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Serotonina - um complexo, enigmático biomarcador na Perturbação do Espetro do Autismo

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Aos meus pais, por tudo.
Aos meus amigos Leonor, Eduardo e Hugo, pelo apoio.

Serotonin - a complex, puzzling biomarker in Autism Spectrum Disorder

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Abstract

Background: Hyperserotonemia has been reported in Autism Spectrum Disorder (ASD) patients and their relatives. Due to serotonin's heuristic potential, this narrative review aims to discuss the biological plausibility of the serotonergic dysfunction hypothesis in ASD, as well as relevance and clinical implications of using serotonin as a biomarker.

Methods: Human and animal studies addressing serotonergic dysfunction in ASD were surveyed, as well as articles reporting blood serotonin measurements in ASD patients and first-degree relatives. No time limit was applied, exclusively articles written in English were included.

Results: Several lines of evidence confirm the involvement of the serotonergic system in ASD. Altered maternal and placental serotonin levels may influence the embryo, thus supporting the developmental hyperserotonemia model of autism. Hyperserotonemia is recorded in more than 25% of the ASD population and is thought to be a familiar trait, possibly related to platelet's handling of serotonin with several serotonergic genes conceivably implicated. Existing data highlights the reliability of hyperserotonemia as a biomarker and denotes serotonin as a potential nexus between the microbiome-gut-brain axis and ASD.

Conclusions: The serotonin system plays a preponderant role in neurodevelopment, thus dysfunction of this neuromodulatory network might be implicated in ASD. Serotonin measurement would provide insights about pathophysiologic mechanisms and risk factors, identify homogenous subsets of patients with a delineated developmental trajectory, facilitate a more accurate diagnosis, while predicting treatment response, adverse reactions and help developing novel therapies. Further investigation is advised, as it will promote a faster translation of these basic science findings to clinical practice.

Keywords

Autism Spectrum Disorder (ASD), Autism, Serotonin (5-HT), Hyperserotonemia, Biomarker

List of Abbreviations

5,7-DHT – 5,7-dihydroxytryptamine; **5-HIAA** – 5-hydroxyindoleacetic acid; **5-HTP** – 5-hydroxytryptophan; **5-MT** – 5-methoxytryptamine; **5-HT$_2$r** – 5-HT$_2$ Receptor; **5-HT$_{2A}$r** – 5-HT$_{2A}$ Receptor; **5-HT** – Serotonin / 5-hydroxytrypamine; **5-HTTLPR** – Serotonin Transporter-Linked Polymorphic Region; **Anti-MBP** – Anti-Myelin-Basic Protein; **AUC** – Area Under the Curve; **AADC** – Aromatic L-amino-acid decarboxylase; **ADHD** – Attention Deficit Hyperactivity Disorder; **ASD** – Autism Spectrum Disorder; **BBB** – Brain Blood Barrier; **CGRP** – Calcitonin Gene-Related Peptide; **CNS** – Central Nervous System; **CSF** – Cerebrospinal Fluid; **DNVs** – De novo Variants; **DSM-5** – Diagnostic and Statistical Manual of Mental Disorders Fifth Edition; **ENS** – Enteric Nervous System; **EC** – Enterochromaffin Cells; **GI** – Gastrointestinal; **HPLC** – High-Performance Liquid Chromatography; **HLA** – Human Leucocyte Antigen; **IL-2** – Interleukin-2; **IL-6** – Interleukin-6; **LSD** – Lysergic Acid Diethylamide; **MRI** – Magnetic resonance imaging; **MIA** – Maternal Immune Activation; **MT** – Melatonin; **MAO** – Monoamine Oxidase; **MAO-A** – Monoamine Oxidase A; **MAO-B** – Monoamine Oxidase B; **NAS** – N-acetylserotonin; **NK** – Natural Killer; **NAc** – Nucleus Accumbens; **OCD** – Obsessive-Compulsive Disorder; **OT** – Oxytocin; **p38-MAPK** – p38 mitogen-activated protein kinase; **PVN** – Paraventricular Nucleus; **PRP** – Platelet-Rich Plasma; **PPP** – Platelet-Poor Plasma; **PE** – Preeclampsia; **PKC** – Protein Kinase C; **PKG** – Protein Kinase G; **ROC** – Receiver Operating Characteristic; **RAVs** – Recessively Acting Variants; **SSRIs** – Selective Serotonin Reuptake Inhibitors; **SERT** – Serotonin Transporter; **SCFA** – Short-Chain Fatty Acids; **TDT** – Transmission/Disequilibrium Test; **TRP** – Tryptophan; **TPH** – Tryptophan-hydroxylase; **VPA** – Valproic Acid; **WB5-HT** – Whole-Blood Serotonin

Contents

1. Introduction

The fifth edition of the Diagnostic and Statistical Manual of Mental Disorders (DSM-5) adopts the umbrella term “Autism Spectrum Disorder” (ASD) to describe a group of neurodevelopmental conditions characterized by persistent impairment in social interaction and communication, as well as stereotyped behaviors and restricted patterns of interests (American Psychiatric Association, 2013; Lai, Lombardo, & Baron-cohen, 2013). ASD prevalence is expected to be at least 1.5% in developed countries with an apparent increase among those without intellectual disability and a sex ratio tilted towards male predominance, even though the disparity is attenuated as symptom severity increases (Lyall et al., 2017; Werling & Geschwind, 2013). Presentation usually occurs during the first years of life and often remains pervasive into adulthood, causing a significant impact on psychosocial functioning (American Psychiatric Association, 2013). Currently, ASD diagnosis is solely based on clinician observation, aided by standardized diagnostic tools and caregivers reports (Lord, Elsabbagh, Baird, & Veenstra-Vanderweele, 2018).

Heterogeneity is a hallmark in ASD, denouncing its underlying etiopathogenesis complexity (Masi, DeMayo, Glozier, & Guastella, 2017). Genetics is thought to play a critical role with a heritage percentage estimated in 83% (Sandin et al., 2017). Seemingly, ASD has also been linked to some specific genetic syndromes (Richards, Jones, Groves, Moss, & Oliver, 2015). Furthermore, innumerous environmental factors have been implicated, particularly those interfering with the prenatal and perinatal period (Lyall et al., 2017). This intricate interaction between genetic and environmental factors translates into a variety of phenotypes, different levels of severity and concomitant presence of physical and mental health conditions with an impact on prognosis, hence the term spectrum (Masi, DeMayo, Glozier, & Guastella, 2017; American Psychiatric Association, 2013). In fact, more than 70% of those affected have comorbidities reported, namely gastrointestinal (GI) conditions, sleep and sensory disorders, epilepsy, as well as other concomitant psychiatric disorders such as Depression, Anxiety, Obsessive-Compulsive Disorder (OCD) and Attention Deficit Hyperactivity Disorder (ADHD) (Lai, Lombardo, Auyeung, Chakrabarti, & Baron-Cohen, 2015; Interagency Autism Coordinating Committee, 2017).

The diagnostic challenge, multifactorial etiology and clinical heterogeneity prompted interest into the pursue for possible biological markers in ASD. Biomarker research aims to provide insights about possible pathophysiologic mechanisms and risk factors involved (risk biomarkers), while

complementing an earlier and more reliable diagnosis (diagnostic biomarkers) (Lassere, 2008; Voineagu & Yoo, 2013). Besides, biomarkers would also permit to foresee prognosis in terms of progression, identify homogenous subsets of patients with a delineated developmental trajectory, while predicting treatment response and potential adverse reactions to psychopharmacological drugs (prognostic biomarkers) (Voineagu & Yoo, 2013).

The majority of the replicated biomarkers in ASD overlap with endophenotypes, that satisfy heritability indicators, thus providing superior reliability and biological plausibility when compared to pure biomarkers. The most consistent biomarkers/endophenotypes described across literature concern i) biochemical biomarkers (namely serotonin (5-HT) and urinary solutes, such as p-cresol and p-cresyl sulfate); ii) morphological biomarkers (head circumference and dysmorphic features, such as abnormal cephalic index and palate dysmorphology); iii) hormonal biomarkers (for instance, oxytocin (OT) and melatonin (MT)); iv) immunological biomarkers; v) brain imaging biomarkers; and vi) neuropsychological biomarkers (Ruggeri, Sarkans, Schumann, & Persico, 2014).

Behavioral changes induced by serotonergic drugs, such as lysergic acid diethylamide (LSD), raised the attention to the involvement of 5-HT in psychiatric disorders, with its interference in central processes subsequently proved (Anderson, Horne, Chatterjee, & Cohen, 1990). Additionally, 5-HT was demonstrated to play a preponderant role in embryogenesis and neurodevelopment, spurring interest into the potential implication of the 5-HT system in neurodevelopment disorders, which comprehends ASD (Anderson et al., 1990). The first association between the 5-HT system and ASD was hypothesized in 1961 when Schain and Freedman described elevated blood 5-HT levels in a subset of children diagnosed with “infantile autism”(Schain & Freedman, 1961). This finding, termed hyperserotonemia, is considered the first biomarker in ASD and it has been consistently reported in more than 25% of the pediatric autistic population, perhaps being the most commonly observed biochemical finding in neuropsychiatry (Montgomery et al., 2018; Ruggeri, Sarkans, Schumann, & Persico, 2014).

Due to 5-HT’s heuristic potential, this narrative review aims to discuss the biological plausibility of the serotonergic dysfunction hypothesis in ASD, as well as the relevance and clinical implications of using 5-HT as a biomarker in this disorder.

2. Methods

On the 15th of November of 2019, an extensive literature search was conducted in PUBMED database, regarding human and animal studies addressing serotonergic dysfunction in ASD, as well as reports on blood 5-HT measurements in ASD patients and their relatives. The search terms used were as follows: (("autism spectrum disorder" OR "autism spectrum condition" OR "autism" OR "asd" OR "pervasive development disorder") AND ("serotonin" OR "5 hydroxytrypamine" OR "5 ht") AND ("biomarker" OR "biological marker" OR "hyperserotonemia" OR "elevated blood serotonin")). No time limit was applied, exclusively articles written in English were accepted.

Titles and abstracts of the studies identified were submitted to prior selection and those concerning information on i) ASD biomarkers, including 5-HT; ii) Serotonergic dysfunction in ASD, across several areas of investigation, with particular emphasis in Neurodevelopment, Genetics, Pharmacology and Immunology; and iii) 5-HT measurements in ASD patients and/or their first-degree relatives, were fully analyzed. The reference lists of these studies were examined to target additional articles. Furthermore, relevant publications obtained through hand-search were also incorporated. Overall, this narrative review includes a total of 140 articles.

3. The 5-HT System: An Overview

In order to dissect into the phenomenon of hyperserotonemia and serotonergic dysfunction in ASD, it is vital to acknowledge the dynamics of the 5-HT system in humans, as well as other mammalian species, and how neural activity is organized in neurotypical individuals.

5-HT is synthesized from L-tryptophan (TRP), an essential amino acid. There are two main sites of production, peripherally, in the GI system, especially in the enterochromaffin cells (EC), responsible for 95% of total 5-HT body production, and centrally, in the serotonergic neurons, predominantly located in the raphe nuclei of the brain-stem (Berger, Gray, & Roth, 2009; Janušonis, 2014).

The brain-blood barrier (BBB) is permeable to TRP, even though its entry is restricted by competition with other amino acids (Janušonis, 2014). On the contrary, 5-HT is unable to cross the mature BBB, which emphasizes the compartmentalization of 5-HT production. In addition, two isoforms of the tryptophan-hydroxylase (TPH) were identified, the TPH1 and TPH2 - while TPH1 operates at the periphery, TPH2 acts mostly at the central nervous system (CNS) (Walther & Bader, 2003). This enzyme is responsible for the conversion of TRP into 5-hydroxytryptophan (5-HTP), which constitutes the limiting step of this two-step synthetic pathway, culminating in the production of 5-hydroxytrypamine (5-HT) by the aromatic L-amino-acid decarboxylase (AADC) (Muller, Anacker, & Veenstra-Vanderweele, 2016). It should be noted that it occurs conversion of 5-HT into MT via the serotonin-N-acetylserotonin-melatonin pathway (Pagan et al., 2014). MT is accountable for the circadian and seasonal rhythms, modulation of the immune system function, as well as neurodevelopment and plasticity (Anderson, 2015). Moreover, TRP degradation via the kynurenine pathway is thought to diminish 5-HT availability and predispose to immunological, neurodegenerative and psychiatric disorders (Badawy, 2017; Quak et al., 2014).

As a well-known neurotransmitter, 5-HT plays a pleiotropic role in the CNS, modulating nearly all neuropsychological processes, due, in part, to the idyllic localization of the serotonergic neurons (Berger, Gray, & Roth, 2009). These neurons, organized into nine clusters known as rafe nuclei (B1-B9), send projections to almost all brain regions (Muller et al., 2016). The more caudal (B1-B5) project into the spinal cord, while the rostral groups mainly send projections to forebrain structures, thus creating the most widely distributed biogenic indolamine network (Muller et al., 2016; Yang, Tan, &

Du, 2014). In addition, brain territories express several 5-HT receptors in a subtype-specific fashion (Yang et al., 2014).

In the synaptic site, 5-HT binds to receptors that initiate intracellular signaling pathways and culminate in distinct behavioral and physiologic effects (Yang et al., 2014). 5-HT receptors are classified in seven subfamilies (5-HT_1 to 5HT_7), that encompass fourteen genetically different receptor subtypes (5-HT_{1A}, 5-HT_{1B}, 5-HT_{1D}, 5-HT_{1E}, 5-HT_{1F}, 5-HT_{2A}, 5-HT_{2B}, 5-HT_{2C}, 5-HT_3, 5-HT_4, 5-HT_{5A}, 5-HT_{5B}, 5-HT_6 and 5-HT_7) (Yang et al., 2014). Further processing through mRNA editing and alternative splicing enhances, even more, the diversity and complexity of this process (Muller et al., 2016). Detailed descriptions of these receptors and effects are beyond the scope of this review.

At the periphery, 5-HT is responsible for a myriad of physiological functions such as regulation of intestinal motility, platelet aggregation, and vasoconstriction (Yang et al., 2014). The majority of 5-HT is produced by the EC in the gut involving the TPH1, with a small percentage being also synthesized by neurons in the enteric nervous system (ENS), operating the TPH2 in resemblance to the CNS (Janušonis, 2014). Overall, 5-HT derived from the gut fits two purposes: locally it acts as a signaling molecule in the ENS, being taken up by neuronal and non-neuronal cells present in the gut that express the serotonin transporter (SERT); when entering the systemic blood circulation, most of the 5-HT is cleared by the liver, lungs and other organs, while the remnant 5-HT is sequestered by blood platelets, that express SERT, but do not synthetize 5-HT (Janušonis, 2014). Several studies have reported unexplained similarities between platelets and neurons since platelets are formed by megakaryocytes derived from the mesoderm and neurons have an ectodermal origin (Padmakumar, Van Raes, Van Geet, & Freson, 2019). This process occurs continuously, causing free 5-HT in the blood plasma to fluctuate when comparing different peripheral sites. Generally, free plasma 5-HT is measured in the distal venous blood after the hepatic and pulmonary circulation (Janušonis, 2014). Thus concentrations are low, with estimations varying according to technical procedures, but definitely inferior to 1000ng/L in healthy subjects (Anderson, 2007; Janušonis, 2014). The concentration of 5-HT in blood platelets is considerably higher, hence whole-blood serotonin (WB5-HT) levels are analogous to the 5-HT taken up by platelets (Janušonis, 2014). In neurotypical individuals, WB5-HT levels are estimated to be between 187-297μg/L and 0.4 μM (McBride et al., 1998; Melke et al., 2008; Janušonis, 2014).

A key point in 5-HT regulation levels is the extracellular clearance, in which SERT plays a preponderant role, accounting for the active transport of 5-HT from synapses into presynaptic neurons and from plasma into platelets (Jaiswal, Guhathakurta, et al., 2015). SERT is also expressed by other peripheral cells such as B-lymphocytes, causing 5-HT signaling to influence the immune system (Veenstra-Vanderweele & Blakely, 2012). Intriguingly, the placenta expresses high levels of SERT proteins as well (Veenstra-Vanderweele & Blakely, 2012).

The SERT regulatory network undergoes protein kinase C (PKC)-, protein kinase G (PKG)-, p38 mitogen-activated protein kinase (p38-MAPK)-mediated regulation and involves a protein complex composed by αIIbβ3 integrin, protein phosphatase 1 (PP1), protein phosphatase 2A (PP2A) and the 5-HT_{2A} receptor (5-HT_{2A} r) (Gabriele, Sacco, & Persico, 2014).

Degradation and inactivation of 5-HT are mainly operated by monoamine oxidase A (MAO-A), leading to the production of 5-hydroxyindoleacetic acid (5-HIAA) (Muller et al., 2016). This metabolite can be found in the cerebrospinal fluid (CSF), reflecting 5-HT function in CNS, with average concentrations approximately estimated in 122 nM (Narayan, Srinath, Anderson, & Meundi, 1993; Janušonis, 2014). Externally to the CNS, it can be measured in the urine, with concentrations around 3.5 μg/mg creatinine (normalized value, expressed in per mg creatinine) (Minderaa, Anderson, Volkmar, Akkerhuis, & Cohen, 1987; Janušonis, 2014). Urinary levels of 5-HT can also be detected, circa 0.1 μg/mg creatinine (Anderson, Minderaa, Cho, Volkmar, & Cohen, 1989; Janušonis, 2014).

4. 5-HT in Neurodevelopment

In the primordial stages of pregnancy, the embryo 5-HT levels rely on exogenous sources of maternal origin (Harrington et al., 2013). The maternal pancreas contributes to blood 5-HT levels, while the hemochorial placenta synthesizes and releases 5-HT that influences the fetal forebrain 5-HT levels (Muller et al., 2016).

Serotonergic neurons are among the first to emerge during neurodevelopment in the human brain, endowing the embryo to synthesize 5-HT independently from the mother by the fifth week of gestation (Harrington et al., 2013; Montgomery et al., 2018). By the tenth week of gestation, significant neuronal multiplication occurs, and axon projections begin to spread from the midbrain raphe nuclei to all brain regions, as previously described (Montgomery et al., 2018). 5-HT transporters and receptors are present by the fourth month of gestation (Harrington et al., 2013).

It is essential to highlight that blood 5-HT levels do not reflect brain 5-HT in the mature brain, due to the compartmentalization of 5-HT production mentioned previously. Nonetheless, the immature BBB is permeable to 5-HT until two years of age. At the same time, brain 5-HT levels reach a peak, followed by a progressive decline until steady adult levels are achieved, equivalent to half of that peak (Hadjikhani, 2010).

As a signaling molecule, 5-HT plays a critical role during brain development, by regulating pre- and postnatal processes such as neurite outgrowth, neuronal differentiation and organization, synaptogenesis, and neurogenesis (Madden & Zup, 2014). 5-HT is also implicated in maturational events, namely developmental apoptosis, and dendritic arborization, while interfering with other neurotransmitter systems, for instance, dopamine and OT (Harrington et al., 2013).

Additionally, 5-HT wields an intrinsic negative feedback mechanism on 5-HT neurons development through 5-HT_{1A} receptors. The central nucleus of the amygdala and the paraventricular nucleus (PVN) of the hypothalamus are highly innervated by 5-HT terminals, with consequent release of calcitonin gene-related peptide (CGRP) and OT via $5HT_{1A}$ and $5HT_2$ receptors (Hadjikhani, 2010).

The amygdala is thought to be a key element in ASD, as it is responsible for social cognition, perception of eye-gaze direction, and emotion expression, that is compromised in ASD. Volumetric magnetic resonance imaging (MRI) data indicates that the volume change of the right amygdala seen in children with ASD correlates with the aptitude to establish appropriate eye contact (Barnea-Goraly et al., 2014; Yang et al., 2014). In addition, CGRP projections to the amygdala contribute to

conditioned responses to acoustic and somatosensory stimuli, as well as to fear conditioning in social interaction (Yang et al., 2014). OT is a notable protagonist in social learning and affiliative behavior(Ruggeri, Sarkans, Schumann, & Persico, 2014). Interestingly, plasma OT levels of ASD individuals have been demonstrated to be negatively correlated with WB5-HT blood levels (Hammock et al., 2012; Ruggeri, Sarkans, Schumann, & Persico, 2014).

5. Evidence of Serotonergic Dysfunction in ASD

Available evidence across the reviewed literature regarding serotonergic dysfunction in ASD will be the object of discussion, with a special highlight for the phenomenon of hyperserotonemia of autism. Four major subjects will be addressed: i) Hyperserotonemia in ASD; ii) Abnormal Maternal and Placental 5-HT Levels; iii) 5 Immunoregulation and 5-HT: Immune Dysfunction in ASD; and iv) 5-HT and the Microbiome-Gut-Brain Axis in ASD. Collected data is presented in *Figure1.*

5.1 Hyperserotonemia in ASD

5.1.1 Hyperserotonemia in ASD Patients

After Schain and Freedman's first observation of hyperserotonemia in ASD individuals, several studies have recurrently reported abnormal serotonergic blood measures. 5-HT measurements in ASD patients reported across the analyzed literature are presented in *Table1*, as well as the measurement conditions and main conclusions of each study. While the vast majority of these studies are congruent with the initial hyperserotonemia findings, two studies reported normal or low mean 5-HT levels, however small samples of participants were used (n=18 and n=10, respectively), as well as platelet-poor plasma (PPP) 5-HT measurements, therefore omitting 5-HT derived from platelets.

In a recent meta-analysis, Gabriele and colleagues underpin the reliability of elevated 5-HT blood levels as a biomarker in ASD. An increase of 23% and 29% in WB5-HT and platelet-rich plasma (PRP) levels was observed in ASD individuals, respectively, when compared to controls. Both biomaterials demonstrated high sensitivity (28% for WB5-HT and 22% for PRP). Even though 5-HT measurements do not seem to be affected by the type of measurement technique (fluorometric assays or high-performance liquid chromatography (HPLC)) nor normalization by platelet count, the authors recommend using whole-blood measurements, normalized by platelet number and quantified by HPLC, in order to standardize future assessments (Gabriele, Sacco, & Persico, 2014). Moreover, WB5-HT seems to be negatively correlated with body mass, with data suggesting its inclusion as a covariate in platelet 5-HT levels studies (Albay, Chen, Anderson, Tatevosyan, & Janušonis, 2009).

5.1.2 Hyperserotonemia in First-Degree Relatives

Blood 5-HT levels have been demonstrated to be elevated, not only in ASD patients but also in their first-degree relatives, with high 5-HT levels demonstrating segregation within families, thus pointing towards the possible presence of a familial trait (Abramson et al., 1989). These results have been replicated in several studies (Cook et al., 1990; Leventhal, Cook, Morford, Ravitz, & Freedman, 1990; Bijl et al., 2015).

Additional data identified two subgroups within the hyperserotonemic first-degree relatives, one with decreased platelet 5-HT_2 receptor (5-HT_2r) binding and another with elevated platelet 5-HT uptake, therefore suggesting familial heterogeneity (Cook et al., 1993).

5.1.3 Theorized Mechanisms for Hyperserotonemia

Although the mechanisms underlying the elevation of 5-HT levels in ASD remain elusive, possible explanations have been postulated, including an increased exposure of the platelet to 5-HT or alterations in the platelet's handling of 5-HT (Anderson, Hertzig, & McBride, 2012).

Across the literature, the hypothesis of decreased catabolism of 5-HT has been assessed by measurements of monoamine oxidase (MAO) activity and monoamine substrates and metabolites (Anderson et al., 1990). Anderson and colleagues findings, regarding *in-vivo* MAO activity, imply that MAO functioning is not altered in autism, which is consistent with one recent study that examined whether platelet hyperserotonemia was associated with increased gut 5-HT synthesis, altered catabolism or altered MT production by measuring 5-HT, 5-HIAA and 6-sulfatoxymelatonin urinary excretion, concluding that the catabolism of 5-HT does not differ between normo- and hyperserotonemic individuals (Anderson et al., 1990; Mulder et al., 2009). However, it is not possible to exclude greater exposure of the platelet to 5-HT as a cause of hyperserotonemia from these results (Mulder et al., 2009).

Both 5-HIAA and 5-HT urinary levels have been used in an attempt to assess platelet 5-HT exposure, however, PPP 5-HT levels appear to deliver the most direct available index *in vivo* (Anderson et al., 2012). PPP 5-HT levels in ASD subjects have been demonstrated to be similar to those observed in controls, including in hyperserotonemic individuals, thus suggesting that

hyperserotonemia is not due to increased exposure of the platelet to 5-HT, but is more likely related to the platelet's handling of 5-HT (Anderson et al., 1990; Anderson et al., 2012). Predictably, results from Gabriele and colleagues meta-analysis corroborate this lack of disparity in PPP 5-HT levels, which emphasizes the necessity to motivate research on the platelet, as neither peripheral 5-HT synthesis nor catabolism seem to be altered in ASD individuals (Gabriele, Sacco, & Persico, 2014; Anderson et al., 2012).

In the last decades, investigation concerning the platelet 5-HT_2r shows inconsistent data. McBride and colleagues reported a reduced number of 5-HT_2r binding sites in platelets of young autistic adults (McBride et al., 1989). Later, Cook and coworkers determined a negative correlation between 5-HT_{2A}r and platelet 5-HT levels within first-degree relatives of ASD subjects (Cook et al., 1993). Hranilovic and colleagues, when testing the activity of SERT, monoamine oxidase B (MAO-B), and 5-HT_{2A}r, suggested a possible mechanism of 5-HT_{2A}r downregulation in response to upregulation of monoaminergic synthesis/degradation (Hranilovic et al., 2009). On the other hand, Perry and collaborators demonstrated no differences in terms of 5-HT_{2A}r binding sites number in autistic children and their first-degree relatives, which is congruent with recent findings of Aaron and colleagues that show no disparities in terms of 5-HT_{2A}r platelet binding nor a predictable relationship with 5-HT levels in autistic subjects (Perry, Cook, Leventhal, Wainwright, & Freedman, 1991; Aaron et al., 2019). Moreover, on the opposite spectrum, Kazek and colleagues noticed a higher expression of 5-HT_{2A}r mRNA in platelets of autistic children (Kazek et al., 2010). Discrepancies across studies might be due to sample selection, since it is essential to take into account that 5-HT levels tendentially decline with age and 5-HT_{2A} receptors number follow a similar decrease, thus complicating comparability between studies (Kazek et al., 2010).

When speculating potential mechanisms for the hyperserotonemia in autism, one reasonable hypothesis worth denoting is the existence of SERT dysfunction, due to its primary role as a regulator of peripheral and central 5-HT homeostasis (Chen et al., 2017). Platelet membranes of autistic subjects seem to express higher levels of SERT, while SERT affinity for 5-HT suffers no alteration, therefore culminating in amplified 5-HT internalization (Marazziti et al., 2000).

5.1.4 Serotonergic Genes

Even though estimates point towards the involvement of more than one thousand genes in ASD etiology, generating great challenges when it comes to identifying individually significant risk *loci*, investigation concerning genetic variations in SERT trafficking has received notable attention (Chen et al., 2017).

The SLC6A4 gene, situated on the chromosome 17q11.1-q12, encodes for both neuronal and peripheral SERT and has been implicated in 5-HT levels determination, with specific variants being associated to an increased risk for hyperserotonemia in ASD patients, probably by the interaction of these alleles with other genes or environmental factors, thus producing abnormally high 5-HT levels in this subgroup of ASD patients (Coutinho et al., 2004; Jaiswal, Guhathakurta, et al., 2015). The most common SERT coding variant identified in ASD subjects, SERT Ala56 (from the conversion of the amino acid Gly to Ala), has been successfully propagated in mice, causing them to exhibit hyperserotonemia, p38 MAPK-dependent hyperphosphorylation, elevated hippocampal 5-HT clearance, increased sensitivity of 5-HT_{1A} and 5-$HT_{2A/2C}$ receptors in the CNS, as well as abnormal behavior, compatible with ASD (Veenstra-Vanderweele et al., 2009; Veenstra-Vanderweele et al., 2012; Robson et al., 2018). Interestingly, the use of α-isoform-specific p38 MAPK inhibitors, able to penetrate the CNS and counterbalance SERT activation by the p38 MAPK, normalizes the phenotype described above, suggesting that p38 MAPK influences SERT Ala56 (Robson et al., 2018). This variation has also been showed to confer an alteration to SERT conformation that can affect transport function, regulation, and eventually, disease risk for some individuals (Quinlan et al., 2019). Moreover, it has been demonstrated that SERTAla56 suffers epistatic interactions that can modulate its functional impact, especially in terms of social behavior (Kerr et al., 2013).

One polymorphism in SLC6A4 that affects gene expression levels, the serotonin transporter-linked polymorphic region (5-HTTLPR), is worth highlighting (Harrington et al., 2013). An excess of the long/long 5-HTTLPR genotype in autistic individuals has been reported, which would, at least in part, provide a molecular justification for high platelet 5-HT content in ASD (Yirmiya et al., 2001). Nevertheless, results across studies are inconsistent, with some finding no association between 5-HTT gene polymorphisms and blood 5-HT levels in ASD, while others do not exclude upfront the possibility of increased uptake by platelets in hyperserotonemia, thus appealing to take into

consideration the effects of 5-HTTLPR genotype on 5-HT uptake when performing platelet 5-HT measures in ASD, albeit the absence of association verified (Betancur et al., 2002; Anderson et al., 2002). Meanwhile, others consider that SERT promoter alleles, by themselves, do not carry an associated risk for ASD, but rather modify the severity of autistic behaviors in terms of social and communication interactions (Tordjman et al., 2001).

Additional mapped genes, regarding hyperserotonemia and ASD, consist on the *ITGB3* gene, the *MAOB* gene and the *HTR2A* gene. The *ITGB3* gene, that encodes to the integrin β3 subunit, appears to interact with SLC6A4 gene, in addition to its independent association with 5-HT levels, hence implying a common genetic mechanism for hyperserotonemia in ASD (Coutinho et al., 2007; Carneiro, Cook, Murphy, & Blakely, 2008). Also, integrin β3 knocked-out mice manifest alterations in social interaction and repetitive behaviors, characteristic of ASD individuals (Carter et al., 2011). Recent findings support the involvement of *rs55827077* as the functional *ITGB3* gene promoter variant contributing to hyperserotonemia in ASD (Gabriele et al., 2019). Encoding to the MAO-B enzyme, the *MAOB* gene has been suggested to increase ASD risk in males, perhaps by interfering with 5-HT metabolism and behavior through a sex-specific regulatory effect (Chakraborti et al., 2016). Collected data concerning the *HTR2A* gene, that encodes to the $5HT_{2A}$ receptor, shows no evidence of unequal transmission of haplotypes through the transmission/disequilibrium test (TDT), even though it is not possible to exclude a parent-origin effect, due to low power of detection (Veenstra-Vanderweele et al., 2002).

In a recent study, Chen and colleagues hypothesized that *de novo* variants (DNVs) and recessively acting variants (RAVs) might also play a role in predisposing for hyperserotonemia and ASD. Through a whole-exome sequencing approach, this investigation group identified a novel gene, *USP15*, involved in the TBG-β pathway and cell junction function, which contributes to 5-HT-related ASD risk, thus emphasizing 5-HT as an effective endophenotype to dissect ASD genetics (Chen et al., 2017). It is fundamental to denote that abnormalities in platelet 5-HT levels do not unavoidably correlate with variations in brain 5-HT levels. However, they do mirror the dysfunction of brain expressed genes that can interfere with neurodevelopment and function, ultimately leading to autistic symptomatology (Coutinho et al., 2007).

5.2 Abnormal Maternal and Placental 5-HT Levels

The external dependency of 5-HT during the early stages of neurodevelopment indicates that altered maternal or placental 5-HT system function may influence the developing embryo (Muller et al., 2017). Muller and colleagues showed that maternal SERT Ala56 genotype impacts offspring placental 5-HT levels, forebrain 5-HT levels, and neurodevelopment, which may increase the risk for ASD, by altering the maternal prenatal environment, namely by metabolic changes and altered immune signaling in the placenta (Muller et al., 2017). Additionally, one recent study demonstrated that mean maternal WB5-HT is associated with neurodevelopmental outcomes in offspring with ASD, which implies that maternal genotype and serotonergic system impacts maternal WB5-HT levels and offspring brain development and behavior. Interestingly, the lowest maternal WB5-HT levels were observed in children with the highest severity phenotype, who most commonly exhibit *de novo* gene disrupting variants, suggesting that the maternal serotonergic system influence on ASD risk differs across ASD subpopulations and might play a more preponderant role in those with a greater contribution from polygenic risk and less from *de novo* mutations (Montgomery et al., 2018).

Evidence suggests that maternal 5-HT is able to cross the placenta since injection of radiolabeled ($_{14}$C) 5-HT into pregnant mice is detected in the fetal brain, even though in small quantities (Koren, Pfeifer, & Sulman, 1966). Within the placenta, particularly in the plasma membrane of the trophoblastic cells, 5-HT prompts autocrine and paracrine effects by the 5-HT$_{2A}$ receptors and perhaps the 5-HT$_{1A}$ receptors (Rosenfeld, 2019). SERT regulates extracellular 5-HT and might avoid vasoconstriction of the placental blood vessels induced by 5-HT (Hadden et al., 2017; Rosenfeld, 2019). Metabolization is carried out by MAO-A, and quantitative and qualitative disruptions of this enzyme are linked to Preeclampsia (PE), leading to hyperserotonemia in the placenta and consequently the embryo (Gujrati, Shanker, Vrat, Chandravati, & Parmar, 1996; Carrasco et al., 2000; Rosenfeld, 2019).

Equilibrium in 5-HT signaling is fundamental for normal placental structure and function (Rosenfeld, 2019). Actually, defects in placental 5-HT have been associated to fetal growth restriction, anxiogenic behaviors and ASD (Yang et al., 2014, Ranzil et al., 2019; Hendricks et al., 2003; Wang, Hausknecht, Shen, & Haj-Dahmane, 2018; Rosenfeld, 2019). In the ASD setting, PE is a candidate risk factor, even though the basis of this relation remains unclear (Rosenfeld, 2019). Furthermore, the

conditions of both hypo- and hyperserotonemia have been implicated as potential risk factors for ASD (Rosenfeld, 2019; Yang et al., 2014).

5.2.1 Hyperserotonemia: The Developmental Hyperserotonemia Model of Autism

Patricia Whitaker-Azmitia was the first to postulate the Developmental Hyperserotonemia Model of Autism, advocating that at early stages of development, when the BBB is not entirely formed, high levels of 5-HT in the blood can enter the brain of the developing fetus, instigating a loss of 5-HT terminals through a negative feedback mechanism (Whitaker-Azmitia, 2005; Hadjikhani, 2010). As hyperserotonemia suppresses 5-HT terminals, it reduces OT levels in the PVN of the hypothalamus and elevates CGRP in the central nucleus of the amygdala, as previously described. Additionally, Whitaker-Azmitia assumes that hyperserotonemia occurs throughout development since 5-HT might also be involved in neurochemical imprinting, a phenomenon that occurs when behavioral changes in the adult animal arise from deviations in neurochemistry formation during development, despite normal neurochemistry until adulthood is attained, which might explain the co-existence of high blood and low brain 5-HT levels observed (Whitaker-Azmitia, 2005; Yang et al., 2014). Pharmacological manipulation to elevate 5-HT levels during the prenatal period in animal models induce a loss of 5-HT terminals as adults, thus supporting this hypothesis (Cabrera-Vera, Garcia, Pinto, & Battaglia, 2000; Whitaker-Azmitia, Zhang, Clarke, 1994; Harrington et al., 2013).

In utero exposure to drugs affecting 5-HT release have been associated with ASD such as thalidomide, valproic acid (VPA), cocaine, alcohol, and selective serotonin reuptake inhibitors (SSRIs), albeit the controversial results across literature for this class of antidepressants (Harrington et al., 2013; Hadjikhani, 2010; Yang et al., 2014). Several animal models of autism were created resorting to 5-HT agonists or medications that increase 5-HT, reproducing some of the biological alterations and social behaviors encountered in autistic individuals (Harrington et al., 2013).

For that matter, Madden and Zup detected disruptions on social play behavior in juvenile rats after administration of a non-selective 5-HT receptor agonist, 5-methoxytryptamine (5-MT), suggesting that hyperserotonemia during the perinatal period might disturb typical neuronal organization, thus leading to neurochemical and behavioral alterations as seen in ASD. Also, eighteen days postnatally, an abnormal number of oxytocinergic cells in the lateral and medial PVN was

apparent in treated females, but not in treated males, as well as relative changes in 5-HT_{1A} and 5-HT_{2A} receptors expression, on the tenth and eighteenth day postnatally, in males and females, respectively, concluding that these differences are probably due to interaction between 5-HT and gonadal hormones (Madden & Zup, 2014). In a subsequent study, the same authors complement these findings by adding that, in contrast to males, females can regulate 5-HT receptors in response to hyperserotonemia, in a manner that stimulates OT cell survival and functionality, thus promoting neural protection (Edwards, Madden, & Zup, 2018). While supporting the contribution of the serotonergic system to ASD etiology, these studies indicate that 5-HT plays a crucial role in neurodevelopment in a sexually dimorphic manner, which is particularly pertinent given the marked male sex bias in ASD prevalence (Madden & Zup, 2014; Edwards et al., 2018). Still, on the topic of possible sexual dimorphism in ASD, Shuffrey and colleagues showed that hyperserotonemia is more often seen before puberty, with males being significantly more likely to present this biochemical alteration, compared to females (Shuffrey et al., 2017).

Additionally, exposure to VPA prenatally seems to induce glutamatergic synaptic transmission and spontaneous firing in 5-HT neurons localized in the dorsal raphe nucleus, leading to amplification of the serotonergic tone in these neurons, as well as stereotypic and anxiety-like behaviors, which is in concordance with the behaviors and hyperserotonemia encountered in ASD (Wang, Hausknecht, Shen, & Haj-Dahmane, 2018).

5.2.2 Hyposerotonemia: The Reverse Scenario

Interestingly, one study reveals an increase in 5-HT axons in *post-mortem* brain tissue from children and adult donors with ASD, when compared to controls (Azmitia, Singh, & Whitaker-Azmitia, 2011). This finding seems contradictory to the model stated above, as it would be expected to verify a compensatory decline in 5-HT axons, nonetheless this increase could be justified if transient situations of hyposerotonemia occur (Yang et al., 2014).

Moreover, when studying mothers without established psychiatric diagnoses, Connors and colleagues reported significantly lower plasma 5-HT levels in mothers of children with ASD, in comparison to mothers with neurotypical offspring, thus implying that low maternal plasma 5-HT might also disrupt neurodevelopment (Connors et al., 2006; Harrington et al., 2013). However, this study

was sharply criticized, due to the fact that plasma 5-HT levels were much higher than previous reports on PPP, making it problematic to guarantee what was actually being measured, thus conclusions ought to be prudently extrapolated from these results (Harrington et al., 2013).

Nevertheless, one animal model of autism is created based on 5-HT depletion through injections of the serotonergic neurotoxin 5,7-dihydroxytryptamine (5,7-DHT) (Boylan, Blue, & Hohmann, 2007). Since 5-HT has been shown to stimulate 5-HT terminal growth indirectly and directly inhibit this process, the appearance of animal studies suggesting that either excess or insufficient levels of 5-HT might culminate in abnormal brain development is predictable (Gaspar, Cases, & Maroteaux, 2003; Whitaker-Azmitia, 2001; Harrington et al., 2013).

5.3 Immunoregulation and 5-HT: Immune Dysfunction in ASD

Immune anomalies are commonly observed in ASD individuals, such as identification of autoantibodies against neuronal antigens, an atypical profile of cytokines and chemokines, and abnormal cellular function (Ruggeri, Sarkans, Schumann, & Persico, 2014; Jaiswal, Mohanakumar, & Rajamma, 2015). Increasing evidence points to the existence of a complex interplay between the 5-HT, immune, and central nervous systems. Accordingly, anomalies in this network may induce behavioral perturbations and psychiatric conditions, including ASD (Jaiswal, Mohanakumar, & Rajamma, 2015).

5.3.1 Hyperserotonemia Prompts Immune Dysfunction in ASD

Hyperserotonemia directly impacts innate and adaptative responses (Jaiswal, Mohanakumar, & Rajamma, 2015). Certain ASD patients with hyperserotonemia report i) antibodies against 5-HT_{1A} receptors; ii) absence of interleukin-2 (IL-2) receptors on the lymphocyte cell surface and iii) reduced natural killer (NK) cell activity in rats, whereas low 5-HT levels are associated with higher NK cell activity (Singh, Singh, & Warren, 1997; Todd & Ciaranello, 1985; Abramson et al., 1990; Gabrilovac, Čičin-Šain, Osmak, & Jernej, 1992; Jaiswal, Mohanakumar, & Rajamma, 2015).

Additionally, findings linking 5-HT to asthma pathophysiology, through T_H1-type cytokines reduction, can be transposed to ASD, making it possible the involvement of hyperserotonemia in autoimmune induction through a similar mechanism, leading to T_H1/T_H2 imbalance in favor of T_H2

induced inflammation, especially since autoantibodies against neuronal antigens are fortuitously identified in ASD subjects (Ménard, Turmel, & Bissonnette, 2007; Cohly & Panja, 2005; Jaiswal, Mohanakumar, & Rajamma, 2015). One study explored this possibility by measuring serum anti-myelin-basic protein (anti-MBP) autoantibodies in autistic children, and no association was found. However, the authors emphasize the utility of including serum 5-HT levels in immune studies (Mostafa & AL-Ayadhi, 2011). Furthermore, additional data suggest that 5-HT is able to instigate delayed-type hypersensitivity responses and that human leukocyte antigen (HLA) genes are correlated to serum 5-HT levels in ASD patients, both vital mechanisms for autoimmunity induction (Askenase et al., 1991; Warren et al., 1992; Bhanja & Mohanakumar, 2010; Jaiswal, Mohanakumar, & Rajamma, 2015).

5.3.2 Interaction Between Two Biomarkers: Interleukin-6 and 5-HT

Another biomarker of growing interest in ASD is interleukin-6 (IL-6). When comparing to neurotypical individuals, autistic subjects show a significant increase of IL-6 plasma levels (Emanuele et al., 2010; Ashwood et al., 2011; Malik et al., 2011). This elevation has also been found in *post mortem* brain samples, in the anterior cingulated gyrus and CSF from ASD patients (Wei et al., 2011; Li et al., 2009; Vargas, Nascimbene, Krishnan, Zimmerman, & Pardo, 2005; Yang, Liu, Sang, Zhu, & Du, 2015). Interestingly, 5-HT is capable of regulating IL-6 secretion via 5-HT_{2B} receptor activation and ERK1/2 pathway, whilst induction of IL-6 gene expression is associated with 5-HT_{7} stimulation in human microglial cells (Li et al., 2013; Mahé et al., 2005). Overall, these findings suggest an interaction between IL-6 and the serotonergic system.

5.3.3 SERT: A Connecting Factor Between Inflammation and Neurodevelopment

Specific functional variants of SERT have been proved to lead to immune dysfunction through upregulation of SERT activity, which reduces 5-HT availability for immune functions and culminates in decreased T-cell proliferation and T cell receptor expression (Carneiro et al., 2009; Jaiswal, Mohanakumar, & Rajamma, 2015). Furthermore, Zhu and colleagues demonstrated that SERT activity is regulated by proinflammatory cytokines via the p38-MAPK signaling pathway (Zhu, Carneiro, Dostmann, Hewlett, & Blakely, 2005; Jaiswal, Mohanakumar, & Rajamma, 2015). This

mechanism was later observed in mice displaying behavioral perturbations compatible with ASD, in association to hyperserotonemia and increased sensitivity of 5-HT receptors (Veenstra-Vanderweele & Blakely, 2012; Jaiswal, Mohanakumar, & Rajamma, 2015).

An extension of this understanding to the neurodevelopment period can be presumed as Kannan and colleagues advocate that maternal intrauterine inflammation and fetal brain development are linked via the serotonergic system, based on the fact that endotoxin administration in pregnant mice disclosed decreased conversion of TRP to 5-HT, alterations in cortical 5-HT levels, diminished SERT expression in the somatosensory cortex, increased apoptotic cells in the ventrobasal thalamus and abnormal thalamocortical development in the fetus, which is primarily regulated by 5-HT (Kannan et al., 2011; Jaiswal, Mohanakumar, & Rajamma, 2015).

5.3.4 Inflammation and the Gut-Brain Axis: An Environmental Risk Factor for ASD

Recent evidence suggests that prenatal exposure to fever or infection, mainly of viral origin, might function as an environmental risk factor for ASD, especially in children that report concomitant GI conditions (Israelyan & Margolis, 2018).

During inflammatory processes, platelets release 5-HT at inflammation sites, which is internalized by lymphocytes via SERT in a second instance. As referred previously, the SERT protein is naturally expressed in lymphocytes. Conditions of hyperserotonemia foment the expression of pro-inflammatory cytokines, which in turn upregulate SERT activity, as demonstrated by Zhu and colleagues (Zhu, Carneiro, Dostmann, Hewlett, & Blakely, 2005; Jaiswal, Mohanakumar, & Rajamma, 2015). Inflammatory insults such as maternal infection, maternal immune activation (MIA) and stress-mediated immune responses jeopardize the efficacy of the placental barrier, thus favoring the passage of toxic microbial metabolites generated along these inflammatory processes, from maternal to fetal blood (Hsiao, McBride, Chow, Mazmanian, & Patterson, 2012; Jaiswal, Mohanakumar, & Rajamma, 2015). This passage elicits proinflammatory cytokines and increased activation of SERT in the fetal brain, which consequently induces hyperserotonemia. Postnatally, lactation might serve as the administration route for toxic metabolites, secondary to, for instance, maternal drug abuse, smoking, or infectious diseases (Jaiswal, Mohanakumar, & Rajamma, 2015).

In developing infants, still with an immature BBB, GI infections alter gut microbiota and intestinal permeability, which in turn may induce immune dysfunction and hyperserotonemia in the gut, ultimately impacting neurodevelopment and leading to behavioral perturbations compatible with ASD via the microbiome-gut-brain-axis, defined as this bidirectional communication between the ENS and the CNS (Jaiswal, Mohanakumar, & Rajamma, 2015; Israelyan & Margolis, 2018). The relationship between maternal infection, immunity, and the gut-brain axis have been explored in an MIA mouse model, which is known to display ASD features, by mimicking a viral infection (Israelyan & Margolis, 2018). Further considerations regarding 5-HT, the microbiome-gut-brain axis, and GI abnormalities in ASD are addressed in the next section.

5.4 5-HT and the Microbiome-Gut-Brain Axis in ASD

As co-occurrence of two or more diseases seemingly unrelated in terms of etiology is rare, the high incidence of GI problems reported in ASD patients, that occur at a four-fold higher rate when compared to the general population and include various modes of GI conditions and inflammatory bowel disease, indicates a possible common mechanism for the behavioral and systemic abnormalities (Kazek et al., 2010; Lim, Lim, Choi, & Ko, 2017; Israelyan & Margolis, 2018). Even though this association remains unclear, abnormalities in the gut-brain axis are thought to be implicated in ASD, as well as dysregulation of factors that affect both the ENS and CNS development (Israelyan & Margolis, 2018).

Since 5-HT and its modulators play a vital role in the ENS and CNS dynamics and are determinant during the neurodevelopment period, serotonergic dysfunction may be a plausible nexus for the gut-brain axis (Israelyan & Margolis, 2018). Interestingly, Robson and colleagues found that mice carrying the SERT Ala56 gene variant report GI perturbations and display restored colonic motility after SERT inactivation through p38-MAPK inhibitors (Robson et al., 2018). Moreover, modulation of the 5-HT_4 receptor, which has been implicated in ENS neurogenesis and colonic motility, might be a possible mechanism to bypass the GI abnormalities associated to the SERT Ala56 gene variation (Liu, Kuan, Wang, Hen, & Gershon, 2009; Manabe, Wong, & Camilleri, 2010; Israelyan & Margolis, 2018). Recently, Margolis and co-workers verified that administration of prucalopride, a selective 5-HT_4 agonist, during critical periods of neurodevelopment, normalized enteric neuronal

numbers in Ala56-expressing mice, while providing long-term reassortment of colonic motility (Margolis et al., 2016; Israelyan & Margolis, 2018).

Also, emerging evidence supports the influence of gut microbiota on the immune system, metabolism, and neurodevelopment via the gut-brain-axis, as well as the impact on the 5-HT synthesis, which seems to lead to GI discomfort and altered mental status (Lim et al., 2017).

Several studies have implicated alterations in gut microbiome composition in ASD pathophysiology (Interagency Autism Coordinating Committee, 2017). Hsiao and coworkers demonstrated the existence of GI barrier defects and microbiota alterations in an MIA mouse model. Furthermore, oral administration of human commensal *Bacteroides fragilis* ameliorated communicative, repetitive, anxiety-like, and sensorimotor behavior. MIA mice also displayed a significant increase in serum indolepyruvate levels, a central molecule in TRP metabolism, which was also corrected with *Bacteroides fragilis* administration (Hsiao et al., 2013).

Seemingly, Lim and colleagues resorted to a VPA-induced mouse model of ASD to demonstrate that prenatal exposure to environmental risk factors can modify the abundance of many gut microbiome taxa postnatally, especially concerning the *Prevotellaceae* family, with results suggesting that this dysbiosis might determine alterations in metabolic and 5-HT pathways since hyperserotonemic mice also showed a concomitant increase in the short-chain fatty acids (SCFA)-producing pathway and increased abundance of the spore-forming acetic-acid bacteria, *Clostridiales Tissierellaceae Sporanaerobacter*, known inducers of 5-HT production (Lim et al., 2017).

However, no precise microbial composition has yet been described for ASD, probably because the majority of these studies reporting differences in gut microbiota composition evaluate dissimilar and small size samples with different analytical methods, which allied to the phenotypical heterogeneity seen in ASD, exponentiates these methodological issues and foments the need to address this matter with large-scale studies that take into consideration ASD phenotypes (Israelyan & Margolis, 2018).

6. Clinical Implications and Research Perspectives

6.1 Clinical Utility of Biomarkers in ASD

In the ASD setting, biomarkers would be a valuable asset in clinical practice, as for allowing to i) stipulate risk assessment at birth for siblings of children diagnosed with ASD, fomenting the creation and application of preventive measures; ii) anticipate diagnosis with higher accuracy, particularly between 12 and 30 months of age; iii) foresee developmental trajectories; iv) predict treatment response and v) identify individuals prone to specific adverse reactions instigated by psychopharmacological drugs (Ruggeri, Sarkans, Schumann, & Persico, 2014).

Furthermore, endophenotypes such as 5-HT could provide the means to determine homogeneous subgroups of ASD patients biologically, while contributing to the understanding of underlying pathophysiological mechanisms involved, as well as genetic and environmental factors implicated, that once identified might be addressed by personalized molecular therapies for partial or complete restitution (Yoo, 2015; Ruggeri, Sarkans, Schumann, & Persico, 2014).

6.2 Serotonergic Dysfunction: A Rationale for Novel ASD Therapies and Risk Factors Identification

Pharmacological ASD treatment is primarily used for associated symptoms, since efficacy for core autistic neurobehavioral symptoms has not been recognized, therefore urging the need to develop novel modalities of treatment (DeFilippis & Wagner, 2016). Several dysfunctional mechanisms and pathways on the serotonergic system dynamics stated above will be addressed in this section, due to the promising applicability of these basic science findings to develop innovative ASD therapies and to identify potential risk factors.

6.2.1 Selective 5-HT Reuptake Inhibitors

Due to the presence of repetitive, ritualistic behaviors and insistence on restricted routine patterns, antidepressants have been contemplated for ASD treatment, including SSRIs (DeFilippis & Wagner, 2016). Through SERT binding, SSRIs inhibit the 5-HT reuptake at the presynaptic neurons, therefore increasing the extracellular 5-HT levels (Harrington et al., 2013). Several studies have

shown that SSRIs especially minimize interfering repetitive thoughts behavior and aggression, as well as improving social interaction and language use in adults with ASD, however, data seems to be inconsistent in terms of efficacy (Mcdougle, Volkmar, Susan, & Study, 1996; Hollander et al., 2005; King et al., 2009). Interestingly, Walsh and colleagues' findings on the necessity of 5-HT in the nucleus accumbens (NAc) for normal sociability establish a possible rationale for pharmacologically targeting this mechanism in ASD, for instance, with SSRIs (Walsh et al., 2018). Another drug that is being tested for therapeutic utility is 3,4-methylenedioxymethamphetamine (MDMA), which elicits 5-HT efflux from synaptic terminals via SERT (Heifets & Malenka, 2016).

Still, regarding the SSRIs in the clinical setting, it should also be highlighted that, as it has been referred, situations of both hypo- and hyperserotonemia during the neurodevelopment period can potentially be implicated in ASD etiology through several plausible, yet unclear, mechanisms.

Various observational studies report associations between the use of antidepressants during pregnancy and ASD in offspring, even though the nature of this relationship can either be causal or confounded by indication, since there is an increased documented risk for ASD in children of mothers with psychiatric conditions, regardless of antidepressant treatment (Hagberg, Robijn, & Jick, 2018; Rai et al., 2017).

Conversely, considering that all antidepressants, including SSRIs, are able to cross the placental barrier, thus being available to the developing fetus and generate an increased exposure to 5-HT levels, it raises safety questions concerning these psychopharmacologic drugs and the inherent possibility for ASD risk in offspring of depressed medicated mothers. However, even when an association between antidepressant use during pregnancy and ASD in offspring is established, it points towards a small absolute risk, considering that, hypothetically, even if no pregnant women took antidepressants, only a trivial number of cases would potentially be prevented. Therefore, apart from fomenting investigation on possible underlying biological and modifiable mechanisms in ASD etiology, it is also essential to take into account this evolving, yet inconsistent, evidence when prescribing and advising depressed pregnant women, by weighing up the benefits and risks of this clinical decision (Rai et al., 2017).

6.2.2 The p38α MAPK Pathway

As previously discussed, the SERT Ala56 gene variant has been showed to induce hyperserotonemia and abnormal behavior compatible with ASD in mice, likely through the p38α MAPK pathway. The use of p38 MAPK inhibitors prompts normalization of behavioral phenotype and colonic motility in mice carrying this genetic variant and presenting concomitant GI perturbations, thus suggesting that inhibition of the p38α MAPK pathway might attest therapeutically benefit in ASD, as it arises as a promising neurotherapeutic target (Robson et al., 2018).

6.2.3 Modulation of the Microbiome-Gut-Brain Axis

Even though many questions remain to be answered, recent investigation regarding the serotonergic system as a nexus between the microbiome-gut-brain axis and ASD has revealed exciting insights that revolutionize the underlying concept of ASD as a disease that encompasses the gut and ultimately influences the immune, metabolic and nervous systems (Hsiao et al., 2013). For that matter, given the current evidence, microbiome-mediated therapies may be a potential and effective treatment in ASD, still requiring further safety and efficacy studies in humans (Israelyan & Margolis, 2018). Seemingly, this principle might as well be transposable to other neurodevelopmental disorders.

Furthermore, pharmacological agonism of the 5-HT_4 receptor appears to be a promising approach, given the gut phenotype reassortment in the SERT Ala56 mice, allied to the fact that 5-HT_4 receptors are expressed in CNS regions implicated in ASD, thus making this strategy worthy of exploration in subsequent human studies (Margolis et al., 2016; Samuels et al., 2016; Israelyan & Margolis, 2018). Also, due to the pleiotropic distribution of the 5-HT system, efforts should be undertaken to establish selective and effective 5-HT_4 agonists, as nonselective 5-HT_4 agonists have been associated to a higher frequency of cardiovascular adverse events in clinical trials (Tack et al., 2012; Israelyan & Margolis, 2018). Novel and more selective 5-HT_4 agonists have shown a superior safety profile. However, the risks and benefits of their use will have to be pondered in the clinical setting (Tack et al., 2012).

6.3 Recent ASD Biomarker Research: Correlations/Associations Between Biomarkers and Multiple Biomarker Assessment

Recent ASD biomarker research has combined efforts to scrutinize correlations and associations between biomarkers or assess multiple biomarkers simultaneously (Bridgemohan et al., 2019). Several studies have followed this line of investigation concerning 5-HT. For instance, Wassink and collaborators recognized an association between the SLC6A4 promoter polymorphism and overgrowth of the cortical grey matter in autistic children, establishing a possible connection between compromised 5-HT signaling during development and head circumference, a heritable trait in the general population used as a proxy for brain size and a recognized ASD biomarker (Wassink et al., 2007).

Additionally, a recent study reporting hyperserotonemia, increased levels of N-acetylserotonin (NAS) in platelets and lower levels of MT in ASD subjects, points out the disruption observed in the serotonin-N-acetylserotonin-melatonin pathway as a highly sensitive and specific biomarker in ASD, while providing a rationale for MT therapy when associated with sleep problems (Pagan et al., 2014).

Yang and coworkers verified that the combination of WB5-HT and plasma IL-6 elevation offered the best sensitivity and specificity in terms of ASD diagnosis (Receiver Operating Characteristic (ROC) analysis expressing an area under the curve (AUC) of 0.96), in comparison to the isolated effect of each variable, while showing a positive correlation with symptom severity (Yang et al., 2015). Further investigation of the topic will probably offer insights concerning innovative pharmacologic treatments.

6.4 Integration of ASD Biomarker Research with Clinical Care: The Translational Research Model

Apart from recognizing the need to stimulate investigation in ASD biomarkers, the Developmental-Behavior Pediatrics Research Network denotes the sparse involvement of developmental behavior pediatric subspecialists in research as a fundamental aspect preventing the progression of the field (Blum et al., 2012). In order to overcome this concern, recent studies have combined efforts towards applying a translational research model, integrating biomarker and ASD research with clinical care.

A pilot study evaluated the feasibility of conducting biomarker research within a subspecialty clinical visit for children diagnosed with ASD, as well as the influence on clinical care perceived by parents and clinicians. Seven biomarkers were obtained (growth measurements, head circumference, neurologic and dysmorphology examinations, digit ratio (2D:4D) measurement, platelet blood 5-HT, and urinary MT sulfate excretion levels), together with cognitive level, demographic features, educational placement and child behavior assessment. The most substantial challenge encountered was the recruitment of participants, due to problems in reaching families by phone and parent apprehension about the blood draw requirement. Although the study clinicians had standardized instruction, further research training might have been pertinent to guarantee the reliability of the results. Another predictable limiting factor was the need for infrastructure to support this type of research, demonstrating that model adaptation according to the specificity of clinical sites is fundamental. Interestingly, the clinical visit was not negatively affected by research activities, and both participants and clinicians acknowledged positive effects such as the opportunity to perform a more exhaustive examination and to have comprehensive discussions with families, leaving the authors to suggest future studies on the effect of clinic-based research on the clinician-patient relationship (Sices et al., 2017).

One supplementary publication examined potential correlations among biomarkers collected in the pilot demonstration previously described. The outcomes established were as follows: i) group means indicated hyperserotonemia; ii) higher income was associated with higher platelet blood 5-HT levels and urine MT sulfate excretion levels; iii) MT correlated negatively with age and neurologic problems; iv) dysmorphic status correlated with higher reported stereotyped behavior and inappropriate speech, and v) participants had excessive rates of medical and psychiatric comorbidities. These results exemplify the potential of this approach to produce fundamental data concerning several biomarkers and functional areas within a single heterogeneous clinical population, even though known limitations must be taken into consideration such as the explanatory nature of the study, sample size, and heterogeneity. In order to excel in these adversities, the authors advance that it would be profitable in future studies to use large multisite samples and focus on subgroups of patients that share specific symptomatology (Bridgemohan et al., 2019).

The presented approaches are new attempts to engage the clinicians in the process actively and integrate collected data by using diverse samples of patients. Moreover, transposition to other medical conditions also marked by heterogeneity might be profitable.

7. Conclusions

The present narrative review provides compelling evidence that serotonergic dysfunction is present in ASD, at least in a subpopulation of patients. This dysfunction may contribute to the heterogeneous behavioral phenotypes seen in ASD, through a complex interplay between the 5-HT system and the immune system, from an early stage of neurodevelopment, in which 5-HT plays a determinant role. An altered maternal and placental 5-HT function may influence the developing embryo, thus supporting the Developmental Hyperserotonemia Model of Autism.

Hyperserotonemia has been consistently recorded in more than 25% of the ASD population and is also considered a possible familial trait. Existing data highlights the reliability of elevated blood 5-HT levels as a biomarker in ASD, namely by WB5-HT and PRP measurements. The underlying mechanism for this phenomenon seems to be related to the platelet's handling of 5-HT, with several serotonergic genes possibly implicated.

5-HT as a biomarker/endophenotype would be a valuable asset in clinical practice, as it may provide information about possible pathophysiologic mechanisms and risk factors, as well as identification of homogenous subsets of patients with a delineated developmental trajectory, facilitating a more reliable and earlier diagnosis, prediction of treatment response and potential reactions to psychopharmacological drugs, while helping to develop novel therapies for ASD.

Recent data concerning the serotonergic system as a nexus between the microbiome-gut-brain axis and ASD revolutionizes the concept of ASD as a disease that comprehends the gut and ultimately influences the immune, metabolic and nervous systems, in which dysbiosis appears to play a determinant role with promising therapeutical perspectives. In terms of treatment, the necessity of 5-HT in the NAc for normal sociability establishes a possible rationale for pharmacologically targeting this mechanism with SSRIs. Seemingly, inhibition of the p38α MAPK pathway might also attest therapeutically benefit in ASD.

The translational research model appears to be an exciting approach to propel ASD research, since biomarker research is focusing on correlations and associations between biomarkers or multiple and simultaneously biomarker assessment.

Further investigation concerning the 5-HT system in ASD is strongly advised, as it will expand our understandings about the adaptive role of this intricate neuromodulatory network and eventually promote a faster translation of these basic science findings to the clinical practice.

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Conflict of Interest

The authors declare no conflict of interest.

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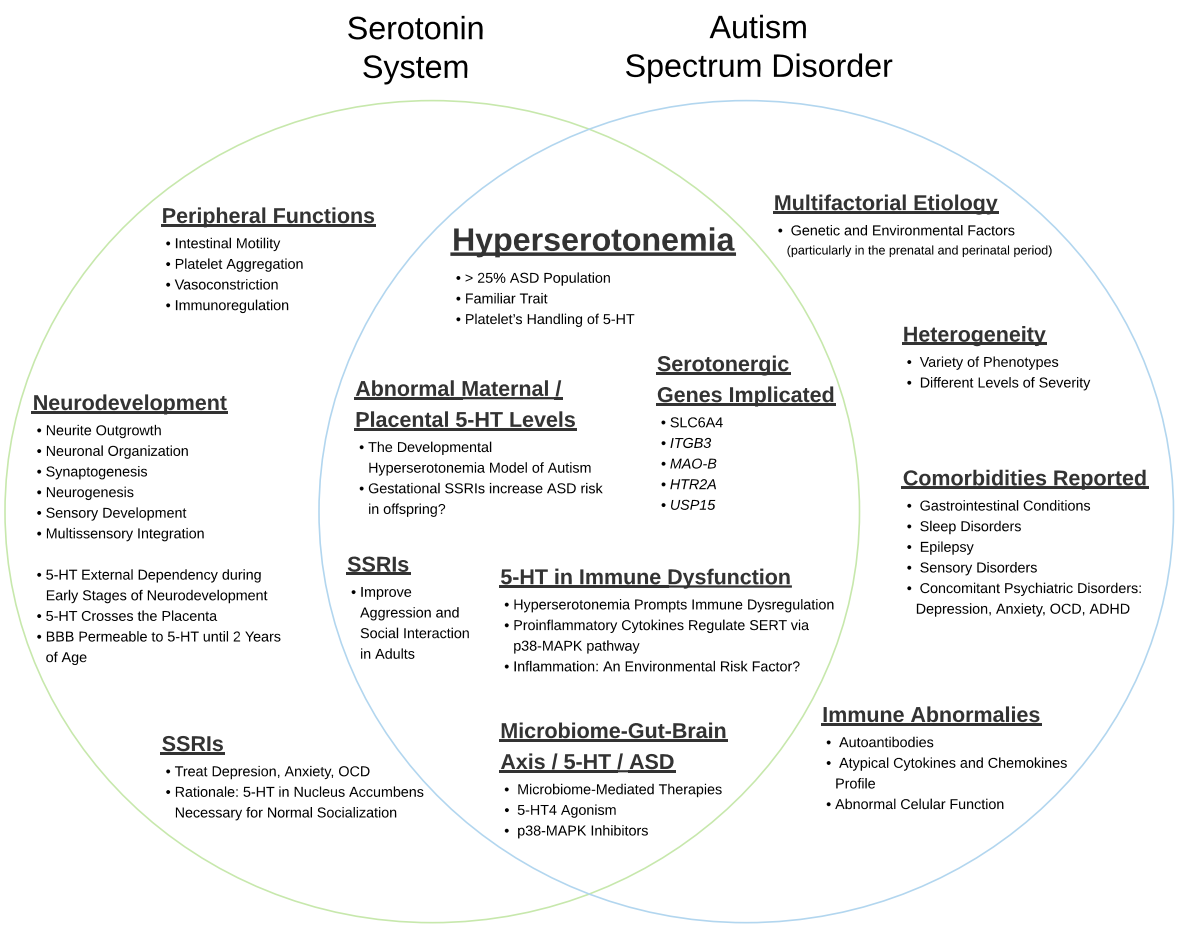

Figure1

The Serotonin System and ASD - Venn Diagram

Considerations and functions of the serotonin system, especially concerning the neurodevelopment period, are displayed in the circle on the left. ASD research findings or clinical aspects are presented in the circle on the right. The overlapping area demonstrates the collected evidence regarding the serotonin system involvement and dysfunction in ASD across the reviewed literature.

Adapted from (Muller et al., 2016).

Table1

Serotonin Measurements in ASD Patients

n – Number; 5-HT – Serotonin; *SD – Standard Deviation; WB –Whole-Blood; PRP –Platelet-Rich Plasma; PPP – Platelet-Poor Plasma; PLT – Platelets; FL – Fluorometric; HPLC – High-Pressure Liquid Chromatography*

Reference		ASD Population (n)	Mean 5-HT Levels ± SD / (Range) Quantification Method | Biological Sample	Main Outcomes / Conclusions
Hanley, Stahl, & Freedman, 1977		n= 27	134.5±56.9 ng/mL FL | WB	• Hyperserotonemia; • Mean WB5-HT is significantly elevated in 30% of ASD population.
Anderson et al., 1987		n= 21	205 ± 16 ng/mL HPLC | WB	• Hyperserotonemia; • ASD subjects show a 51% increase in 5-HT blood levels compared to normal subjects, while mean whole blood tryptophan levels and platelet counts are similar between groups.
Minderaa, Anderson, Volkmar, Akkerhuis, & Cohen, 1987		n= 14	163±86.3 ng/mL HPLC | WB	• Hyperserotonemia; • Elevated WB5-HT levels in ASD might be caused by increased 5-HT gut production.
Abramson et al., 1989		n= 57	399±210 ng/mL FL | WB	• Hyperserotonemia; • Elevated 5-HT levels appear to segregate in families.
Cook et al., 1990		n= 16	266±122 ng/mL HPLC | WB	• Hyperserotonemia; • The trait of hyperserotonemia and WB5-HT levels are familial.
Leventhal, Cook, Morford, Ravitz, & Freedman, 1990		n= 39	288±144 ng/mL HPLC | WB	• Hyperserotonemia; • Points out the familial relationship of hyperserotonemia within families with autistic children.
Perry, Cook, Leventhal, Wainwright, & Freedman, 1991		n= 11	247±94 ng/mL HPLC | WB	• Hyperserotonemia; • Genetic (paternal-filial) factors may play a role in 5-HT$_2$ binding sites binding sites expression in platelets.
McBride et al., 1998	Prepubertal	Black (n= 18)	323±93 ng/mL HPLC | WB	• Hyperserotonemia; • Hyperserotonemia is not found in mentally retarded or cognitively impaired individuals without autistic features. The importance of matching for age and ethnicity is underscored.
		Hispanic (n=13)	325±121 ng/mL HPLC | WB	
		Caucasian (n=27)	245±78 ng/mL HPLC | WB	
	Postpubertal	Black (n= 7)	198±39 ng/mL HPLC | WB	
		Hispanic (n=0)	-	
		Caucasian (n=12)	187±84 ng/mL HPLC | WB	

Reference	ASD Population (n)	Mean 5-HT Levels ± SD / (Range) Quantification Method | Biological Sample	Main Outcomes / Conclusions
Yang, Liu, Sang, Zhu, & Du, 2015	n= 35	158.96 (67.44–336.27) ng/mL HPLC | WB	• Hyperserotonemia; • The combination of 5-HT and IL-6 produced the best sensitivity and specificity for ASD diagnosis.
Montgomery et al., 2018	n= 181	260.3±110.3 ng/mL HPLC | WB	• Hyperserotonemia; • Maternal 5-HT levels negatively correlate with cognitive abilities in ASD probands.
Coutinho et al., 2004	n= 105	304±207 ng/10_9 PLT HPLC | PRP	• Hyperserotonemia; • SLC6A4 gene determines 5-HT blood levels.
Mulder et al., 2004	n= 81	825±274 ng/10_9 PLT HPLC | PRP	• Hyperserotonemia; • Increased 5-HT levels are present in ASD patients, but not in patients with mental retardation.
Hranilovic et al., 2007	n= 9	534 ± 217 ng/10_9 PLT FL | PRP	• Hyperserotonemia; • 5HT concentrations are negatively related to verbal abilities in adult ASD subjects.
Jaiswal, Guhathakurta, et al., 2015	n= 89	17.06±3.63 nmol/10^9 PLT HPLC | PRP	• Hyperserotonemia; • SLC6A4 markers have specific genetic effect on individual ASD behavioral attributes and it might be through 5-HT content modulation.
Spivak et al., 2004	n= 10	3.2±1.9 ng/mL HPLC | PPP	• Hyposerotonemia; • Adults with ASD have lower PPP 5-HT levels, which inversely correlate with Overt Aggression Score (OAS).
Anderson, Hertzig, & McBride, 2012	n= 18	0.15±0.94 ng/mL HPLC | PPP	• Normoserotonemia; • PPP 5-HT levels are not increased in ASD, suggesting that the hyperserotonemia of autism results from the platelet's handling of 5-HT and not from increased exposure of the platelet to 5-HT.

ANEXO

RESEARCH IN AUTISM SPECTRUM DISORDERS

AUTHOR INFORMATION PACK

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DESCRIPTION

Research in Autism Spectrum Disorders (RASD) publishes high quality empirical articles and reviews that contribute to a better understanding of Autism Spectrum Disorders (ASD) at all levels of description; genetic, neurobiological, cognitive, and behavioral. The primary focus of the journal is to bridge the gap between basic research at these levels, and the practical questions and difficulties that are faced by individuals with ASD and their families, as well as carers, educators and clinicians. In addition, the journal encourages submissions on topics that remain under-researched in the field. We know shamefully little about the causes and consequences of the significant language and general intellectual impairments that characterize half of all individuals with ASD. We know even less about the challenges that women with ASD face and less still about the needs of individuals with ASD as they grow older. Medical and psychological co-morbidities and the complications they bring with them for the diagnosis and treatment of ASD represents another area of relatively little research. At RASD we are committed to promoting high-quality and rigorous research on all of these issues, and we look forward to receiving many excellent submissions.

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Author contributions

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