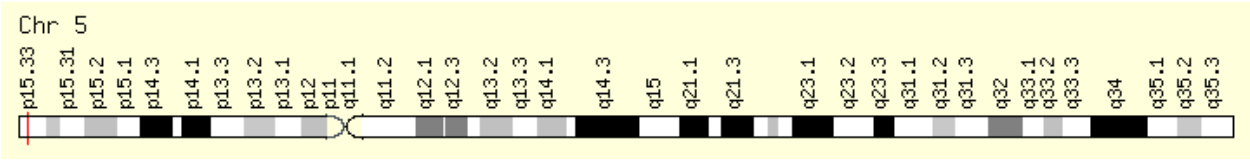
2.3.2.2 SNP19 (rs1076560)

SNP19 (rs1076560, G/T) is an intronic polymorphism located on intron 6 of the *DRD2* gene. SNP19 has a regulatory effect on *DRD2* and effects dopamine availability (Y. Zhang et al., 2007). *DRD2* mRNA in vitro revealed that the intronic SNP19 regulates and adjusts gene transcription and mRNA expression of *DRD2* as well as mRNA splicing of *DRD2* gene (Y. Zhang et al., 2007). The T-allele of SNP19 has been associated with lower expression of the short isoform D2S in comparison to the long isoform D2L. Also, the T-allele of SNP19 in healthy individuals was linked to higher activity of both the striatum and prefrontal cortex and higher performance during memory tasks (Y. Zhang et al., 2007). It was found that patients with PD who are homozygous for the SNP19 G-allele exhibited less gait dysfunction than patients carrying T-allele. However, T-allele carriers were more responsive to treatment of gait dysfunction with dopamine medications (N. S. Miller et al., 2019). Patients with PD who are T-allele carriers exhibited improved performance in motor learning with L-DOPA treatment (Kwak et al., 2013). The prevalence of the SNP19 T-allele in a Han Chinese sample of patients with schizophrenia was higher than that in healthy subjects. Further, the D2L isoform was higher in patients than controls (Zheng et al., 2012).

2.3.3 *SLC6A3* (*DAT1*) Gene

The *SLC6A3* gene, or *DAT1*, is located in the short arm of chromosome 5 at position 15.33 (Vandenbergh et al., 1992). It encodes the dopamine transporter protein (DAT) with 12-spanning hydrophobic transmembrane domains. *SLC6A3* is nearly 53K base pairs long and consists of 15 exons (Shimada., 1991). See figure (2.4) for the location and structure of the *SLC6A3* gene. *SLC6A3* is a member of the SLC6 family with a vast

extracellular loop that contains many sites of N-glycosylation that should be glycosylated to perform the transportation of dopamine (Šerý et al., 2015). The DAT protein is a coupled symporter dependent on Na+/Cl- (L. Iversen, 2000), and it is highly expressed in the striatum presynaptically (Garris et al., 1994). It is responsible for the reuptake of dopamine from the synaptic cleft into presynaptic terminals. It is the central controller of dopamine synaptic concentrations (Kilty et al., 2016). Polymorphisms in the *SLC6A3* gene are found in the promoter, coding, and non-coding regions. The *SLC6A3* gene is implicated with several diseases and movement disorders (e.g., infantile parkinsonism dystonia (Kurian et al., 2009)).



(A)

(B)

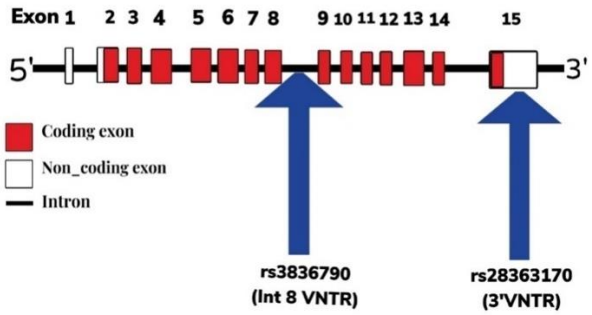


Figure 2.4: (A) Location of SLC6A3 gene (B) Schematic representation of DAT gene, it consists of 15 exons. High portion of exon 15 contribute to the 3'-untranslated region of RNA with a variable number tandem repeat (VNTR). The location of rs3836790 and rs28363170 is indicated.

For instance, by postmortem comparison of brains of patients with PD with normal controls using the binding of [1251]altropane, it was shown that the binding of

DAT in patients with PD was only 13% of that noticed in healthy putamen, 17% of the detected value in the normal caudate nucleus, and 25% of the value noticed in normal nucleus accumbens (Madras et al., 1998). Mutations in the *PARK2* gene (associated with familial PD) can negatively affect the regulation of DAT as the muted parkin protein will not be able to ubiquitylate and degrade the misfolded transporters that aggregate, thus impairing the reuptake of synaptic dopamine (Jiang et al., 2004). Further, α-synuclein, which is also implicated in familial PD, was found to increase the accumulation of DAT by forming a complex with DAT and thus facilitating the reuptake of dopamine and induce degeneration of dopaminergic neurons (Lee et al., 2001). The level of DAT protein differs among individuals according to age and different neuronal pathologies (Volkow et al., 1994). For example, one study found an 84% reduction of DAT in the midbrain in mild PD patients, 78% reduction in posterior putamen, 59% reduction in anterior putamen, and 39% reduction in the caudate nucleus (Frost et al., 1993). Also, a significant decrease in DAT expression has been observed in patients with PD in relation to age (Ishibashi et al., 2014). In a study with Lesch-Nyhan disease, binding to DAT was decreased in both the caudate and putamen in patients more than healthy controls (Wong et al., 1996). Another study found a 70% higher density of the DAT protein in the brain as a function of age in patients with (ADHD) than in controls (Dougherty et al., 1999). In Tourette's syndrome, and by using [123I] beta-CIT binding, it was found that the density of DAT is higher in patients than in the control group (Malison et al., 1995).

Long-lasting use of drugs such as cocaine is associated with elevated DAT (Bowers et al.,1998). Patients with ADHD treated with methylphenidate have shown fewer existing binding sites for DAT than a standard control, and that was associated

with an improvement in their clinical symptoms (Dresel et al., 2000). DAT is considered the main target of ADHD treatments by the inhibition of DAT with several pharmacological agents such as methylphenidate, pemoline, bupropion, and dextroamphetamine (Cook et al., 1995).

2.3.3.1 3'UTR VNTR (rs28363170)

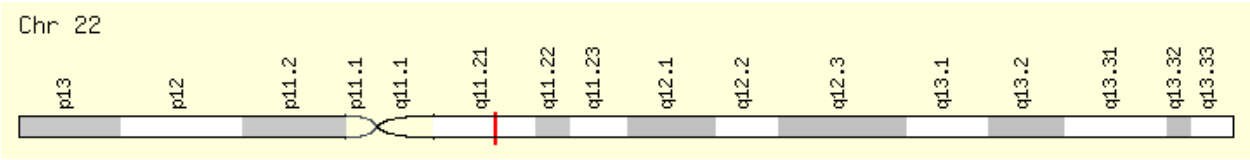
3'UTR VNTR (rs28363170) is an insertion or deletion polymorphism of 40-base pair (bp) variable-number of tandem repeat (VNTR) in the 3'UTR of the *SLC6A3* gene. The VNTR varies from 3-11 copies, but the 9 and 10 repeats (9R, 10R) are the most prevalent alleles of this polymorphism (Sano et al., 1993; Vandenbergh et al., 1992). The variation in SNPs and the length of the tandem repeats in 3'UTR can decrease or increase DAT expression, affecting the diverse expression and density of DAT in the human brain (Fuke et al., 2001; G. M. Miller & Madras, 2002). The expression of the 10R allele is higher than 9R repeat. The 10R allele is linked with higher expression of the DAT protein, thus presumably leading to lower dopamine levels in the synaptic cleft (Dreher et al., 2009). The rs28363170 polymorphism was linked to several diseases such as PD (Le Couteur et al., 1997), (ADHD) (Pattarachotanant et al., 2010; Cook et al., 1995), schizophrenia (Cordeiro et al., 2010), smoking (Sabol et al.,1999), alcohol (Ueno et al., 1999) cocaine addiction (Iii et al., 2016), and Alzheimer's disease (Fehér et al., 2014). Both rs28363170 and rs3836790 exhibited significant effects in studies of L-DOPA response in PD (Guin et al., 2017). For example, rs28363170 was associated with higher effectiveness of L-DOPA and methylphenidate for motor symptoms in patients with PD (Moreau et al., 2015). A meta-analysis found a protective effect of the 10R allele of rs28363170 against PD (Q. Zeng et al., 2021).

2.3.3.2 Intron8 VNTR (rs3836790)

Intron8 VNTR (Int8 VNTR) is located within intron 8 of the *SLC6A3* gene including 30-bp VNTR. It was found that a lower number of repeats in the Int8 VNTR was associated with a higher level of the expression of *SLC6A3* gene transcript in the post mortem tissue (Brookes et al., 2007). The most common alleles have 5- and 6-repeats. In patients with PD, Int8 VNTR 6-repeat homozygotes had better response to L-DOPA and methylphenidate for treating their motor symptoms (Moreau et al., 2015). Further, the 6-repeat variant of the Int8 VNTR has been implicated heroin addiction in an Indian sample (Koijam et al., 2020).

2.3.4 *COMT* Gene

The *COMT* gene is located on the long arm of chromosome 22 at position q11.1-q11.2; it spans nearly 28K base pairs and consists of six exons (Grossman et al., 1992). The location of the *COMT* gene and the schematic structure is illustrated in figure (2.5).



(A)

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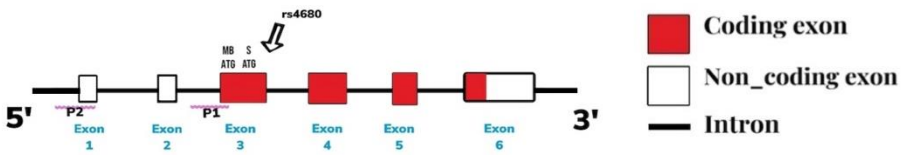


Figure 2.5: (A) Location of the COMT gene. (B) Schematic structure of the COMT gene with its 6 exons. SNP of interest rs4680 in indicated in the diagram.

The *COMT* gene encodes for the enzyme catechol-O-methyltransferase (COMT), which catalyzes the addition of methyl to the phenolic hydroxyl group on catecholamine neurotransmitters dopamine, epinephrine, norepinephrine, and catechol steroids (Weinshilboum et al., 1999). COMT converts dopamine to 3-methoxytyramine (3-MT), which in turn is reduced to homovanillic acid by the monoamine oxidase, and subsequently excreted in the urine (Juárez Olguín et al., 2016). The *COMT* gene encodes two isoforms of the COMT enzyme that are translated from the same gene but with different promoters (Lundström *et al*. 1991). One isoform is the long membrane-bound isoform, and the other is the short soluble isoform (Mannisto and Kaakkola, 1999). The affinity for the membrane-bound isoform to dopamine and norepinephrine substrates is higher than that of the soluble isoform (Lotta et al., 1993). It was found that the membrane-bound isoform is more prevalent in the brain, mainly in the prefrontal cortex (Matsumoto et al., 2003), while the short soluble isoform is detected mainly in the liver, kidney, and adrenal glands (TENHUNEN et al., 1994). COMT plays a role in the metabolism of catechol drugs; COMT deactivates drugs such as L-DOPA (Jo & Palma, 2007; Weinshilboum et al., 1974). COMT inhibitors are commonly prescribed for patients with PD lower the central metabolism of L-DOPA and increase its action (Jo & Palma, 2007). COMT plays a significant role in areas that lack DAT, such as the prefrontal cortex (Gogos et al., 1998). Genetic variations in the *COMT* gene has been examined in several diseases, such as estrogen-sensitive breast cancer (Ahsan et al., 2004) and anxiety (Bækken et al., 2008), but mainly it has been well studied in schizophrenia (Harrison & Weinberger, 2005).

2.3.4.1 Val158Met (rs4680)

Val158Met (rs4680), G>A is a single nucleotide polymorphism that is located in exon 3 at codon 158 in the *COMT* gene. At the biological level, valine exhibits more thermostability of the protein at 37 °C than methionine. Thus, it influences the activity of COMT and impacts the degradation of catecholamines, such as dopamine, in the synaptic cleft (Lachman et al.1996). This polymorphism does not affect mRNA levels, but it influences the abundance of the protein and the enzyme activity (Gogos et al., 1998). The substitution of valine to methionine at codon 158 in the *COMT* gene has been shown to play an essential role in dopaminergic neurotransmission in the prefrontal cortex due to the lack of DAT (Meyer-Lindenberg et al., 2006). Carriers of Val allele (GG) were shown to have a 3-4 fold increase in the activity of the enzyme than carriers of Met allele (AA) resulting in lower dopamine levels specifically in prefrontal cortex and hippocampus (Chen et al., 2004). The A-allele of the Val158Met was expressed more prevalent in patients with PD who experience higher pain levels (Lin et al., 2017). In another study that aimed to determine whether several genes, including *COMT,* affect cognitive decline in PD patients, found that patients with the Met/Met genotype of *COMT* may have higher risk (Paul et al., 2016). In many studies rs4680 was significantly associated with L-DOPA efficacy in PD (Guin et al., 2017). Furthermore, the Met-allele was less prevalent in schizophrenia siblings, indicating that the *COMT* Val-allele could be associated with abnormal prefrontal cortex function and an increased risk for schizophrenia (Egan et al., 2001). This *COMT* polymorphism is highly prevalent in and is considered one of the most studied polymorphisms in neurosciences (Lotta et al., 1995; Palmatier et al., 1999).

Chapter 3

Methodology

3.1 Study Design

This thesis is a case-control study in which patients diagnosed with PD were compared to healthy controls matched for age and sex.

3.2 Study Subjects

The study subjects of this study consisted of 106 individuals: 55 patients with PD and 51 healthy controls. Among the 55 PD patients, there were 22 PD patients with comorbid depression and 33 patients without depression. A clinical neurologist assigned the primary diagnosis. The severity of motor symptoms was assessed using the unified Parkinson's disease rating scale. The PD stage was evaluated using the Hahn & Yahr scale. Patients with PD were assessed for dementia using the mini-mental status examination. All patients with PD underwent a structured clinical interview for psychiatric manifestations. All comorbidities were reported and regressed in the final analysis model. The age of the enrolled participants was range from 55-75 years. Also, age/gender/education-matched healthy subjects were recruited from the same populations the patients come from. The Al-Quds University Research Ethics Committee approved all performed research and according to the Declaration of Helsinki.

3.3 Study Area

The study target population was Palestinian patients of PD that were recruited from neurological clinics and centers throughout the West Bank of Palestine.

3.4 Ethical Considerations

Personal information obtained about subjects in the course of this study did not leave the premises of the Palestinian Neuroscience Initiative at Al-Quds University in any form that would identify individual issues. Subject confidentiality is protected by labeling all electronic files, paper files, questionnaires, and DNA/serum samples in coded form and identifying each participant by an assigned subject ID number. Paper, electronic data, DNA, and serum are kept in locked offices of the Palestinian Neuroscience Initiative. Information about individual subjects were available only to specific key personnel of the study. It did not become part of the subjects' medical records unless a subject request explicitly in writing that we include copies in the medical charts or forward these records to their primary care physician. The key to the coded subject ID numbers identifying the study participants is kept in a separate secure location, with access strictly limited to Dr. Herzallah and specific key personnel. The data obtained from the study procedure were used primarily for research purposes. However, this data may help guide a patient's future treatment after the study, in which case, a patient would be given the option to release the information to a treating clinician. The medical and research records may be audited by the Palestinian government, research lab accrediting agencies in carrying out their duties. Government and lab accrediting agencies may be permitted to make copies of these records. If any parts of a participant's medical records are copied, the lab will make every reasonable effort to remove their name from any copied information.

3.5 Genotyping

3.5.1 DNA Extraction

DNA was isolated from whole blood samples. Master Pure Complete DNA Purification ® kit was used. Ethylenediaminetetraacetic acid (EDTA) Vacutainer tube were used to withdraw 5 ml of blood. Centrifugation was done for 15 minutes at 1000 xg. 600 ul of the buffy coat was transferred into a new tube, then vortex mixed the buffy coat sample. 300 ul of the sample was transferred to another microcentrifuge tubes. 1.2 ml red cell lysis solution was added to each tube. Proteinase K was used to remove and digest the contamination proteins in the preparation of nucleic acid, and to degrades nucleases in the DNA extraction. The tubes were inverted three times to mix and then flicked the bottom of the box. The tubes were incubated at room temperature for 5 minutes, inverted three times to mix then flicked the tubes. Then, the tubes were incubated at room temperature for additional 5 minutes, inverted three times to mix, and then flicked tubes. White blood cells were pelleted by centrifugation at 14000 rpm for 25 seconds. Pulled off most of the supernatant, leaving 25ul of liquid. vortex mixed the tubes to suspend the pellet. White blood cells were resuspended in 600 ul of tissue and cell lysis solution by pipetting the cells up and down 5-7 times. 300 ul of the MPC protein precipitation reagent were added. Vortex mixed the tubes for at least 30 seconds. Centrifugation was done for 10 minutes at 10000 xg in a microcentrifuge. The debris was pelleted. Then, the supernatant was put in a clean microcentrifuge tube, and 750 ul of isopropanol was added. The tube was inverted several 30-40 times. DNA was pelleted by centrifugation at 4 ℃ for 10 minutes at 10000 xg. The supernatant was poured off without dislodging the pellet. DNA was resuspended in 35 ul of Tris-EDTA (TE) buffer and stored at -20 ℃. DNA qualification and quantification (measurement of

purity and concentration) were done for each sample via spectrophotometry. The ratio of absorbencies at 260 nm and 280 nm for DNA samples were within the range $2 \geq A260/ A280 \geq 1.8$.

3.5.2 PCR Primers and Restriction Enzymes

The genotyping of the seven SNPs was done by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP). Primers sequences and restriction enzymes required for the PCR-RFLP of each SNP were taken from previously published studies; see table (3.1) for references of PCR primers. Nucleotide sequences of PCR primers, restriction enzymes, PCR product size, and length of digested fragments are provided in Table 3.1.

Table 3.1: Illustration of selected genes, selected SNPs, the method for genotyping, primer sequence, PCR product, restriction enzymes, length of digested fragments, and primer reference.

Gene	SNP	Method	Primer	Primer sequence	PCR Product (bp)	Restriction enzymes	Length of digested fragments (bp)	Primer reference
DRD1	rs686	PCR-RFLP	Forward Reverse	5'- TGGAGAAGCTGTCCCCAG -3' 5'-GTACCTTAGTTTCiTAATAGCGA-3'	189	Bspl286I	T allele: 189 C allele: 167+22 bp	(Cichon et al., 1994) (Misener et al., 2004)
DRD1	rs4532	PCR-RFLP	Forward Reverse	5'-ACTGACCCCTATTCCCTGCT -3' 5'-AGCACAGACCAGCGTGTTC -3'	207	Ddel	G allele: 146+61 A allele: 146+42+19	(Cichon et al., 1994) (Misener et al., 2004)
DRD2	SNP19 rs1076560	PCR-RFLP	Forward Reverse	5' - AGC ATC TCC ATC TCC AGC TC -3' 5'- AGG CCA TGA GAG ACA AGT CC -3'	323	Hphl	C allele: 175+148 A allele: 175+114+34	(Koehler et al., 2011)
DRD2	C957T rs6277	PCR-RFLP	Forward (1) Forward (2) Reverse	5'- ACC ACG GTC TCC ACA GCA CTC T -3' 5'- ACC ATG GTC TCC ACA GCA CTC T -3' 5'- ATG GCG AGC ATC TGA GTG GCT -3'	196	Taq-alpha I	T allele: 196 C allele: 174+22	(M. M. Hirvonen et al., 2009)
SLC6A3	3'UTR-VNTR rs28363170	PCR	Forward Reverse	5'- TGT GGT GGG AAC GGC CTG AG -3' 5'- CTT CCT GGA GGT CAC GGC TCA AGG -3'	9R= 440 10R= 480			(Tong et al., 2015)
SLC6A3	Int8 VNTR rs3836790	PCR	Forward Reverse	5'- GCT TGG GGA AGG AAG GG -3' 5'- TGT GTG CGT GCA TGT GG -3'	5R=368 6R=398			(Tong et al., 2015)
COMT	rs4680	PCR-RFLP	Forward Reverse	5'- TAC TGT GGC TAC TCA GCT GTG C -3' 5'- GTG AAC GTG GTG TGA ACA CC -3'	237	Nlalll	Val158: G allele: 114+54+41+27 Met158: A allele: 96+54+41+18+27	(Lavigne et al., 1997)

3.5.3 PCR Primer Reconstitution

The tubes of the lyophilized primers were centrifuged, and after that, they were reconstituted with ultrapure nuclease-free water to form a stock solution with 100 pmol/ul of each primer. To make a ten pmol/ul solution, each microliter aliquot from the stock, the solution was diluted with 9 ul nuclease-free water.

3.5.4 PCR Mix

Reactions for PCR-RFLP for each SNP were done in one tube. All PCR reaction components of rs686, rs4532, SNP19, 3'UTR VNTR, Int8 VNTR, rs4680 are illustrated in Table 3.2, while the PCR component for C957T is shown in the Table 3.3.

Table 3.2: PCR components for rs686, rs4532, SNP19, 3'UTR VNTR, Int8 VNTR, and rs4680.

Component	Volume Per Sample	Final concentration
Primer Forward	0.5uL	5 pmol
Primer Reverse	0.5uL	5 pmol
DNA Template	1uL	50 ng
Distilled Water	8uL	-
Green Mix	10uL	1X
Total Reaction Volume	20uL	

Table 3.3: PCR components for C957T.

Component	Volume Per Sample	Final concentration
Primer Forward 1	0.25uL	2.5 pmol
Primer Forward 2	0.25uL	2.5 pmol
Primer Reverse	0.5uL	5 pmol
DNA Template	1uL	100 ng
Distilled Water	8uL	-
Green Mix	10uL	1X
Total Reaction Volume	20uL	

3.5.5 PCR Protocol for Determining the Different Genetic Variants of Selected SNPs in Candidate Genes.

Protocol cycles for PCR amplification for rs686, rs4532, SNP19, C957T, 3'UTR VNTR, Int8 VNTR, and rs4680 are illustrated in the Table 3.4.

Table 3.4: PCR protocol for rs686, rs4532, SNP19, C957T, 3'UTR VNTR, Int8 VNTR, and rs4680.

Genes	Type of Cycle						No. of Cycles
	Initial Denaturation	Denaturation	Annealing	Extension	Final Extension	Cooling	
DRD1 Rs686	95°C*5min	95°C*30sec	57°C*30sec	72°C*45sec	72°C*5min	4°C	35
DRD1 Rs4532	95°C*5min	95°C*30sec	57°C* 30sec	72°C*45sec	72°C*5min	4°C	35
DRD2 SNP19	95°C*2min	95°C*30sec	58°C*30sec	72°C*30sec	72°C*5min	4°C	30
DRD2 C957T	95°C*2min	95°C*30sec	62°C*30sec	72°C*30sec	72°C*5min	4°C	40
SLC6A3 3'UTR VNTR	95°C*5min	95°C*30sec	60°C*30sec	70°C*1min	70°C*5min	4°C	35
SLC6A3 Int8 VNTR	94°C*5min	94°C*45sec	62°C*30sec	72°C*45sec	72°C*5min	4°C	35
COMT Rs4680	94°C*3min	93°C*45sec	55°C*1min	72°C*1min	72°C*5min	4°C	39

3.5.6 Enzyme Digestion Protocol

PCR products for rs686, rs4532, SNP19, C957t, and rs4680 were digested with a proper restriction enzyme. Enzyme digested components are provided in tables 3.5-3.9.

Table 3.5: Enzyme digestion protocol for DRD1 rs686.

Component	Volume Per Sample	Incubation time + Temperature
Bspl286I enzyme	0.2uL	1 hour at 37°C
Buffer	2uL	
Water	12.8uL	
PCR	5uL	

Table 3.6: Enzyme digestion protocol for DRD1 rs4532.

Component	Volume Per Sample	Incubation time + Temperature
Ddel enzyme	0.5uL	1 hour at 37℃
Buffer	2uL	
Water	12.5uL	
PCR	5uL	

Table 3.7: Enzyme digestion protocol for DRD2 SNP19.

Component	Volume per sample	Incubation time + Temperature
Hphl enzyme	0.2uL	3 hours at 37℃
Buffer	2uL	
Water	14.8	
PCR	3uL	

Table 3.8: Enzyme digestion protocol for DRD2 C957T.

Component	Volume per sample	Incubation time + Temperature
Taq alpha I enzyme	0.5	1 hour at 65℃
Buffer	2uL	
Water	12.5uL	
PCR	5uL	

Table 3.9: Enzyme digestion protocol for COMT rs4680.

Component	Volume per sample	Incubation time + Temperature
NIaIII enzyme	0.2ul	3 hours at 37℃
Buffer	2ul	
Water	12.8ul	
PCR	5ul	

3.5.7 Agarose Gel Electrophoresis

We added 3 gm (3%) of agarose powder with 100ml of 1x Tris- acetate-EDTA (TAE). The flask was microwaved until agarose dissolved completely. The solution was checked occasionally to avoid boiling. The flask (Agarose) was cooled until touching the bottom of the beaker with bare hands. At this point, two drops of DNA stain ethidium bromide (2.0 ul) were added. We checked the gel box to confirm it was well

sealed. Gel was positioned inside the electrophoresis chamber and covered with running buffer 1x Tris-borate-EDTA (TBE), and the comb was put. After the gel was solidified (for about 30 minutes), the comb was gently removed by pulling it evenly upward. With the use of a micropipette, 18 ul of the sample mixture of the amplified and digested PCR with R.E. and 5 ul of 50 bp DNA ladder size marker was carefully loaded into the wells. A cover was placed on the electrode box, and the gel was electrophoresed at 100V for 50 minutes. Gel was carefully removed and placed in UV light for the bands to be visualized.

3.6 Genetic Scores

Based on literature-reported functionality of each SNP genetic scores were given to each genotype in each SNP according to the increase or decrease in dopamine level. Each genotype that positively affects dopamine level was given a 1 score, while genotypes that lead to lower dopamine were given a -1 score. Genotype with intermediate effect on dopamine was given 0 scores.

3.7 Statistical Analysis

The differences between the frequencies of genotype and alleles between PD patients and healthy controls were compared through chi-squared test in SPSS version 25. The Hardy-Weinberg equilibrium was calculated for all polymorphisms per gene as follows:

- Frequency of allele 1 (p) = (count genotype 11*2 + count genotype 12)/ (total n*2)
- Frequency of allele 2 (q) = (count genotype 22*2 + count genotype 12)/ (total n*2)
- Frequencies of expected genotype:
    - Genotype 11 = (q^2)*(total n)

- Genotype 12 = (2pq)*(total n)
- Genotype 22 = (p^2)*(total n)

Chi-squared determined the difference between the observed and expected genotypes. P value determined significance according to a Bonferroni correction of alpha. P value < 0.05 was considered statistically significant. Mann-Whitney and Kruskal-Wallis tests were used to score in different groups. In *SLC6A3* (intron8), to make the calculation easier, 3/6 genotypes were added to 5/6 genotypes, and in *SLC6A3*(3'UTR), 8/10 and 9/11 genotypes were included with 9/10 genotype, and 10/11 genotype were included with 10/10 genotype.

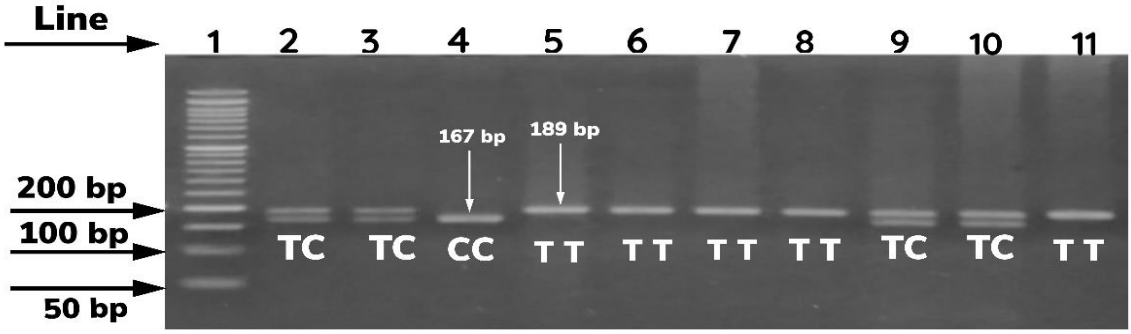
Chapter 4

Results

4.1 RFLP-PCR

In figure 4.1 we illustrate examples from the gel electrophoresis results obtained for *DRD1* gene polymorphisms (rs4532, rs686), *DRD2* gene polymorphisms (rs1076560, rs6277), *SLC6A3* gene polymorphisms (rs3836790, rs28363170) and *COMT* gene polymorphism (rs4680).

(A) *DRD1* rs686



(B) DRD2 rs6277

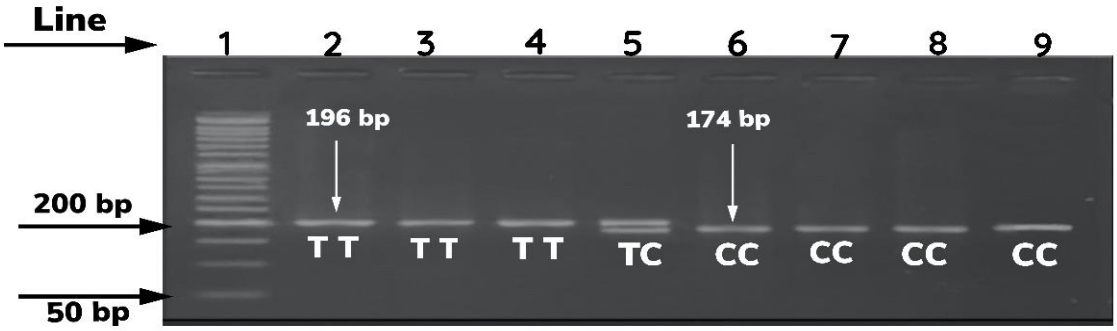


Figure 4.1: Representative photo for gel electrophoreses showing PCR-RFLP of (A) DRD1 gene (rs686), PCR product (189 bp) digested with Bsp1286I restriction enzyme. Line 1 indicates 50 bp DNA ladder, lines (5,6,7,8,11) indicates homozygous TT (189bp), line (2,3,9,10) indicates heterozygous TC (189+167+22) bp, line (4) shows homozygous CC (167+22) bp; (B) DRD2 gene (rs6277), PCR product (196 bp) digested with Taq-alpha I restriction enzyme. Line 1 indicates 50 bp DNA ladder, lines (2,3,4)) indicates homozygous TT (196bp), line (5) indicates heterozygous TC (196+174+22) bp, line (6,7,8,9) indicates homozygous CC (174+22) bp. (Note that the small band not visible in the gels).

4.2 Collective Genotype Differences between Patients with PD and Healthy Controls

As summarized in table 4.1, we conducted chi-squared tests for independence to examine the collective genotype frequency differences between patients with PD and healthy controls. The main significant difference emerged in the *COMT* rs4680, while *DRD1* rs4532, *SLC6A3* rs3836790 and rs28363170 exhibited approaching significance results (with a Bonferroni correction of alpha = 0.007).

Table 4.1: Genotype Frequency comparison for DRD1, DRD2, SLC6A3, and COMT SNPs between patients with PD and healthy controls using chi-squared

Gene/SNP	Genotypes	PD N=55	Healthy N=51	Chi-Squared (X^2)	*P*
DRD1 **rs4532**	AA	15 (27.3%)	4 (7.8%)	7.159	0.028
	GA	12 (21.8%)	17 (33.3%)		
	GG	28 (50.9%)	30 (58.8%)		
DRD1 **rs686**	TT	26 (47.3%)	23 (45.1%)	3.930	0.140
	CT	23 (41.8%)	27 (52.9%)		
	CC	6 (10.9%)	1 (2.0%)		
DRD2 **rs1076560**	AA	0	0	0.053	1.00
	CA	5 (9.1%)	4 (7.8%)		
	CC	50 (90.9%)	47 (92.2%)		
DRD2 **rs6277**	CC	19 (34.5%)	11 (21.6%)	2.219	0.330
	TC	24 (43.6%)	26 (51.0%)		
	TT	12 (21.8%)	14 (27.5%)		
SLC6A3 **rs3836790**	5/5	9 (16.4%)	3 (5.9%)	7.157	0.028
	5/6	18 (32.7%)	29 (56.9%)		
	6/6	28 (50.9%)	19 (37.3%)		
SLC6A3 **rs28363170**	9/9	13 (23.6%)	5 (9.8%)	5.822	0.054
	9/10	31 (56.4%)	27 (52.9%)		
	10/10	11 (20%)	19 (37.3%)		
COMT **rs4680**	AA	4 (7.3%)	21 (41.2%)	18.375	<0.0001
	GA	39 (70.9%)	19 (37.3%)		
	GG	12 (21.8%)	11 (21.6%)		

4.3 Genotype Differences between Patients with PD and Healthy Controls

Using chi-squared test for goodness-of-fit for each of the genotypes, we compared patients with PD and healthy controls (with a Bonferroni correction of alpha = 0.017 per

SNP). See Table 4.2. In the *DRD1* gene, the AA genotype of rs4532 was more frequent in patients with PD than healthy controls. In the *SLC6A3* gene Intron8, the 5/6 genotype was more frequent in healthy control. In the 3'UTR SNP of *SLC6A3* gene, the 10/10 genotype was more prevalent in healthy controls than PD patients. For the *COMT* gene, the AA genotype is more frequent in healthy controls, while the GA genotype is more frequent in PD patients.

Table 4.2:Chi-squared comparison of frequency of genotypes between patients with PD and healthy controls.

Gene/SNP	**Genotype**	**PD N=55**	**Healthy N=51**	**Chi-squared (X^2)**	**Odds Ratio (95% Cl)**	***P***
DRD1	AA	15 (27.3%)	4 (7.8%)	6.790	4.406 (1.353-14.351)	0.009
rs4532	GA	12 (21.8%)	17 (33.3%)	1.766	0.558 (0.235-1.326)	0.184
	GG	28 (50.9%)	30 (58.8%)	0.669	1.378 (0.639-2.970)	0.413
DRD1	TT	26 (47.3%)	23 (45.1%)	0.050	0.916 (0.427-1.968)	0.822
rs686	CT	23 (41.8%)	27 (52.9%)	1.314	1.565 (0.726-3.373)	0.252
	CC	6 (10.9%)	1 (2.0%)	3.435	0.163 (0.019-1.407)	0.114
DRD2	AA	0	0			
rs1076560	CA	5 (9.1%)	4 (7.8%)	0.053	0.851 (0.215-3.362)	1.000
	CC	50 (90.9%)	47 (92.2%)	0.053	1.175 (0.297-4.641)	1.000
DRD2	CC	19 (34.5%)	11 (21.6%)	2.196	0.521 (0.219-1.242)	0.138
rs6277	TC	24 (43.6%)	26 (51.0%)	0.573	1.343 (0.625-2.887)	0.449
	TT	12 (21.8%)	14 (27.5%)	0.454	1.356 (0.558-3.293)	0.501
SLC6A3	5/5	9 (16.4%)	3 (5.9%)	2.896	3.130 (0.797-12.292)	0.089
rs3836790	5/6	18 (32.7%)	29 (56.9%)	6.246	0.369 (0.167-0.813)	0.012
	6/6	28 (50.9%)	19 (37.3%)	1.999	1.747 (0.804-3.794)	0.157
SLC6A3	9/9	13 (23.6%)	5 (9.8%)	3.592	2.848 (0.936- 8.667)	0.058
rs28363170	9/10	31 (56.4%)	27 (52.9%)	0.125	1.148 (0.534-2.469)	0.724
	10/10	11 (20%)	19 (37.3%)	3.883	0.421 (0.176-1.006)	0.049
COMT	AA	4 (7.3%)	21 (41.2%)	16.878	8.925 (2.797-28.483)	0.000
rs4680	GA	39 (70.9%)	19 (37.3%)	12.096	0.244 (0.108-0.549)	0.001
	GG	12 (21.8%)	11 (21.6%)	0.001	0.985 (0.391-2.484)	0.975

4.4 Comparison of Allele Frequencies

In table 4.3 we investigated differences in alleles frequencies in the studied SNPs for 55 patients with PD and 51 healthy controls. Although none of the SNP allelic frequencies

passed the Bonferroni corrected alpha level (0.007), approaching-significance results were observed in the *DRD1* rs4532, *SLC6A3* rs28363170, and *COMT* rs4680.

Table 4.3: Allele's frequencies chi-squared comparisons between patients with PD and healthy controls.

Gene/SNP	Allele	PD N=55	Healthy N=51	Chi-squared (X^2)	Odds Ratio (95% Cl)	*P*
DRD1	A	42 (38.2%)	25 (24.5%)	4.577	1.902(1.051-3.442)	0.032
rs4532	G	68 (61.8%)	77 (75.5%)			
DRD1	T	75 (68.2%)	73 (71.6%)	0.288	0.851(0.473-1.533)	0.591
rs686	C	35 (31.8%)	29 (28.4%)			
DRD2	A	5 (4.5%)	4 (3.9%)	0.051	1.167(0.304-4.470)	1.000
rs1076560	C	105(95.5%)	98 (96.1%)			
DRD2	C	62 (56.4%)	48 (47.1)	1.835	1.453(0.846-2.497)	0.175
rs6277	T	48 (43.6%)	54 (52.9%)			
SLC6A3	5/5	36 (32.7%)	33 (33%)	0.002	0.988(0.555-1.758)	0.966
rs3836790	6/6	74 (67.3%)	67 (67%)			
SLC6A3	9/9	56(53.3%)	37 (38.1%)	4.682	1.853(1.057-3.248)	0.030
rs28363170	10/10	49 (46.7%)	60 (61.9%)			
COMT	A	47 (42.7%)	61 (59.8%)	6.176	0.501(0.290-0.867)	0.013
rs4680	G	63 (57.3%)	41 (40.2%)			

4.5 Genotype Combinations Frequencies

We calculated the difference genotype combinations and compared the genotype prevalence between patients with PD and healthy controls using chi-squared for independence (table 4.4). Significant differences emerged in the *DRD1* rs4532 AA vs. GG+GA, *SLC6A3* rs3836790 6/6+5/5 vs. 5/6, *COMT* rs4680 AA/GG vs. GA and *COMT* rs4680 GG/GA vs. AA. No other genotype combinations among the remaining SNPs showed significant differences between PD and healthy controls. Family-wise Bonferroni correction of alpha at 0.017 was applied to preserve the level of significance.

Table 4.4: Genotype combinations chi-squared comparison of patients with PD and healthy controls.

Gene/SNP	Genotype combinations	PD N=55	Healthy N=51	Chi-squared (X^2)	Odds Ratio (95% Cl)	*P*
DRD1 **rs4532**	AA+GA GG	27 (49.1%) 28 (50.9%)	21 (41.2%) 30 (58.8%)	0.669	1.378 (0.639-2.970)	0.413
	AA GG+GA	15 (27.3%) 40 (72.7%)	4 (7.8%) 47 (92.2%)	6.790	4.406 (1.353-14.351)	0.009
	GG+AA GA	43 (78.2%) 12 (21.8%)	34 (66.7%) 17 (33.3%)	1.766	1.792 (0.745-4.256)	0.184
DRD1 **rs686**	CC+CT TT	29 (52.7%) 26 (47.3%)	28 (54.9%) 23 (45.1%)	0.050	0.916 (0.427-1.968)	0.822
	CC TT+CT	6 (10.9%) 49 (89.1%)	1 (2%) 50 (98%)	3.435	6.122 (0.711-52.737)	0.114
	TT+CC CT	32 (58.2%) 23 (41.8%)	24 (47.1%) 27 (52.9%)	1.314	1.565 (0.726-3.373)	0.252
DRD2 **rs1076560**	AA+CA CC	5 (9.1%) 50 (90.9%)	4 (7.8%) 47 (92.2%)	0.053	1.175 (0.297-4.641)	1.000
	AA CC+CA	0 55 (100%)	0 51(100%)			
	CC+AA CA	50 (90.9%) 5 (9.1%)	47 (92.2%) 4 (7.8%)	0.053	0.851 (0.215-3.362)	1.000
DRD2 **rs6277**	TT+TC CC	36 (65.5%) 19 (34.5%)	40 (78.4%) 11 (21.6%)	2.196	0.521 (0.219-1.242)	0.138
	TT CC+TC	12 (21.8%) 43 (78.2%)	14 (27.5%) 37 (72.5%)	0.454	0.738 (0.304-1.792)	0.501
	CC+TT TC	31 (56.4%) 24 (43.6%)	25 (49%) 26 (51%)	0.573	1.343 (0.625-2.887)	0.449
SLC6A3 **rs3836790**	5/5+ 5/6 6/6	27 (49.1%) 28 (50.9%)	32 (62.7%) 19 (37.3%)	1.999	0.573 (0.264-1.244)	0.157
	5/5 6/6+ 5/6	9 (16.4%) 46 (83.6%)	3 (5.9%) 48 (94.1%)	2.896	3.130 (0.797-12.292)	0.089
	6/6+5/5 5/6	37 (67.3%) 18 (32.7%)	22 (43.1%) 29 (56.9%)	6.246	2.710 (1.230-5.971)	0.012
SLC6A3 **rs28363170**	9/9+ 9/10 10/10	45 (81.8%) 10 (18.2%)	34 (66.7%) 17 (33.3%)	3.200	2.250 (0.916-5.529)	0.074
	9/9 10/10+9/10	13 (23.6%) 42 (76.4%)	5 (9.8%) 46 (90.2%)	3.592	2.848(0.936-8.667)	0.058
	10/10+9/9 9/10	24 (43.6%) 31 (56.4%)	24 (47.1%) 27 (52.9%)	0.125	0.871 (0.405-1.873)	0.724
COMT **rs4680**	GG+GA AA	51(92.7%) 4(7.3%)	30 (58.8%) 21 (41.2%)	16.878	8.925(2.797-28.483)	<0.0001
	GG AA+GA	12 (21.8%) 43 (78.2%)	11 (21.6%) 40 (78.4%)	0.001	1.015(0.403-2.558)	0.975
	AA+GG GA	16 (29.1%) 39 (70.9%)	32 (62.8%) 19 (37.2%)	12.096	0.244 (0.108-0.549)	0.001

4.6 Hardy-Weinberg equilibrium

We calculated the Hardy-Weinberg equilibrium for the studied SNPs for both patients with PD and healthy controls as illustrated in the table 4.5. For healthy controls, the only deviation from the Hardy-Weinberg equilibrium was for the *DRD1* rs686. In PD, however, the results deviated from the Hardy-Weinberg equilibrium for *DRD1* rs4532 and *COMT* rs4680.

Table 4.5: Hardy-Weinberg equilibrium for patients with PD and healthy controls.

Gene	***SNP***	**Groups**	**P-value**
DRD1	rs4532	Healthy PD	0.46 0.00011
	rs686	Healthy PD	0.043 0.76
DRD2	rs1076560	Healthy PD	1.000 1.000
	rs6277	Healthy PD	1.000 0.42
SLC6A3	rs3836790	Healthy PD	0.12 0.066
	rs28363170	Healthy PD	0.38 0.42
COMT	rs4680	Healthy PD	0.14 0.002

4.7 Dopamine Genetic Risk Scores for Patients with PD and Healthy Controls

Genetic risk scores were assigned to different SNPs according to their functionality. The genotypes that lead to high dopamine levels were given a value of 1, those with intermediate dopamine levels were given 0, and SNPs associated with low dopamine levels were assigned a value -1. The sum of scores divided by number of subjects per SNP are summarized in table 4.6. We also plot the distribution of dopamine scores for both patients with PD and healthy individuals in figure 4.2.

Table 4. 6: Sum of scores for DRD1 rs4532, DRD1 rs686, DRD2 rs1076560, DRD2 rs6277, SLC6A3 rs3836790, SLC6A3 rs28363170, COMT rs4680 between PD patients and healthy controls.

Dopamine Genetic Risk Score Sum	***DRD1* rs4532**	***DRD1* rs686**	***DRD2* rs1076560**	***DRD2* rs6277**	***SLC6A3* rs3836790**	***SLC6A3* rs28363170**	***COMT* rs4680**	**TOTAL**
PD	0.2364	0.3636	-0.1273	-0.9091	0.3455	0.0364	-0.1455	-0.2000
Healthy	0.5098	0.4510	0.0588	-0.9216	0.3137	-0.2745	0.1961	0.3333

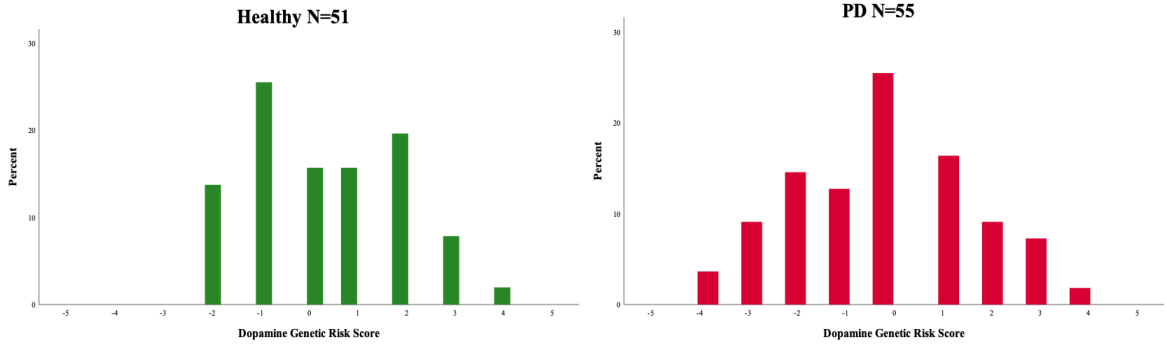


Figure 4. 2: Distribution of dopamine genetic risk scores in PD and healthy.

Finally, we trained a machine learning classifier to examine whether variability in the SNP genotypes and the dopamine risk scores could differentiate patients with PD from healthy controls. We used a backward logistic regression model with the data from the seven SNPs alongside the dopamine risk scores as the input. The model only retained scores of *DRD1* rs4532, *SLC6A3* rs28363170, and *COMT* rs4680 to achieve a 73% classification accuracy of PD (67% sensitivity and 73% specificity) as illustrated in the ROC curve in figure 4.3.

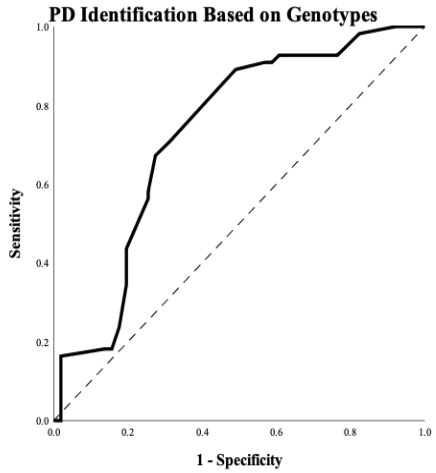


Figure 4. 3: ROC curves of backward logistic regression classification of patients with PD based on dopamine SNP genotypes.

Chapter 5

Discussion and Conclusions

Patients with PD exhibited different genotype and allelic frequencies than healthy individuals in a variety of key SNPs in core dopamine system genes. Although arbitrary assignment of weights did not produce significant differences in the form of dopamine genetic risk scores, machine learning accomplished a 70% accuracy of identifying PD only based on dopamine SNP genotypes.

Dopaminergic innervation is widespread in the central nervous system, and it is involved in numerous primary and peripheral activities. PD is mainly a related dopaminergic disorder that is primarily caused due to striatal dopaminergic depletion in the brain (Beaulieu & Gainetdinov, 2011). PD diagnosis is mainly depending on clinical examination, the neurologist will examine the medical history and check the different clinical features of PD patients, and with the aid of many neuroimaging techniques such as magnetic resonance imaging-MRI, positron emission tomography, single-photon emission computed tomography, and techniques that are based on multimodal neuroimaging. In contrast in less frequently, the diagnose can depend on genetic testing (Massano & Bhatia, 2012; Wize et al., 2018). PD is mainly of sporadic and heredity, with genetic forms of PD only affecting (10-15) % of all patients. However, the resemblance in both familial and sporadic forms can help further understand the physiology and the molecular mechanisms of the disease (Meiser et al., 2013). Although dopaminergic receptors, transporter, and COMT enzyme are widely understood, only a few studies have related their genetic variability as a possible risk factor of PD. In our study, we created a genetic risk score to investigate and address the potential influence

between specific selected naturally occurring SNPs in *DRD1*, *DRD2*, *SLC6A3*, and *COMT* genes as an early marker to detect and predict the risk for developing PD. Our results revealed that SNPs in the *DRD1*, *SLC6A3*, and *COMT* genes exhibited significant genotype and allele differences between patients with PD and healthy controls. The same pattern was evident for both genotype combinations analysis and the Hard-Weinberg equilibrium. Machine learning classification confirmed the significant of *DRD1* rs4532, *SLC6A3* rs28363170, and *COMT* rs4680 genotypes by showing their mathematical importance for differentiating patients with PD from healthy controls.

Our results revealed that rs4680 of *COMT* exhibited significant genotype difference between healthy controls and PD patients. The AA genotype was shown to be more frequent in healthy controls, while the GA genotype is more frequent in PD patients. This result is in line with that carriers of the Val allele (G) were shown to have a 3-4 fold increase in the activity of the enzyme than carriers of Met allele (A) resulting in lower dopamine levels specifically in the prefrontal cortex and hippocampus (Chen et al., 2004). The *COMT* rs4680 polymorphism is considered one of the most studied in neuroscience. Tunbridge et al. screened nearly 23,000 research articles and found strong and medium-high effects for rs4680 on the enzyme activity, stability, and abundance of the COMT protein (Tunbridge et al., 2019). Further, in previous meta-analysis, it was found that there is a significant association between rs4680 of the *COMT* gene and the efficacy of L-DOPA in PD (Guin et al., 2017).

In the *DRD1* gene, the AA genotype of rs4532 was significantly more frequent in patients with PD than healthy controls. This finding corroborates previous reports implicating the A variant of rs4532 in other neurodevelopmental disorders such as ASD (Hettinger et al., 2008). Given the imbalance of D1 and D2 receptor activation as a

consequence of dopaminergic neuron decay, significant SNPs in the *DRD1* genes could play a role in modulating response to dopaminergic treatment. However, this remains an open question for further investigation in the future.

In the *SLC6A3* gene rs3836790 (Intron8), the 5/6 genotype was significantly more prevalent in healthy controls than patients with PD. In rs28363170, the 10/10 genotype was more prevalent, albeit approaching significance, in healthy controls than patients with PD. A previous meta-analysis showed that both rs3836790 and rs28363170 of *SLC6A3* were significantly associated with the efficacy of L-DOPA (Guin et al., 2017).

Although it will be difficult to draw definitive conclusions on PD risk and diagnostics based our limited sample size, our research sheds very important light on the most significant contributing components of the dopaminergic system to PD pathology. These findings can have significant ramification for the development of future pharmacological therapy for PD.

5.1 Conclusions

This present study was carried out to investigate whether selected functional SNPs in dopaminergic genes contribute to the susceptibility of PD. We tested 55 patients with PD and 51 healthy individuals in this study. We reported significant differences between patients with PD and healthy controls in the investigated SNPs in dopamine genes. We designed a machine learning tool that utilizes these genotypes to identify the presence of PD.

5.2 Recommendations

Future work ought to recruit a larger cohort of patients with PD and matched healthy individuals. Genetic analysis could include more SNPs in the studied dopamine receptors and transporters genes. Future research could also focus on other dopamine genes, such as the *MAOA* gene that encodes the monoamine oxidase and other dopamine receptors. Further analysis of different symptoms of PD with linkage to different SNPs could also inform significant developments in the clinical assessment of PD.

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الملخص

الهدف: الهدف من هذه الدراسة هو إنشاء درجة مخاطر جينية للدوبامين لمرض الشلل الرعاشي (باركينسون) إستنادا إلى تعدد الأشكال التي تحدث بشكل طبيعي في جينات الدوبامين الرئيسية المراد التحقق منها.

المواد والأدوات: تعتبر هذه الدراسة دراسة حالة وضبط لمرضى وضوابط مرض باركنسون حيث استهدفت الدراسة 106 فلسطينيًا منهم 55 مريضاً بداء باركنسون و51 شخصًا من الأصحاء. تراوحت أعمار المشاركين بين 55 و75 سنة. تم تحليل الحمض النووي لسبعة من الطرزالجينية المختلفة التي تحدث بشكل طبيعي في الجينات التالية: (rs686, rs4532) *DRD1*، (rs1076560, rs6277) *DRD2*، (rs3836790, rs28363170) *SLC6A3* و *COMT* (rs4680).

النتائج: أظهرت النتائج فروق ذات دلالة إحصائية بين المرضى الذين يعانون من مرض باركنسون والأفراد الأصحاء في النمط الجيني والتردد الأليلي في *D*RD1 rs4532 و *S*LC6A3 rs3836790 و*C*OMT rs4680. كشف تحليل التقليب لتركيبات النمط الجيني وحسابات توازن هاردي واينبرغ عن آثار كبيرة لنفس النيوكليوتيدات. لم تكشف درجات مخاطر الدوبامين الوراثية القائمة على جميع النيوكليوتيدات عن أي فرق بين المرضى الذين يعانون من مرض باركنسون والأفراد الأصحاء بينما حدد مصنف التعلم الآلي القائم 70% من الحالات التي يعاني فيها المرضى من مرض باركينسون على *DRD1* rs4532 و*SLC6A3* rs3836790 و *COMT* rs4680.

الخلاصة: يمكن أن تكون النيوكليوتيدات التي تم التحقق منها في جينات الدوبامين بمثابة تنبؤات وعلامات تشخيصية لمخاطر مرض الرعاش ويمكن أن يتم تطوير النتائج التي توصلنا اليها الى أدوات تقييمية سريرية مستقبلية.