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DNA Banking to Assess Genetic Influences on Schizophrenia

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Abstract

Background: Schizophrenia is among the most prevalent psychiatric disorders globally, with a lifetime prevalence rate of 0.3% to 0.7%, characterized by the heterogeneous presence of positive, negative, and cognitive symptoms that affect all aspects of mental activity. We aimed to describe the genetics of schizophrenia to widening our understanding of the inheritance of this illness.

Methods: This quasi-experimental study was conducted in Razi psychiatric hospital in Tehran province, Iran. Recruitment of the study samples was conducted in Tehran, Iran, among patients with schizophrenia and their families. For this purpose, individuals with schizophrenia in 40 families with at least 1 to 2 affected members were identified and selected based on a clinical interview conducted by a psychiatrist and according to the Diagnostic and Statistical Manual of Mental Disorders, 5th Edition. The clinical and paraclinical data, drug and substance usage, and medical treatments were collected through a standardized clinical questionnaire. Besides, the Global Assessment Scale and the Positive and Negative Syndrome Scale were completed for all study participants.

Results: A total of 22 families had a negative family history, and 1 affected member and the rest of the studied families had a positive family history and at least 2 affected members. In addition, genealogical data (family tree) and lymphoblastic cell categories were developed to examine genes, and subsequent research results will be reported in the future.

Conclusion: As the research continues, the approach to sampling must be modified to ensure that the deoxyribonucleic acid bank is as extensively representative as possible of all schizophrenia cases.

Keywords: DNA Banking, Genetics, Schizophrenia, Family History, Iran

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Introduction

Schizophrenia is among the most prevalent psychiatric disorders globally, with a lifetime prevalence rate of 0.3% to 0.7% (1, 2), being characterized by the heterogeneous presence of positive, negative, and cognitive symptoms that affect all aspects of mental activity. Typically, the onset age of this condition is reported to be late adolescence or early adulthood and it affects men more than women (3). Schizophrenia heritability ranges between 44% and 87%, with a mean value of 81% (4).

Despite progressive medical advances , there is no certain cure identified for this disorder and the available treatment is only limited to managing symptoms and preserving adequate functionality and independence in the affected patients (5). The modern-day psychiatry presented the idea of an association between genetic predisposition and mental illness development. Moreover, epidemiologic research studies employ deoxyribonucleic acid (DNA) banking to investigate genetic risk factors in vari-

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↑What is "already known" in this topic:
Epidemiologic research studies employ deoxyribonucleic acid (DNA) banking to investigate genetic risk factors in various diseases. In this respect, there has been extensive research with favorable outcomes concerning the genetics of schizophrenia.

→What this article adds:
Genealogical data (family tree) and lymphoblastic cell categories were developed to examine genes, and subsequent research results will be reported in the future.

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ous diseases (6). In this respect, there has been extensive research with favorable outcomes concerning the genetics of schizophrenia (7).

Scholars believe that numerous genes affect every illness and that only a small impact is conferred per gene on the phenotype. There is no reported diagnostic predictive value for the individual risk variants. Besides, the availability of enormous epidemiological samples might alter risk estimations in the future (8). There is a shift of knowledge occurring in the genetics model of schizophrenia, where polygenic models are replacing oligogenic ones; however, the genetic architecture of this disorder remains undiscovered (2, 9).

Genome-wide transcription (expression quantitative trait loci [eQTL], currently detected by microarray expression information) and GWAS (Genome-Wide Association Study, DNA variation outputs) data integration, by combining the statistical outcomes and biology accomplishments could help eliminate this gap of knowledge (10). Undiscovered genetic mechanisms might significantly contribute to the heritability of schizophrenia. However, a large body of literature is dedicated to investigating the molecular genetic spectrum in schizophrenia (11). An essential measure is integrating mutations' spectrum detected in schizophrenia with a system that constantly addresses changes in environments and evolution. Genetics and medicalization of mental disorders appear to be strongly correlated. Schizophrenia and other mental conditions are believed to partly arise from genetic deficits; thus, psychiatric medications are widely prescribed to manage biological alterations induced by this disorder, such as decreased levels of neurotransmitters, or deficiencies in neuronal circuits. Thus, it is important to bear in mind that patients encountering a greater genetic contribution to their condition are better responsive to pharmacotherapy (12).

The latest paper, from the Schizophrenia Working Group of the Psychiatric Genomics Consortium, reports an analysis of more than 150,000 people and finds more than 100 genetic regions associated with schizophrenia, laying to rest forever the idea that genetics is not an important cause of the illness. There is also progress in analyzing schizophrenia by DNA microarrays. Based on the outcome of the transcriptome profiling experiments performed to date, it appears that schizophrenia is associated with a global gene expression disturbance across many cortical regions. Deciphering the pathophysiology of mental disorders depends on integrating data from across many research fields and techniques that essentially need available DNA samples and creating cell lines to perform cell culture experiments and develop or evaluate novel drugs to treat this devastating disorder.

The Genetics Research Center of the University of Social Welfare and Rehabilitation Sciences is among the organizations with a high capacity to fulfill the requirements of the Iranian researchers in terms of accessing DNA samples. Patients with different ethnicities are referred to Razi psychiatric center in Tehran from all around the country; there exist some special cases with clinical and research importance. Therefore, investigating the DNA samples of such rare cases could be highly beneficial for various research fields in the future.

The present report focused on the importance of DNA banking in the assessment of genetic influences on schizophrenia. We aimed to describe the genetics of schizophrenia to widen our understanding of the inheritance of this illness. The obtained data will be beneficial for the current and future research on schizophrenia, concerning its prevention, management, and rehabilitation.

Methods

The current study aimed to provide a DNA bank of schizophrenia in the Iranian population. Recruitment of the study samples was conducted in Tehran, Iran, among patients with schizophrenia and their families. For this purpose, individuals with schizophrenia in 40 families with at least 1 to 2 affected members were identified and selected based on a clinical interview conducted by a psychiatrist and according to the Diagnostic and Statistical Manual of Mental Disorders, 5th Edition (DSM-5). This quasi-experimental study was conducted in Razi psychiatric hospital in Tehran province, Iran. The standardized clinical questionnaire used in this study consisted of demographic data, cultural and national origin, the acuteness of disorder, suicidal behavior or hazard by others, life events, familial history of psychiatric and physical diseases, medical data (including height, weight, waist circumference, glucose, triglyceride, cholesterol low-density lipoprotein and high-density lipoprotein, review of all systems, infectious diseases, allergy, and other diseases), developmental disorders, substance use, and medical treatments. Besides, the Global Assessment Scale and the Positive and Negative Syndrome Scale (PANSS) were completed for all study participants. Since genetic assessments must be conducted on both the patients and their families, we also collected samples from the family members of patients with schizophrenia. The informed consent form was obtained from patients or their legal guardians for participation in the study. Additionally, the study participants' family tree was drawn. In the future stages of the research, more samples will be collected to extend the sample size and subsequent research results will be reported.

The inclusion criteria of the current study were as follows: being diagnosed with schizophrenia without a positive family history according to the diagnostic criteria of the DSM-5 and a psychiatrist's endorsement; being diagnosed with schizophrenia with a positive family history according to the diagnostic criteria of the DSM-5 and a psychiatrist's endorsement; providing an informed consent form to participate in the research (by the patients or their guardians); and being a family member of a patient with schizophrenia and providing an informed consent form to participate in the research. The study exclusion criterion was the lack of consent to participate in the research project.

Participants peripheral blood sample (30 mL in 3 tubes without anticoagulant containing 5 Na-heparin, and EDTA (10 mL each), respectively, was collected for the segregation and storage of the serum, the peripheral blood mono-

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nuclear cells (PBMC) (for the preparation of InfoBlast cell category), and the plasma, and the buffy coat. Besides, in the case of healthy/unhealthy siblings, buffy coat separation in a tube containing ethylenediamine tetraacetic acid (EDTA) (10 mL each) was performed. Additionally, blood samples were transferred to the laboratory immediately after sampling. The separation and storage of the PBMC layer were only performed for the infected patients who have been transferred to the emergency room and hospitalized ones. In the case of the affected or healthy siblings, only the separation and storage of the buffy coat and the plasma were performed. After separation, the samples were stored at -80°C according to the protocol. The PBMC was also transferred to liquid nitrogen after 24 hours. In terms of PBMC, storage was done in environments containing FBS 90% and DMSO 10%.

Moreover, lymphoblastic cell lines were created based on the following steps: The growth of B95-8 cells was done to produce the outer liquid layer of the virus, consisting of the following measures: adding RP-10 to B95-8 (human EBV-infected cell category); the 20-mL cultivation of 1x 106 of growing B95-8 cells and heating for 3 days, followed by the bioavailability assessment (more than 95% of cells must be alive), and centrifugation at 1200 rpm surface liquid filter through a 0.45-micrometer filter and storage in liquid nitrogen.

Human PBMC transformation was performed as follows: Heating, washing, and diluting human PBMC; adding 2.5 mL of the liquid outer layer obtained from the stage 1 and heating it in a water bath at 27°C for 2 hours; adding 5 mL RP10, containing 10 micrograms/mL cyclosporine A, and transferring it to a T25 flask and heating it; mixing the cells after 3 weeks and adding 5 mL of it to 2 new T25 flasks; then, depleting 5 mm of RP10 and heating for 1 week (it was possible to count and freeze cells during this period), and performing cell division (1:3) once a week to maintain the level of lymphoblastoid cell line.

Results

In this study, the samples of the plasma serum and the buffy coat were obtained and stored at -80 °C from 40 families that were selected as research samples according to the study inclusion criteria. Twenty-two out of 40 families had a negative family history and 1 affected member, and the rest of the studied families had a positive family history and at least 2 infected members.

At present, the collection of samples and the separation and storage of the plasma, the serum, and the buffy coat have been conducted on 40 families. For 32 samples, the lymphoblastic cell category was also obtained and stored. The genealogical data (family tree) and lymphoblastic cell categories were developed to examine genes. In the future stages of the research, further data will be obtained from new families to extend the sample size, and subsequent research results will be reported in the future.

Discussion

The current research described the establishment of the Iranian schizophrenia DNA bank; it attempted to collect, store, and distribute linked genetic and clinical data from a sample of individuals with schizophrenia and their families. The source for schizophrenia sampling recruitment comprised the largest psychiatric center in Iran, that is, Razi psychiatric hospital in Tehran province, Iran. Also, blood samples were collected from the healthy family members of patients with schizophrenia. All study participants took part in clinical interviews and donated blood samples for the relevant genetic assessments, which led to a comprehensive source of data for DNA banking, including diagnostic and family history information.

There has been remarkable progress in the genetics of schizophrenia. In the past, we lacked a single recognized genetic risk variant or gene, and it was the norm to fail to replicate previous investigations. Accordingly, such a gap of knowledge imposed numerous complications to psychiatric genetics investigators. Currently, there are extensive robustly supported genetic variants, providing evidence for a relationship between more significant characteristics and the risky rare and de novo variants. Advances, such as the possibility to manipulate human cells for differentiation (13, 14), generating pluripotency (15-17), and achieving efficient and precise genome editing (18-20) could significantly contribute to the identification of the risk-related biology of diseases.

Schizophrenia is a heterogeneous and complex condition. Therefore, multiple genetic and environmental characteristics influence its pathogenesis. Subsequently, various clinical and cognitive phenotypes and structural brain alternations could be distinguished for its pathology. The present attempt to DNA banking of schizophrenia aims to provide the foundation for advancing the understanding of this illness by providing a link between the genetic, demographic, and other relevant data (eg, age, gender, negative/positive symptoms, impairments in cognition,) in the affected population. As the results of the currently ongoing research will be reached, experts and scholars could use them to understand significant differences in various groups concerning the genetic characteristics profile of schizophrenia. The DNA banking of schizophrenia in specific populations will also provide opportunities for scientists to evaluate their promising original hypotheses on a representative sample in the case of hard-to-access schizophrenia populations. Another advantage of creating a DNA bank is the possibility of linking different datasets for comparing and understanding rare genetic variations and the genes susceptible to a limited effect, without conducting research on extremely large sample sizes.

The limitations of project implementation included the difficulty in accessing the families of patients with schizophrenia to visit Razi hospital for collecting their blood samples; this was because participants lived in different parts of the country. Also, another issue was that genetic assessments had to be conducted on both the patients and their families.

Conclusion

DNA banking is a valuable and accessible schizophrenia research facility for use by approved scientific investi-

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gators for the prevention, treatment, and rehabilitation of this disorder. As the research continues, the approach to sampling must be modified to ensure that the DNA bank is as extensively representative as possible of all schizophrenia cases.

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Ethical Approval

All procedures performed in the study involving human participants were in accordance with the ethical standards of the institutional and national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. Moreover, participating in the study was voluntary and the study results are available to the study samples upon request.

Informed Consent

The relevant informed consent form was obtained from all study participants.

Conflict of Interests

The authors declare that they have no competing interests.

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