An Observational Analytical Molecular Neuropharmacological and Neuropharmacogenomic Research Study and An Analytical Systematic Review and Meta-analysis on the Pharmacogenomic Mechanisms of Brain Organoids.

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Received: 11-04-2022; Revised: 24-06-2022; Accepted: 30-06-2022; Published on: 15-07-2022.

ABSTRACT

Brain organoids recapitulate in vitro the specific stages of in vivo human brain development, thus offering an innovative tool by which to model human neurodevelopmental disease. Brain organoids can model congenital structural deficits or be subjected to environmental insult. This clinical research, systematic review and meta-analysis were conducted for systematically exploring the molecular neuropharmacological and neuropharmacogenomic mechanisms of the brain organoids, with thorough explanations and analysis of the medical study literature and evidences compiled from the innumerable studies conducted, which explained the multi-dimensional pharmacomolecular significance of brain organoids. The objectives of this study were an observational analytical molecular neuropharmacological and neuropharmacogenomic research study and an analytical systematic review and meta-analysis on the pharmacogenomic mechanisms of brain organoids. The mixed-method study was conducted in accordance with the PRISMA (Preferred Reporting Items for Systematic reviews and Meta-Analyses) Statement and Guidelines, 2009, described by the Cochrane Collaboration in June, 2016. At first, the steps of identification included the records which were identified through database searching and the additional records which were identified through other sources. This led to the steps of screening, which included the screened records after the duplicates were removed. From these screened records, few records were excluded, as per the exclusion criteria. Then, in the eligibility step, the full text articles were assessed for eligibility, from which few full text articles were excluded, according to the exclusion criteria, with adequate reasons. This led to the final inclusion step, where the studies were included in the qualitative synthesis of a systematic review, according to the inclusion criteria, and ultimately the studies were included in the quantitative synthesis of a meta-analysis, with analytical interpretations. An observational analytical molecular neuropharmacological and neuropharmacogenomic research study was also conducted. In this mixed-method study, the systematic review and meta-analysis contributed 3084 refined and relevant medical records, among total 5608 records obtained from the study databases search. It also describes the molecular neuropharmacological and neuropharmacogenomic mechanisms of brain organoids, which elaborated this analytical systematic review and meta-analysis, and observational analytical molecular pharmacological and neuropharmacogenomic research study. To conclude, this clinical research, systematic review and meta-analytical study provided the refined qualitatively synthesised medical records, study literature and databases, with quantitative analytical interpretations, as well as an interpretative analysis on the molecular neuropharmacological and neuropharmacogenomic mechanisms of brain organoids.

Keywords: Systematic Review, Meta-Analysis, Brain Organoids, Neuropharmacogenomics, Pharmacology, Molecular Neuropharmacology, Observational Analytical Clinical Research.

INTRODUCTION

Organoids are three-dimensional cell structures, grown in vitro from the stem cells, mainly isolated from the biopsies or from the pluripotent stem cells, that are extensively similar to the endogenous organs, in both their structural development and functional performance. The organoids are formed of cells which differentiate, undergo spatially restricted lineage...
commitment, and acquire the specific tissue patterning to develop into several endoderm, mesoderm, and ectoderm-derived tissues. These organoids mostly tend to resemble the *in vivo* original organs, with the preservation of their genetic, phenotypic and behavioural traits. These are not only complex structures, but also possess unique capabilities of modeling human organ development and disease, showing wide similarities with the human organ system.

Brain organoids recapitulate *in vitro* the specific stages of *in vivo* human brain development, thus offering an innovative tool by which to model human neurodevelopmental disease. Brain organoids can model congenital structural deficits or be subjected to environmental insult.17

The clinical research, systematic review and meta-analysis were conducted for systematically exploring the pharmacogenomic mechanisms of brain organoids, with thorough explanations and analyses of the medical study literature and evidences compiled from the innumerable studies conducted, thus illuminating on the multi-dimensional pharmacomolecular significance of the brain organoids. The novelty of this study involved the particular focussed exploration of the complex intricacies of the pharmacogenomic constitution and variations of the brain organoids, and the variegated ways of utilising these pharmacogenetically organised structural and functional configurations within these brain organoids in the clinical therapeutic applications and disease modeling, for diseases requiring intensive management, post remaining as co-therapeutic refractory entities. This study also recounts the systematic quantitative chronicle of the extensive clinical research studies conducted, for a better interpretation of the future further medical innovative directions.

**Objectives**

The objectives of this study were an observational analytical molecular neuropharmacological and neuropharmacogenomic research study and an analytical systematic review and meta-analysis on the pharmacogenomic mechanisms of brain organoids.

**MATERIALS AND METHODS**

**Ethical principles**

At first, the Institutional Ethics Committee clearance and approval was taken. The study was conducted in accordance with the ethical principles of Declaration of Helsinki and Good Clinical Practices, contained within the International Council for Harmonization of Technical Requirements for Pharmaceuticals for Human Use (ICH-E6 and ICH-E17), and in compliance with the global regulatory requirements. The regulatory frameworks and general guidelines required for organoids and their clinical applications, for example, drug testing using organoids in Europe, include “Guideline on the principles of regulatory acceptance of 3Rs (replacement, reduction, refinement) testing approaches” by the European Medicines Agency, the regulatory requirements for cell and gene-based therapies, and good manufacturing practices (GMP) of a pharmaceutical drug, for the clinical use of organoids. Informed consent was obtained.

**Study Type**

This study was a multi-variate, observational, descriptive, analytical, qualitative molecular neuropharmacological and neuropharmacogenomic research study and a multi-variate, multi-centre, analytical systematic review and meta-analysis on the pharmacogenomic mechanisms of brain organoids.

**Study Materials**

The study materials consisted of pharmacological clinical research database and medical evidences of global heterogenous research analyses and similar study literature on the molecular neuropharmacological and neuropharmacogenomic mechanisms of brain organoids.

**Study Period**

The study period for this research project and the compilation of the study literature was 1.5 years, from January, 2021 to June, 2022.

**Place of Study**

This research study and the compilation of the study literature was conducted in the Departments of Pharmacology, Clinical Pharmacology, Molecular Pharmacology, Rational Pharmacotherapeutics, Pharmacoepidemiology, Pharmacovigilance, Pharmacogenomics, Evidence-Based Medicine, Clinical Pathology, Molecular Diagnostics, Medical and Reproductive Endocrinology and Diabetology, Clinical Medicine, Regenerative Medicine, Organ Transplantation, and Clinical Research, at Dr. Moumita Hazra’s Polyclinic And Diagnostic Centre, Hazra Nursing Home, Hazra Polyclinic And Diagnostic Centre, Rama Medical College Hospital and Research Centre, Rama University, Mamata Medical College and Hospitals, Mahuya Diagnostic Centre and Doctors’ Chamber, Fortis Hospitals, and Global Institute Of Stem Cell Therapy and Research (GIOSTAR), Institute of Regenerative Medicine (IRM), Institutes, Hospitals and Laboratories.

**Study Procedure**

The study was conducted in accordance with the PRISMA (Preferred Reporting Items for Systematic reviews and Meta-Analyses) Statement and Guidelines, 2009, described by the Cochrane Collaboration in June, 2016. At first, the steps of identification included the records which were identified through database searching and the additional records which were identified through other sources. This led to the steps of screening, which included the screened records after the duplicates were removed. From these screened records, few records were excluded, as per the exclusion criteria. Then, in the eligibility step, the full text articles were assessed for eligibility, from which
few full text articles were excluded, according to the exclusion criteria, with adequate reasons. This led to the final inclusion step, where the studies were included in the qualitative synthesis of a systematic review, according to the inclusion criteria, and ultimately the studies were included in the quantitative synthesis of a meta-analysis, with analytical interpretations.

The study selection criteria were the following:

(a) The inclusion criteria were: The published articles on the molecular neuropharmacological and neuropharmacogenomic mechanisms of brain organoids; the original research studies, systematic reviews, meta-analyses, case reports, case series, narrative reviews, study series, parallel studies and similar kind of studies or reviews, of any or all types, which were either qualitative, or quantitative, or both qualitative as well as quantitative; the publication time-frame within a span of the past 5 years; and any or all types of observational, descriptive and analytical research studies.

(b) The exclusion criteria were: Irrelevant studies; and studies older than 5 years.

Each study was assessed for allocation concealment, blinding, reporting of losses to follow-up or missing outcome assessments, evidence of important baseline differences between the groups, analysis on an intention-to-treat basis and use of a sample size calculation.

An observational analytical molecular neuropharmacological and neuropharmacogenomic research study was also conducted, on the variegated pharmacogenomic mechanisms of brain organoids.

**RESULTS**

(i) The results of this Systematic Review and Meta-Analysis:

In this study, in the systematic review and meta-analysis, in the identification stage, the study literature search on the pharmacogenomic mechanisms of brain organoids, contributed 2167 records in PubMed search, 1266 records in EMBASE search, 1623 records in Scopus search, and 552 records in additional databases search, identified through other sources. The records, after removing 1630 duplicates, were 3978. In the screening stage, the records screened were 3978, with the exclusion of 561 records, according to the exclusion criteria. In the eligibility stage, the full text articles assessed for eligibility were 3417, with the exclusion of 333 full text articles, according to the exclusion criteria. In the final inclusion stage, the records ultimately included in the qualitative synthesis, according to the inclusion criteria, was 3084. These 3084 records were the refined contributions of this systematic review and meta-analysis, on being interpreted and analysed qualitatively and quantitatively. Thus, this systematic review and meta-analyses, on being interpreted and analysed qualitatively and quantitatively.

![](image.png)

**Figure 1:** The Stages in PRISMA (Preferred Reporting Items for Systematic reviews and Meta-Analyses) Statement and Guidelines, 2009
The selected experimental elucidations on the pharmacogenomic mechanisms of brain organoids:

In the clinical research study, from the compilation of pharmacotherapeutic databases and evidences, and the observational analytical molecular neuropathological and neuropharmacogenomic research study, the pharmacogenomic mechanisms of brain organoids was described, in complete details, to explain the qualitative details of the conducted clinical research, systematic review and meta-analysis, to validate the extensive analytical quantifications.

**DISCUSSION**

In this mixed-method study, during the systematic review and meta-analysis, in identification stage, the study literature search on the pharmacogenomic mechanisms of brain organoids, contributed 2167 records in PubMed search, 1266 records in EMBASE search, 1623 records in Scopus search, and 552 records in additional databases search, identified through other sources. The records, after removing 1630 duplicates, were 3978. In the screening stage, the records screened were 3978. From these records, 561 records were excluded, according to the exclusion criteria. In the eligibility stage, the full text articles assessed for eligibility were 3417. From these records, 333 full text articles were excluded, according to the exclusion criteria. In the final inclusion stage, the records ultimately included in the qualitative synthesis, with quantitative analysis, according to the inclusion criteria, was 3084. These 3084 records were the refined contributions of this systematic review and meta-analysis. Thus, this systematic review and meta-analysis contributed 3084 refined and relevant medical records, among total 5608 records obtained from the study databases search. The refined records were further specifically stratified on the basis of well-categorised criteria of the research analysis.

The following selected qualitative experimental elucidations on the pharmacogenomic mechanisms of the brain organoids were described:

In this study, in the clinical research, from the compilation of the pharmacotherapeutic databases and evidences, and the observational analytical molecular neuropathological and neuropharmacogenomic research study, the following details were described.

Genetic engineering and multi-organoid fusion enable the assessment of a broader array of disease mechanisms, such as abnormal interregional development. Various methods can be used to evaluate the developmental changes that underlie the disparate phenotypes observed between normal and diseased organoids. Perhaps the greatest application of organoid technology thus far, in vitro modeling of neurodevelopmental disease enables observation of disease progression throughout neurodevelopment and in conjunction with novel genetic techniques—the opportunity to interrogate underlying pathological mechanisms with previously precluded precision. The versatility of brain organoids permits modeling diseases of either intrinsic (i.e., genetic) or extrinsic (i.e., environmentally mediated) etiology. However, despite recent characterization of functional network development, developmental disorders in which gross structural abnormalities predominate remain the more accessible for in vitro modeling. Autosomal recessive primary microcephaly (MCPH) has been modeled with organoids generated from patient-derived iPSCs carrying mutations in either ASPM, the gene that codes for a protein involved with mitotic spindle function and that accounts for a plurality of MCPH cases, or CDK5RAP2, a gene whose product localizes to the mitotic spindle pole during neurogenesis. Those iPSCs in which ASPM expression was downregulated, predicted to impede neural progenitor proliferation, yielded hypoplastic organoids with fewer proliferative cells, decreased neocortex-like morphology, and diminished neuroepithelial structural integrity. Functional analysis revealed calcium activity in fewer cells than the controls—implicating neuronal maturation impediment—and decreased synchrony. CDK5RAP2-mutant organoids likewise portrayed hypoplasticity with sparse progenitor and neuroepithelial regions. Coincident findings of premature neural differentiation and increased neuron quantity were supported by observation of increased neuronal differentiation upon CDK5RAP2 RNAi-knockdown. Successful phenotypic rescue upon electroporated expression of CDK5RAP2 protein confirmed viable in vitro recapitulation of MCPH.

In several studies, the demonstration of various types of transient and stable approaches for genetic modifications in the brain organoids, have been visualised. The techniques, as per the stage of development for multidimensional applications, include Cas9 nickase KO, Cas9 oligonucleotide knock-in, TALEN inducible gene knock-in, PiggyBac fluorescence and lentivirus fluorescence approaches, at single cell stage, all of which were stable; Sleeping Beauty nucleofection and CRISPR/Cas9 KO nucleofection approaches, at embryoid bodies stage, all of which were stable; adeno-associated virus fluorescence, plasmid gene rescue, plasmid fluorescence, shRNA approaches, at organoids stage, all of which were transient; and lentivirus fluorescence, viral stamping, Cas9 oncogene knock-in and suppressor KO and Sleeping Beauty GFP approaches, at organoids stage, all of which were stable, manifested various degrees of mosaicism and spatial distribution of genetically modified cells, following the above-mentioned types of transfection and genetic modification approaches.

The ultimate application of organoid technology is to use them for organ regeneration and replacement therapies, reducing whole organ transplant requirements and
improving the life quality of patients. The therapeutic use of organoids would be an alternative to the challenging transplantation of organs with a short period of viability outside the body, such as the heart and lungs. Organoids should highly impact regenerative treatments of organs that remain technically non-transplantable, such as the brain. The recent development of edited pluripotent stem cells with targeted disruption of HLA genes by CRISPR/Cas9 technology should also facilitate the generation of immune-compatible healthy organoids for widespread therapeutic purposes.1-7

Therefore, this observational analytical molecular neuropharmacological and neuropharmacogenomic research, as well as systematic review and meta-analysis, provided the refined qualitatively synthesised medical records, study literature and databases, with well-comprehensive records, on the pharmacogenomic mechanisms of brain organoids, with well-specified criteria categorisations.

CONCLUSION

Therefore, in this mixed-method study, comprising of the clinical research, the systematic review and meta-analysis, the systematic review contributed 3084 refined and relevant medical records, among total 5608 records obtained from the study databases search, with specifically categorised research analytical criteria; and the observational clinical research analysed and intensively interpreted the pharmacogenomic mechanisms of brain organoids, which comprehensively clarified and elaborated this entire mixed-method quantitative and qualitative study.

Acknowledgements: My gratitude to the Departments of Pharmacology, Clinical Pharmacology, Molecular Pharmacology, Rational Pharmacotherapeutics, Pharmacoepidemiology, Pharmacovigilance, Pharmacogenomics, Evidence-Based Medicine, Clinical Pathology, Molecular Diagnostics, Medical and Reproductive Endocrinology and Diabetology, Clinical Medicine, Regenerative Medicine, Organ Transplantation, and Medical Academics and Clinical Research, Dr. Moumita Hazra’s Polyclinic And Diagnostic Centre, Dr. Moumita Hazra’s Academic Centre, Dr. Moumita Hazra’s Educational Centre, Hazra Nursing Home, Hazra Polyclinic And Diagnostic Centre, Mamata Medical College and Hospitals, Mahuya Diagnostic Centre and Doctors’ Chamber, Fortis Hospitals, Global Institute Of Stem Cell Therapy and Research (GIOSTAR), Institute of Regenerative Medicine (IRM), Institutes, Hospitals and Laboratories, and, the global electronic medical databases, for the successful completion of this clinical research project.

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