ISSN: 0067-2904

Determining the Genetic Markers of Schizophrenia Using Molecular Diagnosis

Haneen M. Ismaeel[1], Hayba Badro[1], Ali Q. Khazaal[1], Najwa Shihab Ahmed[2]
[1]*Department of Biotechnology, College of Science, University of Baghdad, Al-Jadriya, Baghdad, Iraq*
[2] *Biotechnology Research Center, University of Al-Nahrian, Al-Jadriya, Baghdad-Iraq*

Received: 4/9/2023 Accepted: 7/1/2024 Published: 30/7/2024

Abstract

Schizophrenia poses a considerable disease burden, notwithstanding its relatively low incidence rate. The escalation in population size and mean age has led to a substantial rise in the incidence of illness linked to schizophrenia, particularly in countries with moderate levels of prosperity. Therefore, it is necessary to find a way to detect and recognize the illness as fast as possible to stop the consequences from occurring and stop symptoms from appearing before they even start. Within this study's scope, fifteen distinct primers were selected randomly to determine whether or not they could recognize schizophrenia. To accomplish this, a Randomly Amplified Polymorphic DNA-polymerase chain reaction (RAPD-PCR) was conducted utilizing DNA samples obtained from individuals afflicted with the condition and those in good health. The outcomes of the gel electrophoresis and subsequent analysis revealed that solely three primers including C12, E02 and E03 exhibited monomorphic characteristics. However, it was observed that 80% of the primers, precisely eleven out of fifteen, exhibited polymorphism. The results indicated that among the 15 primers tested, the primer labeled as H01 failed to generate any detectable bands. Subsequent examination of the polymorphic primers revealed that the primer efficiency varied between 0 and 0.0755, while the discrimination power ranged from 0 to 20 percent. The study observed variations in the frequency of disease occurrence between patients and healthy individuals. H05 primer utilization yielded three distinct polymorphic bands exhibiting diverse lengths. The band exhibiting the greatest length measured 1190 bp, whereas the other two bands had 480 and 430 bp lengths respectively. The two polymorphic bands exhibited 100% prevalence in the patient population while being completely absent in the healthy control group. Based on the available data, it appears that utilizing this primer to identify schizophrenia is a valid and valuable claim. Overall, DNA-based diagnostics may overlook a number of difficulties as laboratories have the ability to extract DNA from various sources easily. In this scenario, molecular and genetic techniques may be crucial for diagnosing some diseases.

Keywords: Schizophrenia, Randomly Amplified Polymorphic DNA (RAPD), PCR, Molecular diagnosis

*Email: haneen.m@sc.uobaghdad.edu.iq

تحديد الواسمات الوراثية لمرض الفصام باستخدام التشخيص الجزيئي

حنين مؤيد اسماعيل[1], هيبة قيصر يونان[1]، علي قاسم خزعل[1] , نجوى شهاب احمد[2]

[1]قسم التقنيات الإحيائية، كلية العلوم، جامعة بغداد، بغداد، العراق

[2] مركز بحوث التقنيات الاحيائية، جامعة النهرين، بغداد, العراق

الخلاصة

يشكل اضطراب الفصام (انفصام الشخصية) عبئا مرضيا كبيرا، على الرغم من انخفاض معدل الإصابة به نسبيا. وقد أدى التصاعد في حجم السكان ومتوسط العمر إلى ارتفاع كبير في حالات الإصابة بالأمراض المرتبطة بالفصام، وخاصة في البلدان ذات مستويات الرخاء المعتدلة. لذلك، من الضروري إيجاد طريقة لاكتشاف المرض والتعرف عليه في أسرع وقت ممكن لمنع حدوث عواقب المرض ومنع ظهور الأعراض حتى قبل أن تبدأ. ضمن نطاق هذه الدراسة، تم اختيار خمسة عشر بادئ مختلف بشكل عشوائي لتحديد ما إذا كان بالإمكان استخدامهم مستقبلا كمؤشرات خاصة للتعرف على مرض انفصام الشخصية. ولتحقيق ذلك، تم إجراء تفاعل البلمرة المتسلسل العشوائي (RAPD–PCR) باستخدام عينات الحمض النووي التي تم الحصول عليها من الأفراد المصابين بهذه الحالة أو أولئك الذين يتمتعون بصحة جيدة. كشفت نتائج الترحيل الكهربائي الهلامي والتحليلات الجزيئية اللاحقة أن ثلاثة بادئات فقط والمسماة C12 و E02 و E03 أظهرت انماط تضاعفية غير متغايرة. كما لوحظ أن 80٪ من البادئات، وعلى وجه التحديد أحد عشر من أصل خمسة عشر، أظهرت أنماط تضاعفية متعددة الأشكال. تشير النتائج إلى أنه من بين 15 بادئًا المستخدمة في هذه الدراسة كان هناك بادئ واحد والمسمى H01 لم يظهر أي نمط تضاعفي. وكشف التحليل للبادئات متعددة الأشكال أن كفاءة البادئات تراوحت بين 0 و0.0755، في حين تراوحت قوة التمييز للبادئات من 0 إلى 20 بالمائة. ولاحظت الدراسة اختلافات في وتيرة حدوث المرض بين المرضى والأفراد الأصحاء. أسفر استخدام البادئ H05 عن اظهار ثلاث حزم متعددة الانماط ذات أحجام متنوعة. كانت الحزمة الاكبر بحجم يبلغ 1190 كيلو قاعدة، في حين كانت الحزمتين الآخرتين بحجم 480 و430 كيلو قاعدة. أظهرت هاتان الحزمتان انتشارًا بنسبة 100% في مجموعة المرضى بينما كانتا مفقودتان تمامًا في مجموعة السيطرة. استنادا إلى البيانات المستحصلة من هذه الدراسة، فأنه من الممكن استخدام هذا البادئ في تشخيص مرض انفصام الشخصية. بشكل عام، ان التشخيص المعتمد على الحمض النووي قد يساهم في تجاوز عددا من الصعوبات التي تواجه بعض الاختبارات التشخيصية حيث ان المختبرات لديها القدرة على استخلاص الحمض النووي من مصادر مختلفة بسهولة. في ظل هذه المعطيات، قد يكون استخدام التقنيات الجزيئية والوراثية هي أحد الطرق المهمة في تشخيص بعض الأمراض.

Introduction

Schizophrenia is a highly severe and recurrent mental disease that strikes nearly 1% individuals all over the world [1]. Although characterized by a relatively low incidence rate, schizophrenia represents a considerable disease burden. The escalation in population magnitude and old age has led to a significant prevalence of illness linked with schizophrenia, particularly in countries with intermediate income levels [2]. It was projected in 2013 that the financial cost of schizophrenia in the USA was more than $ 150 billion. The projected economic cost of schizophrenia in the USA increased by twofold, reaching more than $ 340 billion between 2013 and 2019 [3].

Schizophrenia is a psychological disorder that may be passed down from generation to generation and is characterized by cognitive and motivational abnormalities. In many cases, the first signs of schizophrenia don't manifest themselves until the end of the teenage years or the beginning of adulthood [4 – 6]. The pathogenic factors behind schizophrenia remain unclear, albeit it is certain that the disorder is linked to some pathological characteristics that are usually seen in schizophrenia development. According to researchers, exaggerated grey matter loss in

the brain and reduced synaptic density in central nervous system neurons are examples of these pathological features [7]. The deterioration of the cerebral grey matter remains a significant barrier in the context of schizophrenia treatment. As the grey matter deterioration in the brain that results from each mental condition occurrence is perpetual and unalterable, patients will never be able to restore their functioning level before their psychotic event. The correlation between schizophrenia and synaptic loss is in line with the observed reduction in synaptophysin levels and an orientation towards diminishing levels of various synaptic proteins in the brain [8, 9].

The genetic outline of a person is considered to be one of the most important warning signs for schizophrenia. Over the past decade, the investigation into the genetic molecular level has resulted in a number of intriguing advancements. These findings have inspired cautious optimism about identifying the biological roots of schizophrenia [10].
The study area of schizophrenia has significantly advanced with the discovery of molecular and genetic analyses. Feasibility to study DNA loci that cross the genome or a potential genomic variation linked to the illness using the analyses of linkage and association in genetic materials was a significant advancement in this field [11].

Randomly Amplified Polymorphic DNA-polymerase chain reaction (RAPD-PCR), sometimes referred to as arbitrarily primed-PCR (AP-PCR), is a method designed for the quick identification of genomic variation or polymorphism. The method relies on the replication of specific regions of DNA from genomes using brief primers made up of sequences that are randomly generated. The length of the primer can vary from eight to fifteen nucleotides. Various PCR outputs are produced as these arbitrary sequences of primers bind to diverse places in an organism's genome. Thus, a pattern of bands that, in principle, is indicative of the relevant genomic DNA emerges from the separation of the PCR products by DNA electrophoresis [12–15] .

The present investigation employed the RAPD technique to identify genetic markers associated with schizophrenia to develop effective tools for early diagnosis and provide optimal health management to affected individuals and their families. To this end, fifteen distinct random primers were selected for this study. The findings of this research could offer valuable prognostic, diagnostic and therapeutic benefits for schizophrenia patients.

Material and Methods

Study Subjects and DNA Isolation

The research study was carried out with a sample size of 56 participants ranging from 20 to 60 years old. Blood samples were collected from each participant (5 mL in EDTA) and stored at -4ºC. The study sample consisted of 50 individuals diagnosed with schizophrenia, while six healthy individuals were included as a control group. Schizophrenia diagnosis was performed by consultant medical staff at Al-Rasheed Teaching Hospital, Baghdad Iraq. The process of genomic DNA extraction from whole blood was performed following the protocol of the Wizard Genomic DNA Purification Kit (Promega, USA). The isolated DNA was stored at -20°C until further use. The quantity and quality of the DNA samples were evaluated by NanoDrop™ spectrophotometer before performing the RAPD-PCR analysis. Informed consent was obtained from all participants involved in the study, following the approval of the Research Ethics Committee at the College of Science, University of Baghdad (Reference: CSEC/0623/0046 on June 10, 2023.

Randomly Amplified Polymorphic DNA-polymerase Chain Reaction (RAPD PCR)

Fifteen random primers (Alpha DNA, USA) were selected for the DNA amplification by RAPD PCR (Table 1). The DNA amplifications were conducted using a reaction mixture of 25 µl which consisted of 12.5 µL of 1X Go Taq®Green Master Mix (Promega-USA), containing 1.5 mM $MgCl_2$, 10mM Tris-HCl, 50mM KCl, 200 µM of each deoxynucleotide triphosphate (dATP, dCTP, dGTP, and dTTP), 1 unit of DNA polymerase, 1.5 µL (10 pmol/µL) of the primer, 9.5 µL free-nuclease water, and 1.5 µl of (100ng/ul) template DNA. The DNA amplification reactions were conducted in a MultigeneTM Gradient Thermal Cycler (Labnet International, Korea) with the following settings: 1 cycle of initial denaturation at 94°C for 5 minutes followed by 45 cycles, each one consisting of a denaturation step for 1 minute at 94°C, one annealing step for 1 minute at 36°C, and an extension step for 2 minutes at 72°C. An extra extension step was performed for 10 minutes at 72°C. The RAPD-PCR products were subjected to electrophoresis using a 1.5% agarose gel, along with the inclusion of a 1Kb DNA ladder (Promega- USA). Visualization of the products was achieved by staining with 0.5 µg/ml of ethidium bromide for 15-30 minutes. The gel images were digitally recorded using a gel documentation system, and the molecular weight of the RAPD-PCR products was determined by comparing their sizes to those of DNA fragments from a 1 Kb DNA ladder using the computer software Photo-CaptMwt version 10.01.

Analysis of RAPD-PCR Data

Each PCR product generated by a set of primers was set up for RAPD analysis. Initially, the RAPD profiles' DNA bands were analyzed according to their position on the gel and visually assigned a score as present (1) or absent (0) in a table format. The below equation was performed to determine the primer efficiency (PE) [16] .

$PE = n_p/n$

In this equation, n_p represents the number of polymorphic bands for each primer and n represents the total number of amplified bands. As well, the percentage of discriminatory power was calculated as described by Grundman *et al.* 1995 [17].

$Pi= (n_p/n_t) * 100$

Where n_p is the number of polymorphic bands for each primer and n_t is the total number of polymorphic bands of all primers.

The determination of band frequency was conducted solely for amplification profiles generated by polymorphic primers. To achieve this estimation, the number of samples that share a particular band within each respective group was determined.

Statistical Analysis

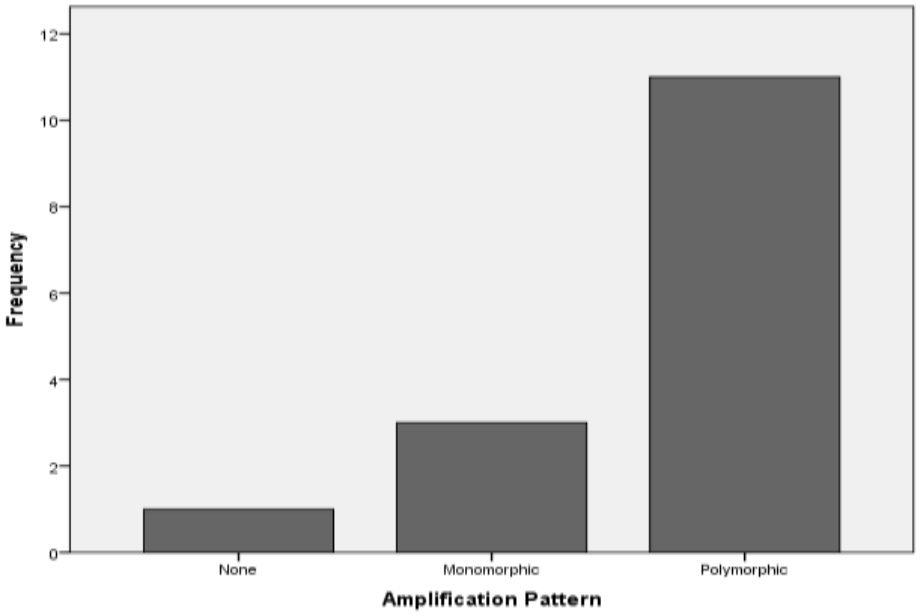
The data was analyzed using the SPSS software (Version 22.0, IBM Company, USA).

Results

In this investigation, 15 different primers were chosen at random to test whether or not they were able to identify schizophrenia. In order to do this, RAPD-PCR was carried out utilizing the DNA obtained from either patients or healthy individuals. This step was performed separately for each primer. Based on the gel electrophoresis results, the findings revealed that, out of the fifteen primers, eleven were polymorphic, three primers were monomorphic, and one primer designated as H01 did not produce any bands at all, (Table 1 and Figure 1).

Table 1: The RAPD-PCR amplification and polymorphism bands for random primers

Primer	Sequence 5'-3'	Amplification Pattern	No. of Amplified Bands	No. of Polymorphic Bands
C11	**AAAGCTGCGG**	Polymorphic	14	8
C12	**TGTCATCCCC**	Monomorphic	6	0
C15	**GACGGATCAG**	Polymorphic	12	3
D01	**ACCGCGAAGG**	Polymorphic	16	12
D05	**TGAGCGGACA**	Polymorphic	14	7
D09	**CTCTGGAGAC**	Polymorphic	8	1
E02	**GGTGCGGGAA**	Monomorphic	6	0
E03	**CCAGATGCAC**	Monomorphic	7	0
F14	**TGCTGCAGGT**	Polymorphic	10	5
F15	**CCAGTACTCC**	Polymorphic	12	3
F16	**GGAGTACTGG**	Polymorphic	12	4
H01	**GGTCGGAGAA**	No amplification	0	0
H05	**AGTCGTCCCC**	Polymorphic	16	3
H07	**CTGCATCGTG**	Polymorphic	15	8
O16	**TCGGCGGTTC**	Polymorphic	11	6
	Total		**159**	**60**



Amplification Patterns		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	No ammplification	1	6.7	6.7	6.7
	Monomorphic	3	20.0	20.0	26.7
	Polymorphic	11	73.3	73.3	100.0
	Total	15	100.0	100.0	

Figure 1: Frequency of RAPD-PCR amplification pattern as no amplification (none), monomorphic and polymorphic.

The amplicons produced from each separate primer were subjected to further analysis which consisted of counting the number of amplified bands and the number of polymorphic bands (Table 1). According to the findings, both D01 and H05 were responsible for producing the highest number of amplicons, each with 16 bands (Table 1). However, the

number of bands generated by primer C12 was the lowest with 6 bands. D01 possessed 12 polymorphic bands (Table 1), making it the primer with the greatest number of polymorphic bands overall. Despite the fact that the primers C12, E02, E03 and H05 shouldn't be used as primers for the diagnosis of schizophrenia, the results of the current study suggest that the remaining primers have the potential to be employed as primers for the molecular diagnosis of this condition.

Additional investigations were conducted and quantified to better understand the features of the random primers including primer effectiveness and percentage of discriminatory power. The value of the above-mentioned variables was zero for monomorphic primers besides the H01 primer (Table 2). Polymorphic primers' range of primer efficiency was between 0.0189 and 0.0755, with discriminating power percentages that varied from 1.7 to 20 (Table 2).

Table 2: The primer efficiency and discriminatory power percentage of the fifteen primers

Primer	Primer Efficiency	Discriminatory Power %
C11	0.0503	13.3
C12	0	0
C15	0.0189	5
D01	0.0755	20
D05	0.0440	11.7
D09	0.0063	1.7
E02	0	0
E03	0	0
F14	0.0315	8.3
F15	0.0189	5
F16	0.0251	6.7
H01	0	0
H05	0.0189	5
H07	0.0503	13.3
O16	0.0377	10

This study investigated the dimensions and occurrence rate of polymorphic amplicons in individuals diagnosed with schizophrenia and those without the disease. The frequency of polymorphic bands was measured for each primer which resulted from varying numbers of bands for both healthy and patient samples (Table 3). The table below represents an illustration of the lengths of polymorphic bands together with the corresponding percentage of polymorphism frequency. For enhanced visualization, the polymorphic band in healthy subjects was marked in light tan color, whereas the polymorphic band in patients was marked in dark grey.

Table 3: Size and frequency of polymorphic amplicons in patients with schizophrenia & healthy controls

Primer	Polymorphic Band Size (bp)	Frequency of Polymorphic Bands (100%)	
		Patient	Healthy
C11	2031	91.6	100
	1920	16.6	16.6
	1500	91.6	100
	1460	83.3	0
	1300	75	100
	1200	91.6	100
	450	58.3	0
	380	25	100
C15	920	0	33.3
	510	33.3	0
	480	66.7	100
D01	1900	16.6	100
	1590	83.3	83.3
	1460	33.3	16.6
	1240	0	50
	1180	8.3	0
	870	66.6	100
	750	50	0
	730	0	100
	660	83.3	100
	500	100	33.3
	470	0	66.6
	400	58.3	0
D05	1320	58.3	50
	920	25	100
	710	0	33.3
	600	66.6	100
	370	41.6	33.3
	190	91.6	66.6
	150	50	16.6
D09	2200	25	0
F14	1780	83.3	100
	1250	25	33.3
	1125	91.3	100
	990	25	0
	840	83.3	100
F15	1750	91.6	66.6
	1250	0	100
	670	25	0
F16	2220	50	100
	900	41.6	0
	860	58.3	100
	820	41.6	0
H05	1190	50	33.3
	480	100	0
	430	100	0
H07	2500	8.3	100
	2070	8.3	100
	1900	8.3	100
	1800	75	100
	830	8.3	0
	580	58.3	50

	540	16.6	0
	490	50	66.6
O16	**2280**	58.3	100
	1085	83.3	100
	850	75	100
	800	83.3	33.3
	570	16.6	16.6
	400	33.3	16.6

Represents a polymorphic band that exists only in healthy subjects.
Represents a polymorphic band that exists only in patients**.**

A representative result of RAPD profiles for the primers C12, E02 and E03 (Monomorphic) is displayed in Figure 2. All samples revealed the same band patterns in both schizophrenia patients and healthy individuals. The findings of this study indicated that the utilization of C12, E02 and E03 primers is not recommended for the purpose of diagnosing schizophrenia.

The amplification amplicons were produced by primer C11, leading to the detection of eight polymorphic bands (Figure 3). The results of this study examined the occurrence of polymorphic bands produced by C11 indicated that five polymorphic bands were detected in each healthy subject (Table 3). The results also revealed that a majority of the patients, precisely 91%, exhibited the production of three polymorphic bands, namely 2031, 1500 and 1200 kb. However, the production of the remaining two bands was observed in 75% and 25% of patients respectively. Additionally, it was observed that two polymorphic bands, labeled as 1460 and 450, were absent in healthy individuals but were present in patients with frequencies of 83.3% and 58.3% respectively. The present study suggests that the aforementioned polymorphic bands, specifically the 1460 kb band, may serve as a potential marker for schizophrenia.

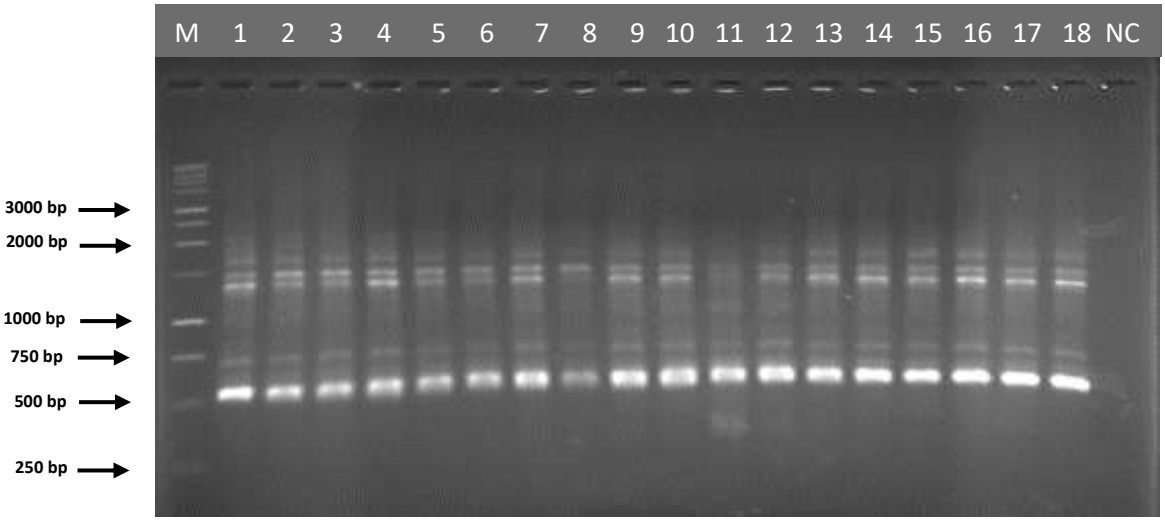


Figure 2: RAPD-PCR amplification products generated by the C12 primer resolved by electrophoresis in 1.5% agarose gel. M: 1Kb ladder, Lanes 1-12: DNA samples from Schizophrenia patients, Lanes 13-18: DNA samples from control subjects and NC: Negative Control.

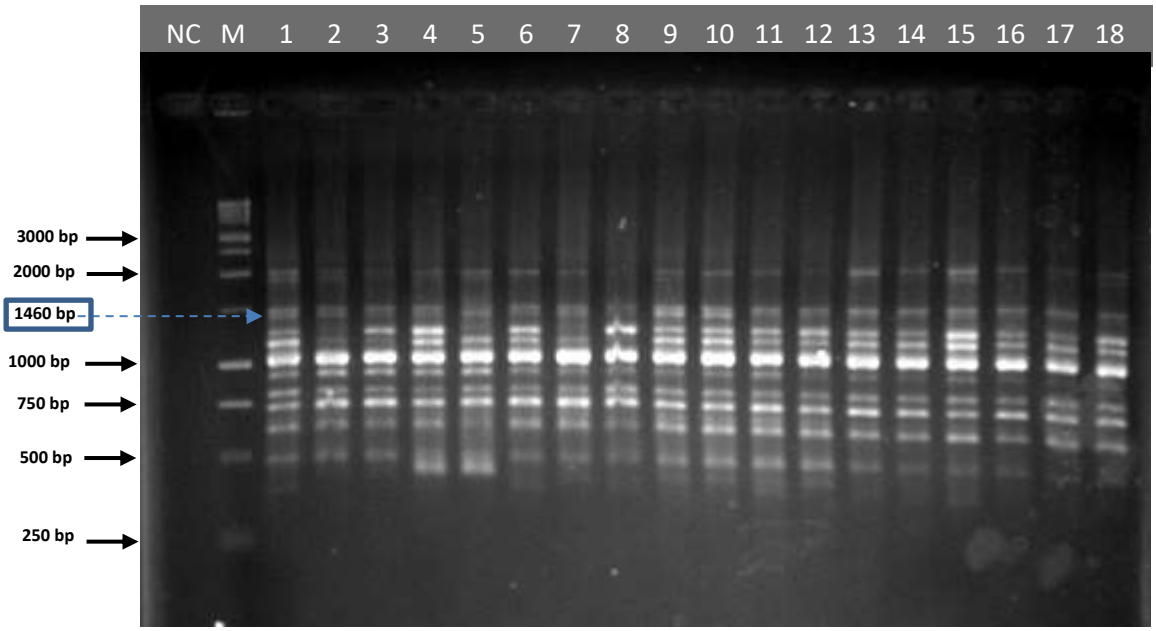


Figure 3: RAPD profile using primer C11. PCR product separated by gel electrophoresis in 1.5% agarose gel. NC: Negative Control, M: 1Kb DNA ladder, Lanes 1-12: DNA samples from Schizophrenia patients, and Lanes 13-18: DNA samples from healthy individuals.

Primer F14 was found to be accountable for the generation of the polymorphic bands, thereby indicating the existence of five distinct polymorphic bands (Figure 4). Based on the findings of the frequency of polymorphic bands produced by F14, it was observed that three bands were generated in each healthy subject (Table 3). The observed polymorphic bands exhibited a high production rate among the patients, with frequencies ranging from 83.3 to 91.3. A single polymorphic band (990 bp) was observed exclusively in patients but not in healthy people. This band was exclusively identified among individuals who had schizophrenia. However, the frequency of this polymorphic band was found to be low, constituting only 25% of patients in this study. These results indicated that F14 may not be a viable option as a primer for diagnosing schizophrenia.

Likewise, the F15 primer yielded a single polymorphic band (670 bp) that occurred in 25% of the patients. However, this band cannot be considered a reliable indicator of schizophrenia, considering its absence in healthy subjects (Figure 5 and Table 3).

Primer F16 exhibited a higher potential to serve as an indicator for the diagnosis of schizophrenia, as evidenced by the generation of two polymorphic bands in the patient group, but not in the control group (refer to Figure 6). Despite the absence of these two polymorphic bands in the healthy population, their frequency of occurrence was observed to be only 41.6%. Therefore, it is not feasible to employ them as markers (Table 3).

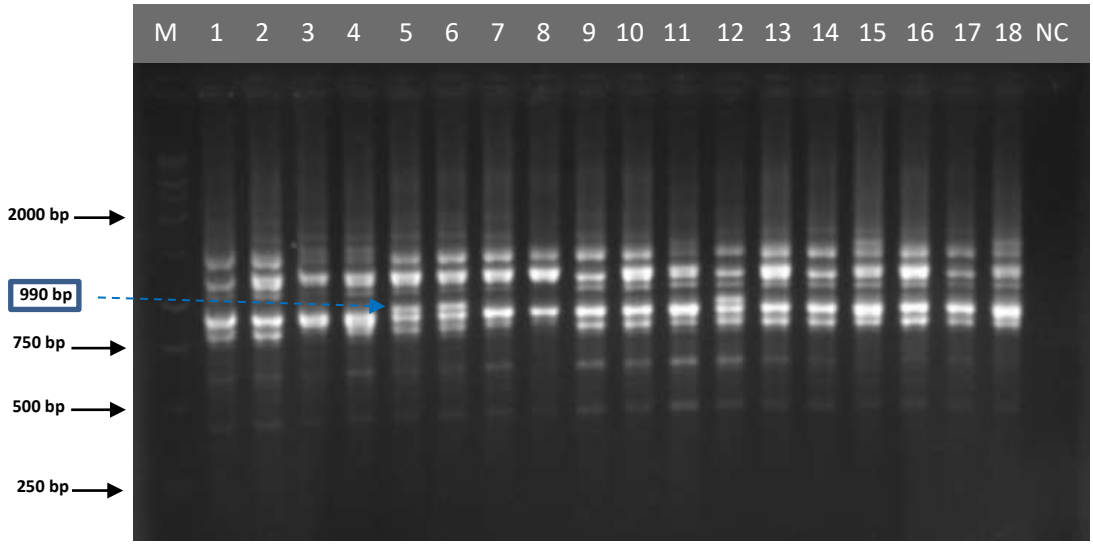


Figure 4: RAPD profile using primer F14. PCR product separated by gel electrophoresis in 1.5% agarose gel. M: 1Kb DNA ladder, Lanes 1-12: DNA samples from schizophrenia patients, Lanes 13-18: DNA samples from healthy individuals, and NC: Negative Control.

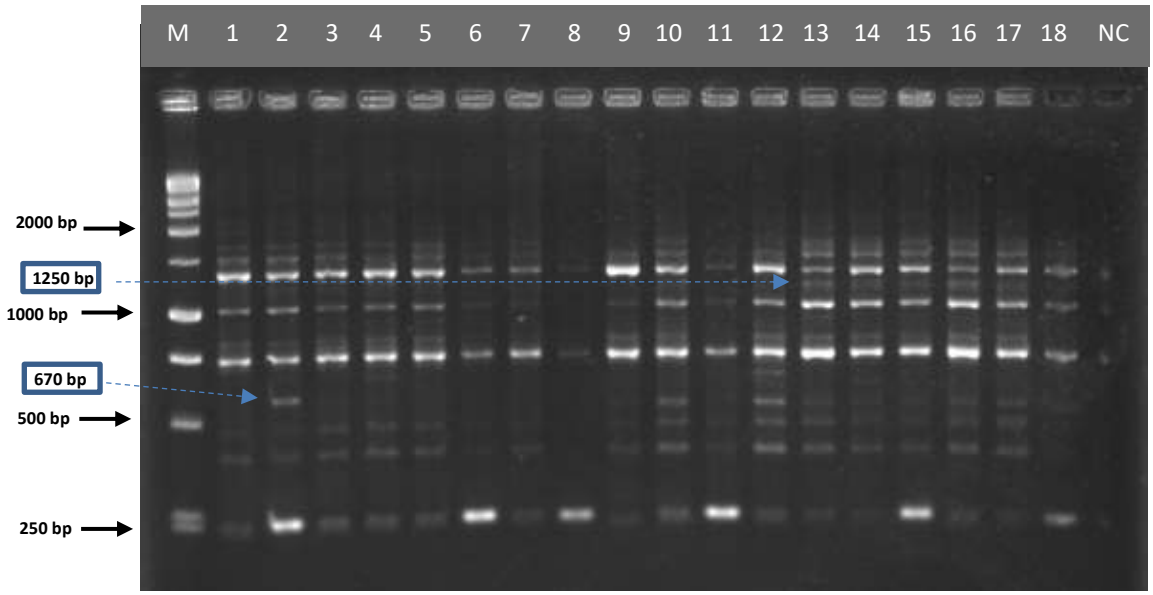


Figure 5: RAPD profile using primer F15. PCR product separated by gel electrophoresis in 1.5% agarose gel. M: 1Kb DNA ladder, Lanes 1-12: DNA samples from schizophrenia patients, Lanes 13-18: DNA samples from healthy individuals, and NC: Negative Control.

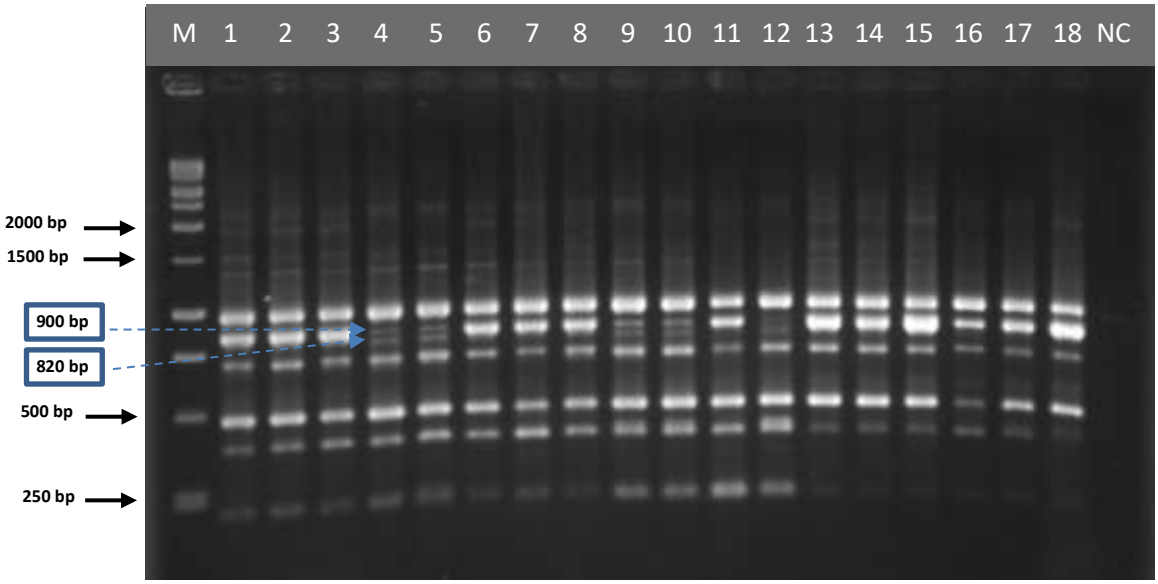


Figure 6: RAPD profile using primer F16. PCR product separated by gel electrophoresis in 1.5% agarose gel. M: 1Kb DNA ladder, Lanes 1-12: DNA samples from schizophrenia patients, Lanes 13-18: DNA samples from healthy individuals, and NC: Negative Control.

Alternately, the H05 primer showed the capacity to function as a reliable signal for the diagnosis of schizophrenia. The H05 primer resulted in the production of three polymorphic bands of varying sizes (Figure 7), containing 1190, 480, and 430 kb respectively. Patients had a frequency of two polymorphic bands (430 bp and 480 bp) that was 100%, while healthy persons had a frequency of 0% (Table 3). Based on the available evidence, it appeared that selecting this primer for schizophrenia detection was an actual and worthy notion.

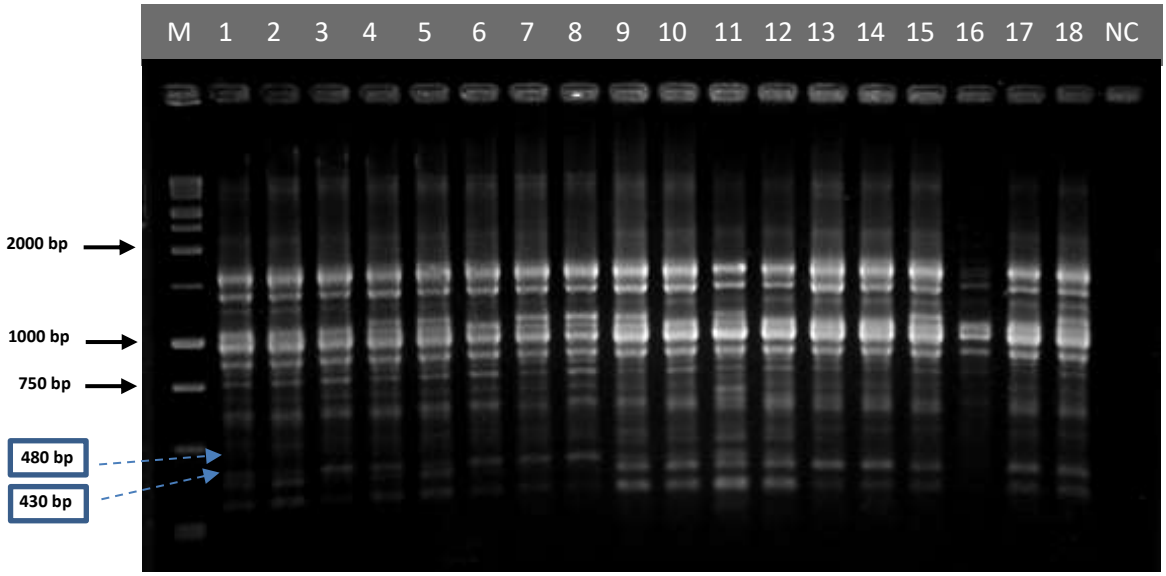


Figure 7: RAPD profile using primer H05. PCR product separated by gel electrophoresis in 1.5% agarose gel. M: 1Kb DNA ladder, Lanes 1-12: DNA samples from schizophrenia patients, Lanes 13-18: DNA samples from healthy individuals, and NC: Negative Control.

Discussion

Schizophrenia frequently appears in late adolescence or shortly after, and is connected to a decreased life expectancy, an increased risk of suicide, serious mental illnesses, and large expenses to both the health care system and society [18, 19]. The treatments are helpful, at least in part, in most individuals; nonetheless, many people continue to have symptoms, and unpleasant effects of therapy are widespread [20]. There is an urgent requirement for developing new treatment targets which is complicated by the lack of pathophysiology knowledge for this condition [21]. More important is a need to locate a method for detecting and identifying the sickness as quickly as possible to avert the occurrence of the disease's complications and may prevent symptoms before they begin.

There has been a longstanding debate regarding the predominant source of the genetic element of multifaceted diseases such as schizophrenia, whether it is primarily due to unusual or frequent DNA modifications [22, 23]. Initial inquiries into the genetic causes of schizophrenia encompassed segregation evaluation and the detection of physiological indicators that demonstrate co-segregation with the condition [11]. Sherrington *et al.*'s results from studying the genetic materials marked the beginning of the quest for a genetic link to schizophrenia. However, it took almost ten years to reach the current level of research in this field [24].

The illness condition of schizophrenia has been postulated to be the result of gene-environment interactions that function in neuronal development throughout early life and adolescence [6]. This theory has been supported by an expanding body of research. While some authors discovered a small number of extremely uncommon variants, none of them had a significant impact on the final results [25], others, in contrast, identified numerous genetic variations that occur frequently with minimal impact on the probability of developing schizophrenia [21]. Moreover, according to the last research, Schizophrenia exhibits a heritability rate ranging between 60-80%, with a significant portion of this being linked to frequent susceptible alleles. The researchers discovered frequent variation connections at 287 different DNA loci using a two-stage genome-wide association analysis that included a sample size of more than tens of thousands of persons with schizophrenia and more than two hundred thousand healthy people. The research showed that, in contrast to other cell types or tissues, the connections were predominantly focused on genes that are expressed within the central nervous system [21]. All this evidence indicates that an individual's genetic profile is a crucial factor in the onset and progression of schizophrenia.

RAPD-PCR utilization has assisted in identifying several types of cancers such as breast [26, 27], colon [28] and prostate [29]. Moreover, RAPD-PCR techniques are appropriate for prompt discrimination and characterization of a diverse range of microorganisms including various types of bacteria such as isolates of rhizobial bacteria [30], fungi such as *Aspergillus fumigatus* [31], and viruses [32]. The present study employed the RAPD-PCR assay to assess the efficacy of fifteen distinct random primers in generating identifiable bands suitable for detecting schizophrenia. The utilization of methods based on molecular and genetic foundations may hold significant importance in this context. Diagnostics relying on DNA can effectively overcome various challenges due to the convenient extraction of DNA from samples in the laboratory.

Various techniques such as allele specific oligonucleotide hybridization and allele specific amplification are utilized to detect genetic variations of several diseases. However, in the

current and other studies, RAPD PCR was the preferred method due to its ability to perform PCR with small quantities of the person's DNA. Basically, as the name suggests, RAPD seeks genetic material for complementary primer sequences and generates DNA segments with diverse sizes [33, 34].

Extensive examination in the present study was conducted on the characteristics of amplicons. Essentially, the first thing explored was the amplification pattern of amplicons. This characteristic is vital since it reveals whether the amplicons that are produced are monomorphic or polymorphic. Principally, a polymorphic pattern indicates that there are distinct differences between healthy individuals and patients, in contrast to a monomorphic pattern which indicates that the bands formed are the same in healthy individuals and patients. In collective cases of RAPD, polymorphic band patterns are the targeted patterns that should be used for diagnostic purposes. Additionally, the number of generated bands that were either monomorphic or polymorphic was counted. Moreover, the primer efficiency and discriminating power percentage were reported as critical primers' qualities.

During the course of the current research, the number of distinct polymorphic bands that were generated by using fifteen different random primers were determined. Next the frequency of these bands in healthy individuals with that of patients was contrasted. The bands between healthy and sick individuals were divided into three distinct categories. Bands that were only detected in healthy persons were neglected. Several bands were formed in patients in addition to healthy individuals. These bands were also disregarded since it was impossible to rely on them in any way. The bands that were solely developed by the patients were used as markers for schizophrenia diagnosis. In this context, the proportion of frequency was considered as the most important component in determining whether or not these bands were valid indicators. Low frequency of a band indicated that it was only present in a small number of patients. For instance, the 1180 kb band in the D01 primer was only present in patients, yet the frequency of its occurrence was 8.3%, indicating that only 8.3% of the examined individuals had this band present in their DNA. Hence, due to this fact, this band could not be relied upon. On the contrary, high frequency of a band indicated that it was present in a significant number of patients. For instance, the frequency of 1460 kb in C11 primer only existed in patients, and since its frequency was 83.3%. This band was considered as an excellent choice for diagnosing schizophrenia. The ideal scenario happens when a band is solely detected in each and every one of the samples of the patients and not in any of the healthy person's samples (that is, its frequency in healthy people is 0%).

The H05 primer showed that it had the potential to act as a reliable signal for the diagnosis of schizophrenia. The use of the H05 primer led to the formation of three polymorphic bands with various sizes (Figure 7). These bands included 1190, 480 and 430 bp respectively. In patients, the frequency of two polymorphic bands (480 bp, and 430 bp) was found to be 100%, nonetheless in healthy individuals the frequency was found to be 0% (Table 3). Based on the data that is currently available, it would seem that selecting this primer for detecting schizophrenia is an excellent choice.

The phenomenon of molecular diagnosis using RAPD PCR presents a multitude of advantages such as enhanced efficiency and optimal cost-benefit ratio. Furthermore, it does not necessitate any specialized understanding of the genetic sequence of the subject organism. The amplification of a DNA segment using identical primers is influenced upon the presence or absence of complementary positions within the primer sequence. In the event of a mutation at the primer's complementary site on the template DNA, these modifications would induce a

number of alterations in a PCR product, leading to a distinct configuration of augmented DNA fragments from the non-mutated sample [35–37]. In brief, the RAPD-PCR test is a valuable, practical and effective approach that can help determine whether or not schizophrenia is present. The current study had notable limitations including a small sample size, particularly the number of healthy individuals. Hence, further investigation is recommended to include a larger sample size in order to validate the results of this study.

Conclusion

The results of our study emphasized the significance of genetic elements in the development of schizophrenia and could aid in the creation of individualized strategies for the diagnosis, prevention, and management of this disorder. It is essential to consider that the current investigation was carried out exclusively on the people of Iraq. To validate and extend the findings, additional research involving larger sample sizes and diverse ethnic groups is necessary. In summary, this research can enhance comprehension of the genetic indicators of schizophrenia and may have the potential to expedite the creation of more precise diagnostic techniques, prognostications, and personalized treatment strategies for this widespread disorder.

Acknowledgement

The authors would like to express their sincere gratitude to the consultant medical staff at the Al-Rasheed Teaching Hospital, Baghdad for their valuable cooperation throughout the course of this study.

Conflict of Interest

The authors hereby state that they have no conflicts of interest to disclose.

References

[1] C. M. Borelli and H. Solari, “Schizophrenia.,” *JAMA*, vol. 322, no. 13. United States, p. 1322, Oct. 2019. doi: 10.1001/jama.2019.11073.

[2] F. J. Charlson, A. J. Ferrari, D. F. Santomauro, S. Diminic, E. Stockings, J. G. Scott, J. J. McGrath, and H.A. Whiteford, “Global Epidemiology and Burden of Schizophrenia: Findings From the Global Burden of Disease Study 2016.,” *Schizophr. Bull.*, vol. 44, no. 6, pp. 1195–1203, Oct. 2018, doi: 10.1093/schbul/sby058.

[3] A. Kadakia, M. Catillon, Q. Fan, G. R.Williams, J. R. Marden, A. Anderson, N. Kirson, and C. Dembek, “The Economic Burden of Schizophrenia in the United States.,” *J. Clin. Psychiatry*, vol. 83, no. 6, Oct. 2022, doi: 10.4088/JCP.22m14458.

[4] V. L. Valaparla, R. Nehra, U. M. Mehta, J. Thirthalli, and S. Grover, “Social cognition of patients with schizophrenia across the phases of illness - A longitudinal study.,” *Schizophr. Res.*, vol. 190, pp. 150–159, Dec. 2017, doi: 10.1016/j.schres.2017.03.008.

[5] I. S. Ramsay, P. A. Lynn, B. Schermitzler, and S. R. Sponheim, “Individual alpha peak frequency is slower in schizophrenia and related to deficits in visual perception and cognition,” *Sci. Rep.*, vol. 11, no. 1, p. 17852, 2021, doi: 10.1038/s41598-021-97303-6.

[6] H. Jaaro-Peled and A. Sawa, “Neurodevelopmental Factors in Schizophrenia.,” *Psychiatr. Clin. North Am.*, vol. 43, no. 2, pp. 263–274, Jun. 2020, doi: 10.1016/j.psc.2020.02.010.

[7] A. Sekar, A.R. Bialas, H. de Rivera, A. Davis, T. R. Hammond, N. Kamitaki, K. Tooley, J. Presumey, M. Baum, V. Van Doren, G. Genovese, S. A. Rose, R. E. Handsaker, M. J. Daly, M. C. Carroll, B. Stevens, and S. A. McCarroll, “Schizophrenia risk from complex variation of complement component 4.,” *Nature*, vol. 530, no. 7589, pp. 177–183, Feb. 2016, doi: 10.1038/nature16549.

[8] E. F. Osimo, K. Beck, T. Reis Marques, and O. D. Howes, “Synaptic loss in schizophrenia: a meta-analysis and systematic review of synaptic protein and mRNA measures,” *Mol. Psychiatry*, vol. 24, no. 4, pp. 549–561, 2019, doi: 10.1038/s41380-018-0041-5.

[9] O. D. Howes and E. C. Onwordi, "The synaptic hypothesis of schizophrenia version III: a master mechanism," *Mol. Psychiatry*, 2023, doi: 10.1038/s41380-023-02043-w.
[10] M. G. Henriksen, J. Nordgaard, and L. B. Jansson, "Genetics of Schizophrenia: Overview of Methods, Findings and Limitations.," *Front. Hum. Neurosci.*, vol. 11, p. 322, 2017, doi: 10.3389/fnhum.2017.00322.
[11] N. Berry, V. Jobanputra, and H. Pal, "Molecular genetics of schizophrenia: a critical review.," *J. Psychiatry Neurosci.*, vol. 28, no. 6, pp. 415–429, Nov. 2003.
[12] J. Welsh and M. McClelland, "Fingerprinting genomes using PCR with arbitrary primers.," *Nucleic Acids Res.*, vol. 18, no. 24, pp. 7213–7218, Dec. 1990, doi: 10.1093/nar/18.24.7213.
[13] B. Krawczyk and J. Kur, "Chapter 16 - Molecular Identification and Genotyping of Staphylococci: Genus, Species, Strains, Clones, Lineages, and Interspecies Exchanges," V. B. T.-P.-T.-M. T. S. Savini, Ed. Academic Press, 2018, pp. 199–223. doi: https://doi.org/10.1016/B978-0-12-813547-1.00016-9.
[14] J. G. Williams, A. R. Kubelik, K. J. Livak, J. A. Rafalski, and S. V Tingey, "DNA polymorphisms amplified by arbitrary primers are useful as genetic markers.," *Nucleic Acids Res.*, vol. 18, no. 22, pp. 6531–6535, Nov. 1990, doi: 10.1093/nar/18.22.6531.
[15] J. M. Butler, "Chapter 16 - Non-human DNA," J. M. B. T.-A. T. in F. D. N. A. T. M. Butler, Ed. San Diego: Academic Press, 2012, pp. 473–495. doi: https://doi.org/10.1016/B978-0-12-374513-2.00016-6.
[16] R. T. and P. K. Archana Sornakili, Prabukarthikeyan Karthikeyan Rathinam, "Comparative Assessment of RAPD and ISSR Markers to Study Genetic Polymorphism in Colletotrichum gloeosorioides Isolates of Mango," *Asian J. Plant Pathol.*, vol. 11, pp. 130–138, 2017.
[17] D. Grundman, H., Scheider, C., Hartung and T. L. Daschner, F.D., and Pitt, "Discriminatory power of three based typing techniques for P. aeruginosa," *J.Clin. Microbiol.*, vol. 33, no. 3, p. (528-534., 1995.
[18] A. Ventriglio, A. Gentile, I. Bonfitto, E. Stella, M. Mari, L. Steardo, and A. Bellomo, "Suicide in the Early Stage of Schizophrenia.," *Front. psychiatry*, vol. 7, p. 116, 2016, doi: 10.3389/fpsyt.2016.00116.
[19] F.-H. Hu, D.-Y. Zhao, X.-L. Fu, W.-Q. Zhang, W. Tang, S.-Q. Hu, W.-Q. Shen, and H.-L. Chen, "Effects of social support on suicide-related behaviors in patients with severe mental illness: A systematic review and meta-analysis.," *J. Affect. Disord.*, vol. 328, pp. 324–333, May 2023, doi: 10.1016/j.jad.2023.02.070.
[20] E. Jääskeläinen, P. Juola, N. Hirvonen, J. J. McGrath, S. Saha, M. Isohanni, J. Veijola, and J. Miettunen, "A systematic review and meta-analysis of recovery in schizophrenia.," *Schizophr. Bull.*, vol. 39, no. 6, pp. 1296–1306, Nov. 2013, doi: 10.1093/schbul/sbs130.
[21] V. Trubetskoy, A. F. Pardiñas, T. Qi, G. Panagiotaropoulou, S. Awasthi, T. B. Bigdeli, J. Bryois, C.-Y. Chen, C. A. Dennison, *et al.*, "Mapping genomic loci implicates genes and synaptic biology in schizophrenia.," *Nature*, vol. 604, no. 7906, pp. 502–508, Apr. 2022, doi: 10.1038/s41586-022-04434-5.
[22] G. Gibson, "Rare and common variants: twenty arguments.," *Nat. Rev. Genet.*, vol. 13, no. 2, pp. 135–145, Jan. 2012, doi: 10.1038/nrg3118.
[23] C. O. Iyegbe and P. F. O'Reilly, "Genetic origins of schizophrenia find common ground.," *Nature*, vol. 604, no. 7906. England, pp. 433–435, Apr. 2022. doi: 10.1038/d41586-022-00773-5.
[24] R. Sherrington, J. Brynjolfsson, H. Petursson, M. Potter, K. Dudleston, B. Barraclough, J. Wasmuth, M. Dobbs, and H. Gurling, "Localization of a susceptibility locus for schizophrenia on chromosome 5.," *Nature*, vol. 336, no. 6195, pp. 164–167, Nov. 1988, doi: 10.1038/336164a0.
[25] T. Singh, T. Poterba, D. Curtis, H. Akil, M. Al Eissa, J. D. Barchas, N. Bass, T. B. *et al.*, "Rare coding variants in ten genes confer substantial risk for schizophrenia.," *Nature*, vol. 604, no. 7906, pp. 509–516, Apr. 2022, doi: 10.1038/s41586-022-04556-w.
[26] Z. Z. gataa Allami and M. Dragh, "Detection of Breast Cancer Related Gene Polymorphisms By a New Modified PCR-RAPD-PCR Technique." Research Square, 2022. doi: 10.21203/rs.3.rs-1095491/v1.
[27] Haneen M. Ismaeel, "Identification of Genomic Markers By RAPD-PCR Primers in Iraq Breast Cancer Patients," *Iraqi J. Sci.*, vol. 54, no. 1, pp. 97–104, 2013.
[28] A. Talebi, S. Daneshbakhtiyar, M. Meshkat, and M. Meshkat, "Comparison of DNA Instability in

Adenoma and Adenocarcinoma in Patients with Colorectal Cancer, Using Random Amplified Polymorphic DNA Polymerase Chain Reaction (RAPD-PCR)," *J. Isfahan Med. Sch.*, vol. 35, no. 432, pp. 609–614, 2017.

[29] E. K. S. Zafir H. Ghali, Intisar H. Ahmed and B. K. S. S. A. Badry A. Al-Ani, "Assessment of genomic instability in blood and tissues from prostate cancer patients by random amplified polymorphic DNA analysis," *Biotechnol. Res. Cent.*, vol. 3, no. 2, 2009.

[30] N. J. Al-judy and E. Majeed, "Morphological , Biochemical and Molecular Characterization of Ten Rhizobial Bacteria Isolates .," *Iraqi J. Sci.*, vol. 54, no. 2, pp. 280–287, 2013.

[31] O. E. Valencia-Ledezma, C. A. Castro-Fuentes, E. Duarte-Escalante, M. G. Frías-De-León, and M. D. R. Reyes-Montes, "Selection of Polymorphic Patterns Obtained by RAPD-PCR through Qualitative and Quantitative Analyses to Differentiate Aspergillus fumigatus.," *J. fungi (Basel, Switzerland)*, vol. 8, no. 3, Mar. 2022, doi: 10.3390/jof8030296.

[32] T. Hu, Y. Zheng, Y. Zhang, G. Li, W. Qiu, J. Yu, Q. Cui, Y. Wang, C. Zhang, X. Zhou, Z. Feng, W. Zhou, Q. Fan, and F. Zhang, "Identification of a novel Getah virus by Virus-Discovery-cDNA random amplified polymorphic DNA (RAPD).," *BMC Microbiol.*, vol. 12, p. 305, Dec. 2012, doi: 10.1186/1471-2180-12-305.

[33] A. R. Prabhakar, G. Sreeja, and S. V Naik, "DNA finger printing of S. Mutans present in the saliva of caries active children and those associated with intellectual disability - An RAPD analysis.," *Saudi Dent. J.*, vol. 31, no. 4, pp. 424–430, Oct. 2019, doi: 10.1016/j.sdentj.2019.04.009.

[34] I.-M. Redmo Emanuelsson, P. Carlsson, K. Hamberg, and D. Bratthall, "Tracing genotypes of mutans streptococci on tooth sites by random amplified polymorphic DNA (RAPD) analysis.," *Oral Microbiol. Immunol.*, vol. 18, no. 1, pp. 24–29, Feb. 2003, doi: 10.1034/j.1399-302x.2002.180104.x.

[35] M. A. El-Haggar, Y. A. Mahgoub, H. M. Aly, N. M. Ghazy, F. K. El-Fiky, and A. M. El-Hawiet, "DNA barcodes, ISSR, RAPD and SCAR markers as potential quality control tools for molecular authentication of black and white mulberry," *Food Control*, vol. 152, p. 109821, 2023, doi: https://doi.org/10.1016/j.foodcont.2023.109821.

[36] S. Panigrahi, P. Velraj, and T. Subba Rao, "Chapter 21 - Functional Microbial Diversity in Contaminated Environment and Application in Bioremediation," S. Das and H. R. B. T.-M. D. in the G. E. Dash, Eds. Academic Press, 2019, pp. 359–385. doi: https://doi.org/10.1016/B978-0-12-814849-5.00021-6.

[37] H. Hadrys, M. Balick, and B. Schierwater, "Applications of random amplified polymorphic DNA (RAPD) in molecular ecology.," *Mol. Ecol.*, vol. 1, no. 1, pp. 55–63, May 1992, doi: 10.1111/j.1365-294x.1992.tb00155.x.