The Use of iPSC-Derived Neural Organoids to Investigate Autism Spectrum Disorders

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Abstract— **Autism spectrum disorders (ASD), characterized by impaired social communication, behavioral abnormalities, and restricted interests, affect an estimated 1 in 59 children worldwide. This review provides a comprehensive background on ASD, its historical context, genetic bases, and the influence of epigenetic and environmental factors. Neural organoids, 3D models that mimic human brain structure and functionality, are utilized with intrinsic organoid protocols and guided differentiation to gain insights into ASD modeling, risk gene identification, and environmental effects. Neural organoids also offer promise in drug screening and personalized treatment development for ASD. Despite limitations and ethical concerns, neural organoids stand as a transformative tool in understanding the molecular and cellular bases of ASD, ultimately paving the way for more effective interventions and support for affected individuals.**

I. Introduction

Autism spectrum disorders (ASD) are complex neurodevelopmental disorders characterized by impaired social communication, behavioral abnormalities, and restricted interests. Understanding the underlying molecular and cellular changes associated with ASD is crucial for advancing our knowledge of the disorders and developing effective interventions. In recent years, the development of neural organoids, three-dimensional models that mimic the structure and functionality of the human brain, has provided researchers with a powerful tool to study neurobehavioral disorders such as ASD.

A. Background on Autism Spectrum Disorders (ASD)

ASD, characterized by impacted communication, social interaction, and behavior, are a highly prevalent group of neurodevelopmental disorders, affecting an estimated 1 in 59 children [1]. Individuals with ASD may have difficulties with social interactions such as understanding nonverbal cues or engaging in conversation. They may also exhibit repetitive behaviors or restricted interests. ASD is considered a spectrum disorder due to the wide variation in severity of symptoms, ranging from mild to severe, with some symptom overlap across the spectrum, but also potential differences between mild and severe cases [2].

While individuals with mild ASD may have social difficulties, such as with initiating and maintaining conversations, understanding social cues, or interpreting emotions, they may still desire social interaction and have some capacity in adaptive functioning. People with mild ASD often have good language skills, although they may struggle with certain aspects of communication, such as understanding non-literal language. Intellectual functioning is often within the average or above-average range. They may excel in certain areas of interest or have specific talents. Individuals with mild ASD may exhibit repetitive behaviors that do not significantly interfere with their daily activities.

In severe ASD, social interaction may be extremely limited or absent. Communication difficulties are more pronounced, with limited or no verbal language. In severe ASD, intellectual disability may be present, and individuals may have significant cognitive impairments across multiple domains. Challenging behaviors can be more pronounced and disruptive, including self-injury, aggression, tantrums, or meltdowns.

1. History

ASD was first described by Kanner (1943) in a detailed report of 11 children with similar unusual behaviors, including improper language, indifference to other people, and obsessive tendencies [3]. As the 20th century progressed, the initial rarity of diagnoses of ASD became much more prevalent, with 1 in 59 individuals being diagnosed on the spectrum [4]. This increase can be partially attributed to the increasing awareness of mental health issues in the past century as well as scientific acknowledgement of a spectrum of neurodevelopmental disorders beyond the 1950s' automatic categorization of schizophrenia [4].

Current Diagnostic and Statistical Manual of Mental Disorders (DSM) criteria deems only two broad features as characteristic of an ASD diagnosis: deficits in social communication and social interaction across multiple contexts; and restricted, repetitive patterns of behavior, interests, or activities [5]. Due to the generality of these definitions, ASD often overlaps with other conditions, such as motor abnormalities, gastrointestinal issues, epilepsy, and intellectual disorder.

The causes of ASD are not fully understood, but it is believed to be caused by both genetic and environmental factors. About 10-20% of ASD cases can be attributed to a known genetic cause, and patients with similar mutations may be diagnosed on very different levels of the spectrum [1]. Considering the nature of its etiology, or the in-depth causation of a disease, ASD does not specifically "target" any particular demographic. ASD can affect individuals of any gender, race, ethnicity, or socioeconomic background. However, research has indicated that autism is diagnosed more frequently in males than females, with three times as many males diagnosed than females. This gender disparity may be due to a withstanding lack of diverse research, and research is ongoing in terms of possible sex-linked genes.

2. Genetics of ASD

High-throughput genotyping, or simultaneous genotyping for hundreds or thousands of markers in hundreds to thousands of individuals, of single-nucleotide polymorphisms (SNPs) using microarrays has enabled genome-wide association studies (GWAS) of ASD to detect any variants associated with the disorder. Several GWAS have investigated the link between common genetic variants and ASD diagnosis, identifying two

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significant risk loci at chromosome 5p14.1 and within gene *MACROD2* [37]. However, replication of these loci has been challenging despite the high heritability and prevalence of the disease. Sample sizes in ASD GWAS are relatively small compared to studies on other common diseases, and larger sample sizes are needed to identify additional loci as ASD cohorts expand.

As said above, the effect size of individual polymorphisms in ASD is small based on GWAS: genetic linkage analysis has identified significant signals at specific loci, but common variants responsible for the linkage signals have not been identified. The search for rare deleterious variants that confer greater risk in ASD has thus been fueled by smaller-scale genetic models in families and the need to explain genetic heterogeneity. Strategies such as homozygosity mapping in families, identification of inherited variants on the X chromosome (often linked to fragile X mutations), and searching for rare knockout variants have yielded new leads in the search for inherited variation in ASD [7]. Similarly, many cases of autism appear to be caused by several abnormal genes acting in concert, indicating chromosomal aberration [6].

Rare *de novo* genetic variants, which arise from mutations in the parental germline or early somatic cells but are not present in either parent, are one genetic cause of ASD that is not accounted for in heritability estimates. *De novo* copy number variants (CNVs) and single-nucleotide variants (SNVs) have been found to play a role in ASD susceptibility. Recent advancements in sequencing technologies have enabled the study of *de novo* SNVs, revealing several new ASD susceptibility genes, such as *CHD8*, *DYRK1A*, *GRIN2B*, and *TBR1* [6].

ASD biomarkers are found in various pathways involved in growth regulation and protein synthesis. ASD has been connected to transcription factor families such as TCF; as well as related signaling pathways such as Wnt/β-catenin [6] (involved in embryonic development, tissue homeostasis, cell proliferation, gene expression, and cell fate decisions); mTOR [6] (involved in regulating protein degradation via the protein complex mTORC1, downstream from proteins coded by ASD risk genes); serotonin and oxytocin signaling; and neuron-glia signaling [6].

3. *Epigenetics and Environmental Factors of ASD*

Even in familial ASD cases that follow typical inheritance patterns, where a single gene mutation is responsible for the condition, the penetrance (i.e. the likelihood of expressing the disorder) is reported to be less than 50%. This suggests that environmental factors and epigenetic factors, which involve modifications to gene expression without altering the underlying DNA sequence, may play an important role in explaining some aspects of the etiology of ASD [8].

Epigenetic alterations refer to non-permanent changes in gene expression regulation that affect DNA shape and configuration, rather than the DNA sequence itself. These alterations can impact the transcription of specific genes by modifying the accessibility of chromatin threads, which carry genetic information. These alterations are seen in the form of DNA methylation, histone modifications, acetylation, phosphorylation, and noncoding RNA-related factors. DNA methylation, a mode of gene repression that involves the addition of methyl groups to DNA, is particularly prevalent in ASD [38]. For instance, DNA methylation of the brain-derived neurotrophic factor (*BDNF*) gene has been linked to autism and other neurological diseases [8].

Dysregulation of growth factors has been found in a number of adults with ASD. These growth factors are involved in neuronal growth, differentiation, proliferation, and survival in the course of neurodevelopment and can also modulate axonal and dendritic outgrowth [38].

Severe maternal viral infection in the first trimester of pregnancy and bacterial infection in the second trimester is also highly associated with a diagnosis of ASD. Maternal use of valproic acid during pregnancy could also affect the production of GABAergic neurons via the blocking of histone deacetylase. Gamma-aminobutyric acid (GABA) is an amino acid that functions as the primary inhibitory neurotransmitter for the CNS. It functions to reduce neuronal excitability by inhibiting nerve transmission. GABA is thought to play a major role in controlling nerve cell hyperactivity associated with anxiety, stress, and fear, and thus its dysregulation is associated with the symptoms of ASD [43].

Research on oxidative stress (imbalance between the production of reactive oxygen species (ROS) and the body's ability to neutralize them) in ASD has also revealed a potential role in the condition's neurobiology [9].

While ASD is mostly caused by the interaction between genes *and* the environment, many environmental factors alone play a role in its etiology. Various prenatal factors have been associated with an increased risk of ASD. As stated above, these include maternal exposure to certain medications (e.g., valproic acid and thalidomide) [10], maternal use of tobacco or alcohol during pregnancy, maternal infection (e.g., rubella, cytomegalovirus), and certain maternal health conditions (e.g., diabetes, obesity). Other examples include phenylketonuria, hypoxia during birth, and exposure to air pollution, pesticides, and heavy metals [11].

B. *Background on Neural Organoids*

Neural organoids, also called brain or cerebral organoids, are 3D models that mimic the structure and functionality of the human brain. They are derived from induced pluripotent stem cells (iPSCs) and can self-organize and differentiate into various cell types, superficially modeling the cellular diversity and complexity found in the real organ. Therefore, neural organoids provide a valuable tool for studying human development, disease modeling, drug screening, and personalized medicine.

1. *History*

Due to the complexity and inaccessibility of human brains, postmortem and surgical samples were often used in neural research. However, these methods were

typically inconsistent due to variability in genetic and environmental background and issues with tissue processing and preservation. Hence, animal model organisms, such as mice, have been extensively utilized to investigate brain development and function. However, there are notable differences in the developmental processes and structures of the human brain and that of mice (e.g. longer cell cycle times of neural progenitors and greater complexity of progenitor types in humans) [12].

The discovery of human pluripotent stem cells (hPSCs), which have the ability to differentiate into any cell type in the body, has provided exceptional prospects for investigating the intricacies of human brain development and understanding the underlying mechanisms of human disorders. The first extracted type of hPSC, embryonic stem cells (ESCs), discovered in 1998 [13], raise ethical concerns because their derivation requires the destruction of a human embryo. However, the 2007 discovery of iPSCs opened new doors into disease modeling and regenerative medicine [14]. iPSCs are generated from adult cells through reprogramming into an undifferentiated embryonic state.

Presently, there are *in vitro* protocols that allow for the differentiation of hPSCs into diverse human neural cell types, utilizing a 2D monolayer culture system, which offers several benefits, including uniform accessibility to growth/differentiation factors, feasibility, and scalability [39]. However, the 2D culture also presents various limitations, due to its lack of proper representation of very complex growth processes (cell-to-cell or cell-to-extracellular matrix interactions, spatial gradient of growth factors, cell polarity). Therefore, the necessity for a model that better represents the human brain development became even more dire.

The first neural organoid was created by Lancaster and Knoblich (2013) [15]. Their findings demonstrated the successful generation of 3D brain-like structures in the laboratory using iPSCs. These brain organoids exhibited the ability to self-organize and differentiate into various cell types found in the human brain, mimicking the early stages of brain development. The study provided valuable insights into the complex processes of human brain development and offered a novel experimental model for studying neurological disorders and diseases in a more accurate and ethical manner.

2. *Neural Organoid Protocols*

Generally, intrinsic organoid protocols artificially mimic the intrinsic patterning that guides cells into differentiation during embryonic development [16]. In intrinsic organoid protocols, specific conditions and factors are provided to the organoids that enable them to generate diverse brain regions. Exact protocols to achieve this self-organization may vary among research groups, and ongoing advancements in the field continually refine and improve the methods used to generate brain organoids. However, the creation of an organoid can be very broadly condensed into the following four steps:

1) Obtain hPSCs: hPSCs (iPSC or ESC) have the potential to differentiate into various cell types.

2) *Formation of embryoid bodies:* The pluripotent stem cells are aggregated in suspension and cultured to form 3D structures called embryoid bodies to initiate the differentiation process.

3) *Neural induction:* The embryoid bodies are exposed to specific signaling molecules or growth factors that promote the differentiation of stem cells into neural progenitor cells (NPCs), precursors of neurons and glial cells.

4) *Culture and maturation:* The NPCs are then cultured in conditions that support their growth and maturation, allowing them to organize and develop into more complex structures resembling brain tissue.

Oftentimes, scientists apply directed differentiation methods to guide the formation of specific brain regions within the organoids. By exposing the NPCs to precise combinations of signaling molecules (Steps 3 and 4 above), researchers can coax them to develop into ultra-specific brain cell types found in particular regions of the brain. Key signals used in neural induction include BMP inhibition, Wnt inhibition, Activin/Nodal and FGFs, SHH inhibition, Dual-SMAD inhibition, Notch signaling, and small molecule compounds. These signals are discussed further in Section II.

Besides the signaling molecules of neural induction, Matrigel or other extracellular matrix-like scaffolds can be used to support the self-organization and structural development of neural organoids. Co-culture with specific cell types is also seen: by introducing other cell types found in the brain, such as glial cells or neurons with specific genetic mutations associated with neurodevelopmental disorders like ASD, scientists can enhance the complexity and relevance of brain organoid models [17]. Finally, in some protocols, organoids are transferred to an air-liquid interface culture, where the upper portion is exposed to air, encouraging the development of neuronal connections and cellular maturation [17].

3. *General Application to Neurological Diseases*

Neural organoids have so far been used to study diseases such as microcephaly, ASD, Alzheimer's, and cancer. By generating organoids from individuals with neurobehavioral disorders or using genome editing techniques to introduce disease-related genetic mutations, researchers can observe and analyze the behavioral characteristics of the organoids. These characteristics include assessing neuronal activity, synaptic connections, and cellular interactions within the organoids. Furthermore, neural organoids can be combined with other techniques like single-cell RNA sequencing (scRNA-seq) or functional imaging to gain deeper insights into the molecular and cellular changes associated with neurobehavioral disorders. By comparing organoids derived from individuals with neurobehavioral disorders to those without, researchers can identify differences in gene expression patterns, neuronal connectivity, and functional activity that may contribute to the disorder.

II. Neural Organoids to Approach ASD

A. Modeling ASD

To develop brain organoids to model ASD, it is crucial to consider the choice of signaling factors and their timing of activation during the generation process [18]. Certain signaling pathways influence the formation of different brain regions and cell types (Table 1).

Signaling Pathway	Influence in Organoid
Wnt pathway	Neural induction and early patterning of the anterior-posterior patterning. Direct differentiation of iPSCs into NPCs and different brain regions, such as forebrain (activation), or midbrain and hindbrain (repression)
Bone Morphogenetic Protein (BMP) pathway	Dorsal-ventral patterning of the developing brain. Specify the locational identity of NPCs in an organoid, contributing to their specificity
Fibroblast Growth Factor (FGF) pathway	NPC proliferation and early brain regulation. Balance between neuronal subtypes (e.g. excitatory and inhibitory neurons). Anterior-posterior patterning during development
Sonic Hedgehog (SHH) and Notch pathways	Medial-lateral patterning and cell fate decisions during neurodevelopment. Differentiation of NPCs into cell types – neurons, glial cells, and differentiated neurons like motor neurons.
Retinoic acid signaling	Differentiation of NPCs into cortical neurons. Formation of cerebral cortex

Table 1. Signaling pathways in formation of neural organoid.

By manipulating these signals in a stepwise fashion, researchers can guide the development of brain organoids and generate specific brain regions or cell types that are relevant to ASD. Doing so has revealed various cellular phenotypes [19] associated with ASD.

B. Identification of Risk Genes

Many studies have employed regional brain organoids to identify risk genes and potential biomarkers for ASD. These studies have led to reports of the identified gene-related transcriptional pathways, neuronal networks, and other associated phenotypes [20]. Note that various types of brain organoids were used in the following discussed studies, each of which correspond to the modeling of different regions of the brain (forebrain, cerebral, telencephalic, cortical, etc.).

In a recent study, hundreds of brain organoids were analyzed, generated from iPSCs of individuals with ASD or CRISPR-edited cell lines, which presented haploinsufficiency of ASD risk genes including *SUV420H1* (also known as *KMT5B*), *ARID1B*, and *CHD8* [21]. *KMT5B* codes for a Histone-lysine N-methyltransferase involved in histone methylation and plays a role in DNA damage repair and gene silencing. *ARID1B* encodes a subunit of the BRG1/BRM-associated factor (BAF) chromatin complex and is essential for brain development and function. *CHD8* encodes the chromodomain-helicase-DNA-binding protein 8, which functions as a transcriptional regulator. *CHD8* is also involved in chromatin remodeling and is frequently associated with ASD due to mutations in this gene in patients with ASD.

CHD8 is one of the most strongly associated genes with ASD. A study focused on cerebral organoids generated from *CHD8* mutant and control hESCs to understand its impact on neurodevelopment related to ASD [22]. They observed changes in gene expression related to Wnt/β-catenin signaling and varying alterations in GABAergic neurons, indicating an imbalance between excitatory and inhibitory neurons. Another finding showed that *CHD8* haploinsufficiency in cerebral organoids disrupted the development of inhibitory and excitatory neurons, resembling the condition seen in patients with macrocephaly [23]. *CHD8* was also found to regulate the expression of ASD-related genes *TCF4* and *AUTS2* [22].

CNTNAP2 is another ASD-associated gene involved in neural processes. Studying forebrain organoids from ASD patients with a *CNTNAP2* mutation revealed increased expression in early-born excitatory neurons and larger organoid volume due to enhanced cell proliferation [24]. Mouse cortical organoids from *CNTNAP2* knockout mice also showed GABAergic neuron defects, possibly caused by downregulated expression of specific transcription factors in the ventricular zone.

Another major study used cerebral organoids from CRISPR-edited iPSCs (edited with ASD-associated 16p11.2 deletions and 15q11–13 duplications) [25]. RNA-seq was then performed on the samples and scRNA-seq data was integrated. The study found that *YPEL3*, *KCTD13*, and *INO80E* genes can be associated as driver genes linked with ASD. Other studies have also found that the neuronal hemizygous deletion of the ASD-related gene *SHANK3* exhibits intrinsic excitatory synaptic deficits [26]. *RAB39b* mutations are also known to result in over-proliferation and differentiation deficits of NPCs in cerebral organoid studies [27].

Organoids have been utilized to study mutations in the *MECP2* gene [28], which is essential for brain epigenetic regulation and gene expression. In addition to the loss of function mutations in *MECP2* that cause Rett Syndrome (RTT), a motor neurodegenerative disorder, duplication of *MECP2* causes a distinct disorder, indicating that the nervous system is very sensitive to *MECP2*, and any disruption in the function of the protein product, MeCP2, can lead to neurological and psychiatric problems. The many clinical features found in RTT and the various clinical problems that arise from disrupting MeCP2 function has led the concept that RTT is a “prototypical” neurological disorder to provide understanding and insight into a vast array of genetically defined and undefined clinical conditions such as idiopathic ASD. While RTT is primarily caused by *MECP2* loss-of-function mutations, the role of *MECP2* in ASD is more complex, with both loss-of-function and gain-of-function mutations reported [42]. The effects of *MECP2* dysfunction on brain development and neural circuits may contribute to the development of ASD.

Altogether, the studies reveal an imbalance between

excitatory and inhibitory pathways in ASD. Common pathways most often see molecular convergence with overexpression of transcription factor families and upregulated GABAergic neuron production.

C. Identification of Environmental/Epigenetic Effects

Considering the complexity of ASD, environmental factors are also known to play a role in its occurrence.

In one study [29], human forebrain organoids (hFOs) were used to investigate the relationship between valproic acid (VPA) [30], an anti-epileptic medication, and ASD risk. iPSCs from healthy individuals were used to create hFOs, which were exposed to a clinically relevant concentration of VPA for three days. Through various analyses, such as proteomics, genomics, electrophysiology, and scRNA-seq, the study identified specific genes (*AMK4*, *CLCN4*, *DPP10*, *GABRB3*, *KCNB1*, *PRKCB*, *SCN1A*, and *SLC24A2*) affected by VPA exposure. These genes overlapped significantly with pathways known to be dysregulated in the organoids of individuals with ASD. Furthermore, VPA exposure disrupted synaptic transmission in the hFOs.

Another study applied brain organoids carrying a heterozygote CRISPR-edited mutation in *CHD8* [31]. The organoids were then exposed to chlorpyrifos (CPF), a neurotoxic organophosphate pesticide, or its metabolite, chlorpyrifos oxon (CPO). The compounds caused detrimental effects on neurite outgrowth, increased oxidative stress, and disrupted neurotransmission after 24 hours of exposure. Overall, the study highlighted the adverse impacts of toxic agents on autistic patients with this gene mutation.

III. Limitations and Concerns

A. Identification of Risk Genes

While more effective than 2D cultures, neural organoids lack the full complexity and cellular diversity found in the actual brain. The intricate interactions between different cell types and regions in the brain are not fully replicated in organoids, which may limit their ability to fully capture the complexity of ASD [32]. One of the primary challenges is to induce the differentiation of specific non-neural cell types within the organoid without introducing additional heterogeneity.

To address this issue, scientists have been exploring various protocols and techniques to coax the organoid's neural stem cells to differentiate into different non-neural cell types intrinsically [40]. By manipulating the signaling pathways and environmental conditions, they hope to induce the generation of diverse cell populations within the organoid. An alternative approach is to artificially combine separately cultured cell types. This method involves separately differentiating various cell types outside the organoid and then assembling them together to create a more heterogeneous organoid. However, while this might be more feasible in the short term, it may not fully recapitulate the natural interactions and complexities of the developing brain [40].

Organoids also do not fully recapitulate the developmental maturity of a real brain. They are more representative of the early stages of human brain development, which may limit their relevance when studying the pathogenesis of ASD that typically manifests later in childhood. The human brain also interacts with a complex and dynamic environment, including the bloodstream, immune system, and other physiological factors. Organoids lack these external factors, which may influence the development and progression of ASD. And while organoids can be genetically engineered to carry specific mutations associated with ASD, the genetic complexity of ASD is vast and involves a combination of multiple genetic and environmental factors. Reproducing this complexity of circuit integration and plasticity accurately in organoids is challenging.

Due to organoids' poor maturity, several studies have delved into synapse formation and calcium signaling in neural organoids. Researchers have explored the presence and dynamics of synapses within organoids, aiming to understand how they form, stabilize, and mature over time. Additionally, calcium ion (Ca++) levels are indicative of neuronal activity and can influence processes like synaptic plasticity. Organoids so far have provided a platform to observe the initial stages of synapse formation and Ca++ signaling, but their simplified structure and lack of long-range connectivity could limit their ability to fully capture the complexity of these processes as they occur in the developing human brain [41].

B. Moral and Ethical Concerns

Obtaining informed consent from human cell donors is crucial, particularly when studying cells from children with ASD or their family members. Neural organoids offer an alternative to animal models [33], but the ethical implications of this choice require careful consideration [34]. Researchers must guard against potential misuse or unintended consequences, such as inappropriate enhancements or invasive experiments. Ethical considerations in ASD research extend beyond the laboratory, with implications for society's perception of the condition, stigmatization, and applications of research findings.

Transparent and inclusive discussions involving researchers, ethicists, individuals with ASD, and their families are essential to establish responsible guidelines and ethical frameworks for using brain organoids in ASD research. Balancing the potential benefits of organoids with upholding ethical standards and protecting the well-being and dignity of those affected by ASD is paramount.

Despite any physical limitations and moral concerns, neural organoids remain a promising tool for ASD research, allowing scientists to explore certain aspects of early brain development, study the effects of specific genetic mutations, and screen potential therapeutic interventions. However, they should be used in conjunction with other complementary approaches to gain a more comprehensive understanding of ASD.

IV. Conclusion and Perspective

Through the study of neural organoids, significant strides have been made in identifying ASD risk genes and

understanding the impact of environmental factors on neural development. The findings have shed light on key signaling pathways involved in the balance between excitatory and inhibitory signaling, providing valuable insights into potential therapeutic targets for ASD treatment. Furthermore, the application of personalized drug testing using patient-specific iPSC-derived organoids shows promise in tailoring unique treatments to individuals with ASD.

Looking to the future, neural organoids can be combined with other models, such as animal studies and patient-derived cell lines, to bridge the gap between cellular-level findings and whole-organism behavior. Animal models, while not perfect representations of human neurodevelopment, still offer valuable insights into the in vivo effects of genetic and environmental manipulations. By conducting parallel studies using both organoids and animal models, researchers can compare and validate their findings, thereby enhancing the translatability of research outcomes to humans.

Furthermore, patient-derived cell lines, obtained from individuals with ASD, can be incorporated into organoid research to investigate combined effects of specific genetic mutations and environmental factors on neural development. This personalized approach allows for a better understanding of the unique genetic underpinnings of each individual's ASD and can help identify potential personalized treatment strategies.

Moreover, brain imaging techniques, such as functional magnetic resonance imaging (fMRI) and positron emission tomography (PET), can also be employed to study brain activity in individuals with ASD and correlate these findings with the cellular-level changes observed in organoids [35]. Integrating these non-invasive imaging modalities with organoid research can provide a more holistic understanding of how alterations at the cellular level manifest as behavioral and functional changes at the macroscopic level.

Efforts to enhance the complexity and maturity of brain organoids are ongoing, through two main methods: vasculature and immune components, which can better replicate the dynamic interactions between neural cells and the surrounding microenvironment. The vascularization of organoids would introduce blood flow, nutrient delivery, and waste removal, more accurately mimicking in vivo conditions–essential for maintaining cell viability and supporting complex cellular interactions. Immune components within organoids can enable the study of neuroinflammation [36], which is implicated in various neurological disorders, including ASD.

Neural organoids have revolutionized the study of ASD, providing a more relevant and sophisticated model for exploring its complex pathogenesis. As this technology continues to advance, its integration with other models, validation of drug targets, and personalized medicine applications hold the potential for groundbreaking discoveries and transformative treatments for individuals and families affected by ASD. By addressing ethical concerns through establishment of global stipulations and refining organoid models, neural organoids are poised to play a central role in shaping the future of ASD research and improving the lives of those living with this challenging neurodevelopmental disorder.

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VI. References

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