A Review of the Placenta and Trophoblast Induced Pluripotent Stem Cells in Autism Spectrum Disorder Research

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Abstract

Autism spectrum disorder (ASD) imparts a tremendous health burden with psychological, social, and economic implications. The biology of ASD is complex involving genetic, molecular, hormonal and immunologic factors however the convergence point of these various factors has not been identified as of yet. Limited evidence exists to suggest that the placenta may play such a governing role in ASD manifestation. The placenta is a neuroendocrine modulator by participating in the fetal hypothalamic pituitary gonadal (HPG) axis and also regulates the intrauterine environment mitigating fetal exposure to damaging factors to modulate the fetal stress response. Placental dysfunction has been associated with developmental abnormality and neuropsychiatric pathology adding to the biologic plausibility of the governing role the placenta may play in ASD development. By using current technology like induced pluripotent stem cells (iPSCs), a practical model system can be created to study ASD providing an alternative method to further research the placenta in ASD development

Keywords: Placenta; Trophoblast; Autism spectrum disorder; Induced pluripotent stem cell; Neuroendocrine regulator; Maternal immune activation

Introduction

Autism spectrum disorder (ASD) poses a great health burden and a tremendous effort towards researching the etiology has been performed. Though a great body of research has identified candidate genes, gene networks, and possible molecular pathways, the underlying regulation of these steps is currently unknown. Broadening ASD research to include environmental exposures and perinatal factors has not identified a convergence point of these various players. Neurobiological findings suggest that ASD pathophysiology may originate during fetal development [1-9]. Given this data, a plausible convergence point appears to be the placenta, which is known to regulate proper fetal development in utero. The placenta could therefore be thought of as the final common pathway in the development of ASD merging pertinent genetic, structural and environmental factors that impact fetal brain development. Targeting the placenta and trophoblast in understanding the complex biology of ASD may therefore be a more effective strategy to guide basic, translational and clinical science research.

One challenge that exists in furthering ASD research is the lack of a standard model system to study the etiology of the disorder. Most research about autism is based on retrospective studies, biological samples taken from autistic adults, or post-mortem brain autopsies. While useful in identifying biomarkers in autistic individuals, there is difficulty in proving causation rather than just correlation. These methods have proven to have limitations decreasing their efficacy in developing a comprehensive ASD model. Induced pluripotent stem cell (iPSC) technology can advance current ASD research by providing a means of circumventing the limitations associated with traditional research paradigms. Recreation of placental tissues from individuals diagnosed with ASD can be achieved by converting cryopreserved peripheral blood cells into iPSCs [10] and then inducing differentiation toward hormone secreting trophoblast cell types [11]. While conditions to induce iPSCs toward trophoblast development remains to be refined, some of the different trophoblast and neural cell features of autism patients can be reflected in such experimental systems [12]. This technology provides a solution to tissue availability and prolonged placental tissue cryopreservation. Use of iPSCs may identify the cellular and genetic pathways related to ASD and can elucidate the epigenetic and synergistic effects of the environment and stress on such pathways within the placenta. A tissue culture model is capable of measuring cellular response to different stimuli including pro-inflammatory cytokines, infectious agents, pesticides and oxidative stresses, all of which can be administered in the trophoblast culture system. The iPSC-trophoblast model is therefore ideal for investigation by creating placental precursor stem cells to examine the trophoblast and placental role in integrating genetic, hormonal, environmental and perinatal contributors to autism.

This article summaries the limited existing data supporting the placental convergence hypothesis. We review the biology and pathophysiology of the placenta with respect to neuropsychiatric outcomes highlighting trophoblast development and function with respect to neuromodulation and brain morphology, demonstrating the placental role in neurodevelopmental environment regulation and explaining differential placental gene expression involved with neuromodulation. We further review existing data discussing the resultant pathology that occurs from placental dysregulation in other organ systems to argue the idea of biologic plausibility of placental

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function in ASD formation. We also briefly review the clinically relevant data regarding the placental role in fetal stress response programming and maternal immune activation. Finally we review the study of iPSCs in ASD research and suggest that iPSCs can also help establish causational data to support the role of the placenta in ASD emergence. If iPSCs are also used to help examine differential gene expression the model system could contribute to efforts for potential reversal of the ASD phenotype.

The placenta governs neural development through hormone secretion

Trophoblasts are the precursors of the placenta and play a regulatory role in fetal neural brain development through a highly coordinated sequence of developmental events. Just 8 days after implantation, the trophoblast cells form the syncytiotrophoblast, the outer multinucleated layer, and the cytotrophoblast, the inner mononuclear layer [13]. The syncytiotrophoblast secretes the hormone human chorionic gonadotropin (HCG) which among its various functions is thought to play a role in neurodevelopment. HCG belongs to the same growth factor family as nerve growth factor and animal studies have demonstrated increased neurite processes and decreased apoptosis in *in vitro* studies of fetal rat brains cultured with HCG. This hormone may also function in behavior regulation as areas within the brain related to behavior including the hippocampus and hypothalamus express high levels of HCG receptors [14]. The syncytiotrophoblast creates an interconnecting network of vascular channels 12 days after implantation so that by day 17, placental circulation is established [15]. Shortly after the establishment of this blueprint of fetal circulation, fetal neurodevelopment begins to take center stage. At 3 weeks of gestation, neural tube formation begins as regulated by modulatory effects from the notochord. *In vitro* studies of early human embryogenesis demonstrates that HCG secreted by the trophoblast influences embryoblast development and growth as well as induces progesterone synthesis, an obligatory role in neural precursor cell and neural tube formation through autocrine and paracrine methods [16]. Furthermore, *in vitro* studies of messenger RNA (mRNA) expression demonstrate that human placental cells produce follistatin, which along with inhibin, exerts an inhibitory effect on placental HCG secretion [17]. *In vitro* and animal studies provide evidence that neural development is influenced by trophoblast cells which are able to create vascular networks. This vasculature composes the proper regulatory hormonal milieu influencing nervous system and fetal brain development which if dysregulated may result in clinical neuropathology [18]. Use of iPSCs may provide a promising tool to advance research in this area. Emphasis on creating induced trophoblast cells as opposed to neural stem cells may offer a unique insight into the neuromodulatory factors occurring in utero at early gestational ages. Trophoblast differentiated stem cells could be used to measure differences in secretion of HCG, follistatin and progesterone between autistic and non-autistic individuals.

The placenta influences neuronal function and brain morphology

Differences in brain functioning including multiregional dysregulation of neurogenesis, migration, maturation, neuronal hyper-reactivity and reduced neural synchronization have been identified in individuals with ASD [12,19]. Evidence suggests that the placenta might be involved in some of these functions through epigenetic mechanisms [20-22]. The establishment of proper neuronal functioning as mediated through migration, organization and myelination begins in fetal life around 12-20 weeks gestation with some processes continuing into

postnatal life [23]. Migrating neurons will await connections from afferent neurons in the thalamus, basal forebrain and brainstem in order to undertake the necessary developmental steps [2] and as the fetal brain undergoes these dynamic processes, the placenta provides both transport of necessary metabolic nutrients and neuroendocrine functions necessary to support fetal brain development [20]. Evidence also exists suggesting the placenta may influence neuroanatomy. Neuronal proliferation begins around 12-16 weeks of gestation [23] and may be mediated early on by placental secreted factors including HCG and follistatin [14]. It is likely that improper secretion of these hormones by the placental trophoblast may result in too many or too few neural connections. Macrocephaly is a noted clinical feature of children with autism and postmortem studies of ASD individuals have revealed an excess amount of neurons in the prefrontal cortex. MRI studies in individuals at risk for psychiatric and cognitive disorders have demonstrated volume reduction in areas of the brain associated with these nervous system processes [3]. Animal models have demonstrated that the onset and duration of placental insult produces varied neuropathological outcomes and similar morphologic and functional changes seen in human neuropathology [2]. As the nervous system undergoes dynamic change throughout gestation and into postnatal life with the rate of neurogenesis equaling approximately 40,000 synapses per second, this process is likely subjected to modulatory factors both essential and detrimental. A trophoblast-differentiated model would allow for a systematic examination of the specific metabolic nutrients and neuroendocrine factors necessary for neural functioning and fetal brain anatomy. Use of iPSCs may aid to clarify the exact pathways responsible for these associations advancing the knowledge provided by current animal and *in vitro* models.

The placenta actively regulates environmental determinants of nervous system physiology

The placenta functions as a transport organ to provide necessary nutrients to the developing fetus. During prenatal development, the placenta serves to maintain homeostasis and control environmental exposures [24,25]. Environmental triggers found to increase the risks of neurodevelopmental disorders during pregnancy include nutrition, stress, infection, and inflammation. The possible insults that can affect the developing fetus are diverse; however the placenta determines whether these insults can be transmitted to the developing fetus [26,27]. The placenta plays a role maintaining the delicate balance of nutrients such as metals which are known to influence fetal brain development. Metallothionein-1 is found within the syncytiotrophoblast and is known to bind heavy metals [28]. Furthermore, human studies have shown differing concentrations of metals by gestational age, suggesting that the placenta may actively regulate the concentrations of certain metals for various neurodevelopmental processes and pathways [28]. Disruption of the delicate balance of metal concentration may lead to neurobehavioral pathology. Lower whole body zinc levels and zinc to copper ratios have been seen in children with ASD [29]. Preconception zinc deficiency has caused aberrant neural tube development in mice [30] and it appears that supplementation of metals in individuals with micronutrient deficiency may modulate placental vascular function in humans [31]. Furthermore, both zinc and copper are regarded as neurotransmitters with high concentrations in the hippocampus. Disruptions in the balance of these metals have been associated in behavior disorders [32]. The enzymes involved in metal processing have been associated with certain types of neuropathology and the placenta is thought to be a key regulator in these metabolic pathways. Copper is oxidized by the protein ceruloplasmin (CP), an iron transporter, and is studied in the etiology of autism, schizophrenia,

and obsessive-compulsive disorder. CP is an acute phase reactant that is regulated by cytokines in response to hypoxia resulting from infection or inflammation. The placenta also has receptors for CP to deliver copper to the fetus [33]. High levels of CP protein are present in placental tissue, specifically in cases of preeclampsia [34]. When comparing autistic individuals to their non-autistic siblings, lower CP levels were detected in autistic children who lost "previously acquired language skills" as compared to those individuals who did not [35]. This suggests that low CP levels might contribute to the clinical presentation of neurodegeneration seen in autistic individuals. The underlying mechanism of this observation is thought to be due to elevated iron concentration that occurs with decreased CP leading to cerebral damage [35]. The data involving metals and brain development both basic science and clinical observational studies seems to support the connection between placental function and neuropathology.

Differential gene expression within the placenta influences neuroendocrine regulation of brain development

Differential gene expression, especially seen from gender differences, has been theorized to explain features of ASD [36-38]. Interestingly, gender differences in gene expression within the human placenta have been identified [39-41] and are associated with clinical outcomes such as differential fetal growth [42] and stress response [43]. Given the genetic heterogeneity of ASD and the numerous implicated genes [44-47] the placenta appears to be a likely a convergence point of differential gene expression. Many of the implicated genes are known to affect components of the hypothalamic pituitary gonadal (HPG) axis, a known neuroendocrine regulator of brain development, of which the placenta is a major component. Alteration in the HPG axis has demonstrated varied neurologic effects primarily through follicle stimulating hormone (FSH). A promising cluster of candidate genes labeled as the Root 66 genes has revealed a non-random association with ASD and the HPG axis. When we cross-referenced these 66 genes, we determined that 50 of them have gene or protein expression within placental trophoblasts or decidua [48]. Furthermore, research involving the use of iPSCs to re-create an "autistic cell" has suggested that ASD likely arises from a common developmental origin [36]. Given that the HPG axis is highly conserved among species, it is very plausible that the conserved pathways of neuronal and endocrine development are regulated by the placenta, which has demonstrated to affect both these aspects.

Placental dysfunction leads to developmental abnormality

The human placenta serves as a regulator of fetal programing in other organ systems, which when altered, results in pathology [49]. Various human and animal studies suggest that the development of many chronic diseases including heart disease, obesity, hypertension and type 2 diabetes originate in prenatal life [50-59]. The cardiovascular health after maternal placental syndromes (CHAMPS) study demonstrated a doubling in the risk of premature cardiovascular disease in women without a previous history of cardiac disease who had a maternal placental syndrome during pregnancy [51]. Furthermore, expression of placental genes has been shown to impact fetal cardiac function and anatomy. One such gene, HOXA13, which is expressed in placental and not cardiac tissue, is involved with cardiovascular development. Knockout HOXA13 mice results in abnormal placental endothelium and is associated with a reduction in ventricular wall thickness leading to embryonic death [50]. There is also evidence to support the idea that vulnerability to disease is "programmed" in fetal life with a growing body of evidence linking intrauterine environment,

neurodevelopment and subsequent neuropsychological outcomes [60]. An iPSC trophoblast model would provide vital real-time data regarding the neuromodulatory role that the placenta plays in both ASD affected and unaffected individuals.

The placenta impacts neuronal cellular structure and function [3]; additionally, clinically relevant physiologic associations between the placenta and fetal brain development such as brain sparing have also been seen. This phenomenon occurs in human developmental pathophysiology when the fetus alters hemocirculation to preserve oxygen and nutrient supply to the brain in the setting of placental insufficiency. Brain sparing occurs regionally and in a hierarchical order with an initial attempt to preserve higher cognitive function areas first, though prolonged placental insult will result in shifting towards preserving survival areas of the brain. This physiologic phenomenon is thought to be a protective mechanism; however, prolonged brain sparing may lead to negative consequences persisting postnatally [61]. Sheep models of chronic placental insufficiency in the second half of gestation demonstrate features of brain sparing and Guinea pig models of chronic placental insufficiency have resulted in schizophrenia-like features comparable to those found in humans [2]. Whether brain sparing from prolonged placental insult is causational in resultant neurocognitive and neuropsychiatric pathology remains unknown, placental insult appears connected to proper neurodevelopment and brain function. Genetic predisposition may influence the likelihood of placental dysfunction resulting in clinical pathology however, further studies are needed. The current limited data seems to support a tight connection between the brain and the placenta during prenatal development [62].

Fetal stress response is prenatally programed by the placenta and may result in neuropsychiatric pathology when disrupted

Mammalian systemic stress responses are controlled by the hypothalamic pituitary adrenocortex (HPA) axis, which exerts its regulatory effects through the concentrations of corticotropin-releasing hormone (CRH) from the hypothalamus, adrenocorticotropic hormone (ACTH) from the pituitary, and cortisol from the adrenal cortex. During pregnancy, the placenta plays a pivotal role in balancing the maternal HPA activities thereby modulating the fetal stress response. The placenta secretes CRH and expresses 11β-Hydroxysteroid dehydrogenase (11β-HSD), which regulates how maternal glucocorticoids enter the fetal bloodstream and cortisol's effects [63,64]. In addition, the placenta has been shown to produce and secrete serotonin which accumulates in the fetal forebrain at mid-gestation [65] connecting the stress response pathways to direct neurologic effects within the developing fetus. These placental mechanisms not only control fetal exposure to maternal stress, but also provide feedback regulation for the maternal HPA axis to attenuate stress level as pregnancy proceeds [66-68]. Genetic variations leading to differential expression of glucocorticoid and CRH receptors associated with depression and stress disorders may impact the maternal HPA axis and fetal stress response [69-71]. Furthermore, sexual dimorphism in the execution of the HPA axis has already been observed in neurodevelopmental abnormalities. Studies in patients with congenital adrenal hyperplasia, a condition of abnormal cortisol metabolism, have shown increased autistic like behaviors in affected females but not in males when compared to controls [37,72]. Hormone activity in the HPA axis can be detected as early as 8-12 weeks of gestation and is regulated by the placenta through feedback loops involving cortisol, a factor for proper maturation of organ systems including the central nervous system [73].

Placental modulation of the stress pathway can also be influenced

by many other maternal factors, one being nutritional status. Murine studies demonstrate the importance of the placenta in protecting the fetal brain [74]. In a dietary deprivation model, changes in gene expression favoring the catabolic pathways were only observed in the placenta without altering the fetal hypothalamus. On the contrary, elevated expression of transporter proteins was observed in the fetal hypothalamus but not in the placenta [75]. This may explain the dire outcomes of human development, including metabolic disease and psychiatric disorders during famine [76]. Maternal diet has been shown to influence placental glucocorticoid metabolism which can predispose offspring to hypertension [77,78], and is also known to reduce placental 11β-HSD expression [79]. Changes in maternal cortisol level or in the placental HPA pathway have not only been linked to adverse birth outcomes, but also in neurodevelopmental outcomes including cognitive and behavioral changes, emotional reactivity and motor dysfunction [80-84].

Maternal Immune activation: linking maternal stress to the development of ASD

In addition to modulating stress response and allocating needed resources to the developing fetus, the placenta also protects the fetus from harmful exposures, especially inflammation. The maternal immune activation (MIA) model is useful in ASD research. The effects of MIA can potentially be mediated by inflammatory cytokines interleukins 1 and 6 (IL-1 and IL-6); maternal IL-6 is affiliated with limiting the signaling of cytokines through the placenta via gene expression (SOCS3). If an infection occurs during a certain time frame, the intensity of MIA can lead to fetal brain damage. In response to an infection, the mother's immune system normally will send out leukemia inhibitory factor (LIF) through the placenta and activate the LIF signaling pathway within the fetus. This "maternal-fetal signal relay" stimulates fetal neurogenesis of the cerebrum, as demonstrated in rat models [85]. A severe MIA reaction causes an increase in leukocytes and IL-6 production in rat models [86] and a decrease in fetal LIF, leading to stunted neurodevelopment. The cerebrum is subsequently damaged due to the lack of LIF in the fetal system and the high levels of IL-6 result in elevated SOCS3 expression which inhibits any further neurogenesis [85]. Placental trophoblast IL-6Rα knockouts had decreased placental and fetal brain inflammation resulting in less irregular behaviors in mice offspring [87]. Similarly, placental and neurodevelopmental damage induced by lipopolysaccharide (LPS) mediated inflammation was shown to be alleviated by an IL-1 receptor antagonist [88].

Given that fetal susceptibility to MIA is observed during mid-gestation, it would be most informative to analyze biochemical changes in trophoblast-differentiated stem cells following activation of the immune system. The MIA model remains to be the most reproducible with phenotypes closely mimicking human ASD in mouse models of autism-like behavior disorders. Monogenic models often have incomplete penetrance making it difficult to link placental dysfunction with the autism phenotype. Changes in gene expression of the fetal brain have been observed in MIA models [89,90] and these models should be used to analyze immediate placental gene expression responses to MIA. Long-term effects of MIA can subsequently be identified through genome wide analysis of epigenetic changes by identifying changes in DNA methylation patterns.

## Molecular and genetic differences should be examined in the placenta

Gene expression analyses of other tissues reveal key changes in pregnancies complicated by ASD including natural killer cells, tissues of the prefrontal cortex, lymphocytes within peripheral blood and other tissues [91-94]. Examination of differences in baseline gene expression or after a stress challenge may identify or better clarify diagnostic and therapeutic targets for clinical use. Prolonged preservation of viable trophoblast tissue is labor intensive and costly. Stem cells from individuals with autism offer an alternative source of cells. A recent study used neuron-differentiated iPSCs from ASD affected persons with deletion or duplication of the 16p11.2 region to analyze the biological mechanisms underlying the macrocephaly and microcephaly phenotypes. This region is associated with a copy-number variant mutation linked to certain neurological disorders. This use of iPSC technology was able to demonstrate that reduced synaptic density, which likely would result in larger scale neuroanatomical changes within the brain as a whole, were a result of mutations in the 16p11.2 region [95]. Similarly, iPSC use may also elucidate the relationship of pathologic manifestations associated with ASD, such as trophoblast inclusions [96,97]. Stem cells can be induced into trophoblast cells, examined during various stages of development when exposed to differing environmental exposures or genetic/epigenetic changes and examined for the presence or absence of trophoblast inclusions. The iPSC model appears to allow for more efficient and effective methods to examine the ASD phenotype from genetic, epigenetic and molecular differences in the placenta and trophoblast which since now has been limited.

Induced pluripotent stem cells as a model system

Induced pluripotent stem cells are becoming more utilized in research for neurological disorders and are gaining more attention in ASD research and treatment. Studying neuron-differentiated iPSCs from patients has contributed to our limited knowledge of ASD pathogenesis and has facilitated drug screening platforms for therapeutics [98]. Previous to iPSC technology, there was a dearth of sufficient human samples of neurons to study these neurological disorders. With iPSCs, researchers can now analyze the mechanisms involved in neuronal cellular development from initial stage to an adult stem cell that may result in neuropsychiatric disorder manifestation [99]. A neuron-differentiated iPSC model system has revealed insights into the role dysfunctional glial cells play in ASD pathophysiology in addition to providing a means to test potential therapeutics [100]. Currently the data examining iPSC technology in the placental trophoblast is limited and even more limited when examining this technology in ASD patients [11-12]. As the placenta plays a major role in fetal development, especially neural development, iPSC technology should not only be examined in neuronal cells but also in placental cells. By discussing the limited data supporting the role of the placenta in ASD and the even more limited research done about iPSC modeling for the connection between the placenta and ASD manifestation, we hope to spark more conversation and action into using this model system.

Of course, despite the usefulness of iPSCs, there are limitations. With iPSCs, there have been noted differences in the transcriptome, proteins, and the epigenome compared to embryonic stem cells. These differences could be due to the reprogramming process iPSCs undergo, which could also affect how efficiently the cells differentiate [12,101-103]. Despite these identified limitations, iPSC technology does appear to be an effective tool to advance our comprehension of ASD. As with any model, understanding the limitations posed by the iPSC system will be vital to draw meaningful conclusions from future research endeavors (Figures 1 and 2, Table 1).
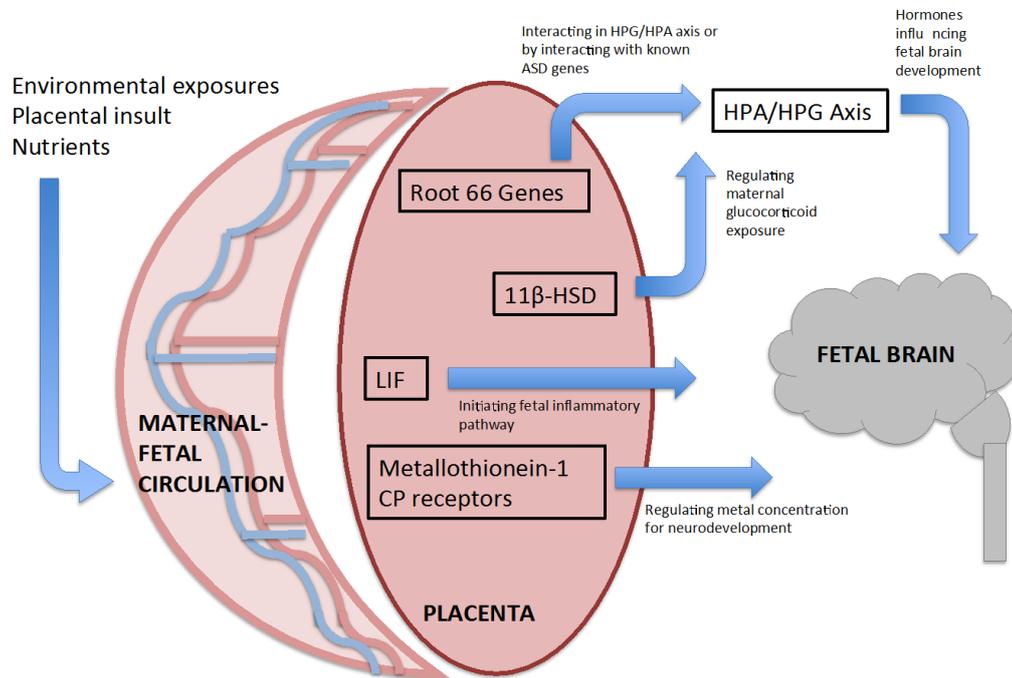
Figure 1: The placenta appears to function in fetal brain development through various proposed pathways including its participation in the HPA/HPG axis and as a regulator of insults and essential nutrients to provide a suitable environment for proper fetal brain development. Pathology including neuropsychiatric outcomes like ASD may result when placental dysfunction occurs.
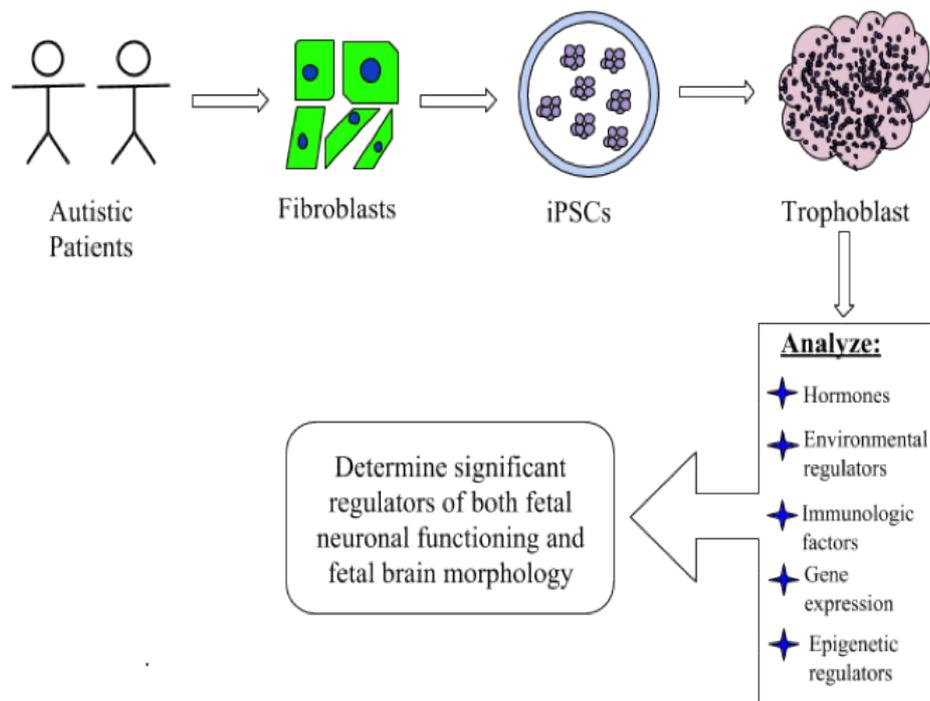
Figure 2: Utilizing iPSC technology, a model system can be constructed to understand trophoblast function in fetal brain development. Fibroblasts from autistic patients are induced into trophoblast cells. These induced cells can be used to measure hormone secretion, gene expression and epigenetics, immunologic factors and environmental exposures. These factors can be compared to non-autistic trophoblast controls to identify significant influencers in ASD pathophysiology.

50 of the Root 66 Genes Detected in Placenta					
Class	**Gene**	**Detection**	**Class**	**Gene**	**Detection**
Known to be Associated with Autism	TCF4	Low	Interact with ASD candidate genes	SIRPA	Medium
	UPF2	Low		SVIL	Medium
Interact with ASD candidate gene	ACSL4	Medium		TDP2	Low
	ASAH1	Low		TIMP2	Medium
	BAX	High		TXN	Low
	BCL6	Low		UBE2D3	Medium
	CAPZ A2	Medium		ZNF644	Low
	CNOT4	High	Related to genes associated with neurological conditions	AGTPBP1	Medium
	COL4ABP	Medium		ARID4B	Low
	CSF2RA	Low		CPD	Medium
	CUL4A	Low		DSE	Medium
	GMPR2	Medium		KIF1B	High
	HNRNPC	High		LAMTOR3	Medium
	HSPD1	Medium		OCIAD1	Medium
	ITGB1	High		PHF20L1	Low
	LAMP2	High		RAB24	Low
	LYST	Low		RAB2A	High
	NFE2L2	High		RBM25	High
	PCNP	High		SLC44A2	Medium
	PFDN5	High		SORL 1	High
	PPM1B	Medium		SUPT4H1	Low
	RBM39	Low		TNRC6A	High
	RBMX	High		VMP1	Medium
	RHEB	Low		WIPF1	Low
	SEPT2	Medium		WLS	Low

Table 1: Classification of the 50 Root 66 genes according to gene function as outlined by Diaz-Beltran et al. (2016). Placental presence and detection level obtained by cross-referencing the Human Protein Atlas and Gene Cards websites.

Conclusion

Efforts have been made to diagnose ASD as early as possible [104], specifically because the burden of disease is extraordinary. In a cost-of-illness analysis, ASD was projected to account for up to 3.6% of GDP in 2025, exceeding the burden of stroke and hypertension [105,106]. Many research modalities have been explored to address this clinical challenge without much avail. Placental contributions to neurobehavioral developmental disorders have largely been neglected until recently. The placenta is a neuromodulator influencing brain morphology, regulating the environment for proper brain development and function, impacts fetal stress response and maternal immune activation. It is part of the HPG and HPA axes by way of its hormonal secretions and its dysfunction has been associated with clinically relevant neuropathological outcomes. Using iPSC technology can advance the placental origin of autism theory and provide new diagnostic and therapeutic markers for treatment. Re-conceptualizing ASD research involves the understanding that placental abnormality is a feature of ASD and that using of iPSC technology can examine the exact genetic, biochemical, and environmental factors that cause ASD development.

Acknowledgement

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