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The pathogenesis of autism in relation to maternal antibodies: from molecule to connectome

Patogenéza autismu vo vzťahu k materským protilátkam: od molekuly až po konektom

Bakalárska práca

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Prehlásenie

Prehlasujem, že som záverečnú prácu spracovala samostatne a že som uviedla všetky použité informačné zdroje a literatúru. Táto práca ani jej podstatná časť neboli predložené k získaniu iného alebo rovnakého akademického titulu.

V Prahe dňa …….

……..

Lívia Rusková

Poďakovanie

Chcem sa poďakovať môjmu školitelovi prof. MUDr. Přemysl Jiruška, Ph.D. za jeho neskonalú trpezlivosť a všetky cenné rady a pomoc, ktorú mi poskytol.

Abstrakt

Autizmus je neurologická choroba ktorá postihuje prevažne chlapcov. Preukazuje sa anti-sociálnym a stereotypným správaním s nízkou úrovňou empatie. Doposiaľ sa pracovalo hlavne s pôvodom genetickým, no posledné roky sa štúdie začali sústreďovať aj na imunitný systém matky. Táto práca zhŕňa hlavne poznatky o materských autoreaktívnych protilátkach ktoré rozpoznávajú špecifické proteíny dôležité pre vývoj mozgu.

Sústreďuje sa na funkciu jednotlivých proteínov v neurogenezii a ich možné prepojenie s patologickým konektomom autistického mozgu.

Kľúčové slová: autismus, materské protilátky, konektom, vývoj mozku,

Abstract

Autism is a neurological disease that affects predominantly boys. It is characterised by anti-social and stereotypical behaviour with a low level of empathy. Most of the studies have been focusing on the genetic aetiology of this disease, however in the past years research has focused on the role of maternal immune system. This thesis summarizes information predominantly about maternal autoimmune antibodies that recognize specific proteins important in neurogenesis.

It is focusing on the function of these proteins in neurogenesis and their possible correlation with the pathological brain connectome in autism.

Key words: autism, maternal antibodies, connectome, neurodevelopment

List of abbreviations

ASD – Autism spectrum disorder

AU – mothers of children with autism

BCSFB - blood- cerebrospinal fluid barrier

BTBR – mutant strain of mice

CNS – central nervous system

CRMP 1/2 – collapsin response mediator protein

CSF – cerebrospinal fluid

FVB – highly social control strain of mice

GP – control group

LDH – lactate dehydrogenase

MR – mental retardation

PFC – prefrontal cortex

STIP 1 - stress-inducible phosphoprotein 1

SVZ – subventricular zone

VZ – ventricular lumen

WT – wild type

Obsah

Introduction

Autism spectrum disorder (ASD) represents a group of complex neuro-developmental conditions characterised by a set of typical features. These include deficits in social-emotional reciprocity, such as verbal or non-verbal communication or developing and understanding relationships, accompanied with repetitive and restrictive behaviour (American Psychiatric Association,2013). Various aetiologies have been associated with this disorder, ranging from genetic to immune disturbances, suggesting that it has also various subtypes. The effect of maternal immune system has been long discussed with relation to autism, in recent years maternal anti-brain antibodies and their corresponding antigens have been detected (Braunschweig et al., 2008). When it comes to the connectome in ASD, , it has been shown that large-scale (whole-brain) connectivity in ASD is altered. While some studies have found over-connected neural systems (Belmonte et al., 2003) other studies demonstrated deficit in connections between multiple areas in the brain (Just et al., 2004) .

The central goal of this work is to examine plausible relationships between the presence of maternal antibodies against foetal brain proteins during brain development and future alternation of the whole-brain connectome in ASD.

In the following chapters, specific proteins that have been so far associated with reactive maternal antibodies will be described and their possible contribution to the pathological connectome of an autistic brain identified.

Autism spectrum disorder

World Health Organisation groups ASD in the ICD-10 under the name “Pervasive developmental disorders“ along with Rett or Asperger syndrome. Autism can also be classified as “childhood” or “atypical”. The difference between them is the onset time of symptoms and the amount of them presented (World Health Institution, 2016).

First described in 1943, the diagnosis of autism and research of its aetiologies have come a long way. First study was purely observational, where the behaviour of 11 children was described not only thanks to direct observation but also because of detailed journals kept by the parents of these children (Kanner, 1943).

The aetiologies of autism have been discussed for a very long time. It is still unclear as to what the underlying causes are. There have been theories expressed both for genetic (Bailey et al., 1995; Klei et al., 2012; Lichtenstein et al, 2010; Rosenberg et al., 2009) and environmental roots. Under “environmental” there can be understood several causes such as: stress during pregnancy (Beversdorf et al., 2005; Ronald et al., 2011), advancing parental age (Croen et al. , 2002; Lauritsen et al., 2005), or gestational diabetes mellitus (P. Krakowiak et al., 2012; Nahum Sacks et al., 2016). It is likely that ASD rises after an interaction of both genetic and environmental factors during neurogenesis.

Brain connectivity in autism

The human brain must work with huge amounts of information constantly. Therefore, it has developed a system, in which its different areas collaborate effectively to give rise to our perception and interaction with the world. The word “connectivity” however, is very broad. There can be several types recognized, such as: local connectivity based on neural communication or long-range connectivity between different parts of the brain. There is also a significant difference between physical connectivity (synapses) and computational connectivity – the information transfer.

We can also come across functional vs. effective connectivity, where functional is basically statistical dependence among neurophysiological events and effective connectivity is what is trying to explain it.

The notion of looking at brain connectivity in patients with autism isn´t something novel. Since the development of various brain-imaging techniques (especially functional magnetic resonance imaging and diffuse tensor imaging), the number of studies has abruptly increased. The results have been inconsistent, but largely agree that patients with autism have a high local connectivity and low long-range one (reviewed in Belmonte, 2004). It is believed that the decreased long-range connectivity limits effective information integration between multiple brain areas of both hemispheres which is essential for higher cognitive functions. What are the exact mechanisms responsible for abnormal axonal connectivity remains an enigma. We can speculate that abnormal connectivity may be a consequence of a pathological process that interfere with axonal growth, sprouting and pruning during brain development. The presence of maternal anti-bodies against the fetal brain during pregnancy can represents one of the pathological processes.

Maternal immune system

Role of maternal immune system in pregnancy

Maternal immune system is in a delicate balance all throughout the pregnancy. There are three stages to be considered. First, highly inflammatory stage during which woman can experience fever and nausea, because the blastocyst is being integrated into the uterus lining. Second is the developmental stage that is also regarded to as anti-inflammatory, since the foetus is developing and there must be a balance, in order that it's not rejected by the mother's body but also protected from various pathogens. Last stage, the delivery, is achieved again by renewed inflammation (reviewed in Mor et al., 2010).

It is during the "anti-inflammatory" stage that problems can occur, as this is the stage when the foetus goes through development including the brain. One of the constituents of immune system that aid to passive foetal protection against pathogens are maternal immunoglobulins of the G subclass (IgG antibodies). They get transferred through placenta starting at around week 18 and can persist in the child up to even 3 months post-natal (Garty et al., 1994; Heininger et al., 2006). While most of these IgG antibodies are immunoprotective, also auto-reactive IgG antibodies can transfer along with them through placenta.

Blood-brain barrier

The blood-brain barrier (BBB) is a special interface between the cerebrospinal fluid in circulation and the brain. This term encompasses mainly special properties of central nervous system (CNS) vessels, along with tight-junctions and specific transporters to make sure the brain environment has stable conditions. Another barrier used by the CNS is the blood-cerebrospinal fluid barrier (BCSFB), which separates blood from the CSF though epithelial cells of the choroid plexus. Even though the barriers should be unbreachable to antibodies, there is a growing evidence that during early stages of development they are not yet fully formed and therefore proteins and antibodies can still pass through. Various environmental factors may increase the permeability of BBB as well (reviewed in Saunders et al., 2012).

One of the earliest detections of IgG antibodies in the cerebrospinal fluid was done by Adinolfi et al., in 1976. Along with IgG also higher levels of alpha fetoprotein and albumin have been observed (Adinolfi et al. , 1976).

Maternal immune system dysregulation and autism

The association of "abnormal" maternal immune system and consequent diagnosis of autism in their offspring has been shown before. It has been even reported that only an activated immune response to a pathogen during pregnancy can lead to developmental changes in the foetus. This been shown by studies examining a correlation between a hospital admission during pregnancy and ASD, showing a higher risk of ASD cases among children of mothers that have been hospitalized due to an infection during pregnancy. (Lee et al., 2015; Zerbo et al., 2015)

The association has been shown for example with human influenza virus in pregnant mice, which resulted in highly abnormal behavioural trends in their offspring, such as lower interest in exploration of novel places and objects, or deficits in social interactions (Shi et al. , 2003). Another examples of diseases associated with autism include measles-rubella-mumps (MMR) antibodies (Singh et al, 2002), polyoma virus (Lintas et al., 2010) or Borna disease virus (Lancaster et al., 2007). This however doesn´t indicate that the pathogens as such are responsible for the correlation, rather the immune system response caused by them.

Immune response to virus infection and inflammation consists of an increase in the level of cytokines and chemokines. These are signal molecules in a cascade that runs after immune activation (Borish et al., 2003). Various studies have found some of them being expressed in a developing and adult brain, suggesting their roles in neurodevelopment, ranging from neuronal and glial migration, cell survival to proliferation of neural precursors (reviewed in Stolp, 2013).

Their disrupted levels have been linked to autism in the past. Jones et al. have found significantly higher levels of these molecules in mid-gestational samples of mothers of children with ASD with intellectual disability (Jones et al., 2017), higher levels of chemokines in amniotic fluids from mothers from a Historic Birth Cohort in Denmark have also been reported (Abdallah et al., 2012). Increased levels of various chemokines and cytokines in dried neonatal plasma samples of children later diagnosed with ASD have also been reported. (Ashwood et al., 2011a, 2011b; Krakowiak et al., 2017) These findings suggest that a maternal immune response during pregnancy, may cause a disbalance in the developing brain and result in an abnormal connectome.

Autoimmunity

The correlation of autoimmune diseases in families and their offspring having autism was started by a case study, presenting a child with ASD from a family with a history of autoimmune diseases. (Money et al., 1971) In a study which consisted of 61 ASD patients and 46 controls, it was reported that the mean number of autoimmune diseases was higher in families with autistic offspring, 46% of the ASD patients had two or more members of the family with autoimmune disorders. The risk of autism was increased from 1.9 to 5.5 as the number of family members with autoimmune disorder has increased from 1 to 3 respectively. (Comi et al., 1999)

Increased chance of ASD in families with autoimmune disease has been confirmed by later studies and meta-analysis. Specific autoimmune diseases that have shown a correlation with autism include: eczema/psoriasis, rheumatoid arthritis, hypothyroidism, type 1 diabetes and systemic lupus erythematosus (Atladóttir et al., 2009; S.-W. Chen et al., 2016; Vinet et

al., 2015; Wu et al., 2015). Maternal asthma has also been implicated with ASD in the offspring (Croen et al., 2018). Most studies have however shown none or very weak association with paternal autoimmune disease in contrast to maternal (Keil et al., 2010). This may suggest that the most important link between these diseases and the resulting ASD diagnosis is the pre-natal environment in which neurogenesis occurs, therefore the state of maternal immune system during pregnancy.

Antibodies

Earlier findings

One of the first studies to connect maternal antibodies with autism was done by Warren et al. in 1990. In a sample of 11 children with ASD, their siblings and 20 controls they have found significantly higher reactivity of peripheral maternal blood against lymphocytes of the autistic children. Throughout the years, various studies have found reactive antibodies in the plasma of mothers of autistic children. Some were anti-MBP (myelin basic protein) (Singh et al., 1993), other targeting brain endothelial cells and nuclei (Connolly et al., 1999) while others associated reactive antibodies with regions such as caudate nucleus, cerebral cortex or cerebellum (Singh et al., 2004).

Dalton et al. have shown that a serum of a mother of three children (1 normal, 1 with autism and 1 with severe specific language disorder) had antibodies reactive to rodent Purkinje cells and other neurons. After injecting mice with this serum, they have exhibited differences in exploration and motor coordination compared to mice injected with control serum (Dalton et al., 2003).

Studies expanding on these findings have uncovered reactivity to specific protein bands. In a study of 29 subjects using archived mid-gestational blood specimens on rodent neuronal tissue, bands at 100-kDa in various areas of autistic subjects have been found. Denser bands were also observed at 73-kDa for both autistic children and their non-autistic siblings than in controls (Singer et al., 2006). Part of these findings has been confirmed in a study with a larger sample – 84 autistic children and two control groups - 49 children with mental retardation or developmental delay (MR) and 160 randomly selected children (GP). By Western-blot,

reactivity to band at 39-kDa was predominantly found in children with autism (7%, compared to 0% in MR children and 2% in GP) with simultaneous reactivity to bands at 39kDa and 73kDa (Croen et al., 2008).

Reactivity to 73kDa has also been observed alongside with 37-kDa bands (Braunschweig et al., 2008). Individual reactions to each band have been observed significantly higher in mothers of children with autism (AU). Reactivity to both bands at the same time was observed only in the AU group. These bands – 37, 39 and 73-kDa have either alone or in combination been associated with problems of development in verbal and non-verbal language, sleep/wake cycle disturbances and a delay in neurodevelopment (Piras et al., 2014). They have also correlated 45 and 62-kDa antibodies to autism severity. 45-kDa has been associated with cognitive impairment, while 62-kDa with motor stereotypes. Both correlate with larger head circumference. These findings correlate with findings by Goines et al., in which the 45-kDa antibodies were more associated with the diagnosis of autism, while 62-kDa with broader autism spectrum disorder diagnosis. (Goines et al., 2011) Reactivity to the 37 and 73-kDa antigens have been associated with impaired use of expressive language, as well as an association with higher scores on the Irritability subscale of the ABC was made. (Braunschweig et al., 2012)

Identified Bands	**Association**	**Study**
37-kDa	-impaired use of expressive language -higher scores on the irritability subscale of the ABC	Braunshweig et al. 2008
39-kDa	-problems with development of verbal and non-verbal language -delay in neurodevelopment	Croen et al. 2008 Piras et al. 2014
45-kDa	-cognitive impairment -larger head circumference	Piras et al. 2014 Goines et al. 2011
62-kDa	-motor stereotypes	Piras et al. 2014 Goines et al. 2011
73-kDa	-problems with development of verbal and non-verbal language -delay in neurodevelopment -higher scores on the irritability subscale of the ABC	Braunshweig et al. 2008 Singer et al. 2006 Croen et al. 2008

Table 1 - summary of identified protein bands against which the antibodies were reactive and associations to specific behaviour or deficit

Animal model studies

Several studies have looked at the effect of maternal antibodies using animal models. As mentioned before, in one study, mice injected with a serum from a mother of 3 children, of which one was diagnosed with ASD, exhibited differences in exploration and motor coordination compared to mice injected with control serum (Dalton et al., 2003). Kim and his colleagues (2016) have used a special strain of mice - BTBR T+tf/J (BTBR) which is a good animal model for autism because of their lower social interactions, unusual vocalisation and highly repetitive behaviour. They have been shown to have much higher levels of IgG in serum, brain deposits as well as anti-brain IgG antibodies than other, highly social strain C57BL/6. BTBR mice had higher levels of brain reactive IgG than FVB (highly social control strain) for whole brain, striatum and substantia nigra. Deposited IgG levels in the hippocampus, cerebellum or cortex were also significantly higher in the BTBR mice (Kim et al., 2016) It has also been proven thanks to animal models that even a single low dose of purified brain-reactive IgG antibodies from mothers of children with ASD produced offspring with anxiety-like behaviour and significant impairments in sensory and motor development (Braunschweig et al., 2012).

Offspring of mice that have been injected with purified IgG antibodies during days 13-18 of gestation from mothers of children with ASD have exhibited increased activity compared to control group as adolescents. As adults, they have displayed a deficit in social interactions and a behaviour similar to anxiety. (Singer et al., 2009) Autistic-like stereotypical behaviour in mice has also been observed, after their mothers have been injected with IgG antibodies on day 14. Alternations to social behaviour have also emerged compared to the control group of mice (Camacho et al., 2014).

Another animal model beside mice and rats that has been used for studying maternal antibodies in relation to autism is rhesus monkey. This model is easier to study as primates are closer in behaviour to humans than mice. One study has used purified IgG antibodies from mothers of children with ASD, injecting 4 pregnant rhesus monkeys and another 4 were injected with IgG from mothers of normally developing children. All were injected with the serum on three days during gestation (days 27,41, and 55). The offspring of monkeys

injected with IgG from mothers of children with ASD have exhibited stereotypical and hyperactive behaviour compared to controls (Martin et al., 2008). Another study using rhesus monkeys was carried out by injecting six doses of purified IgG antibodies during first and second trimesters of their pregnancy. The offspring has been observed for duration of 2 subsequent years. What the researchers have found was that mothers of monkeys that have been administered IgG antibodies from mothers of children with ASD have become more protective of their offspring than control groups in the early development. The IgG-ASD primates have exhibited differences in social behaviour and males have had an enlarged brain volume with biggest difference being in the white matter in the frontal lobes. (M. D. Bauman et al., 2013)

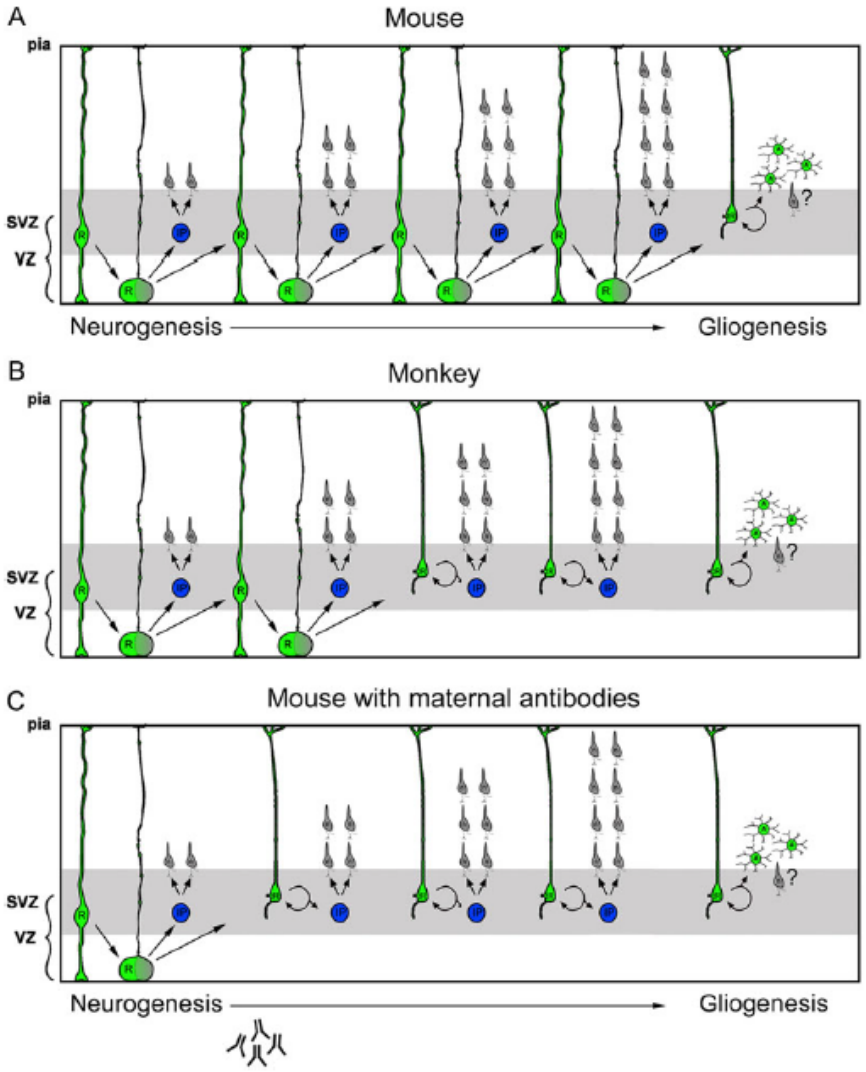

Figure 1 – Scheme showing the impact of maternal antibodies on precursol cell function. (A) – Mouse, Pax6+ RG cells begin moving from the VZ later in neurogenesis (B) – In rhesus monkey they translocate very soon after the start of neurogenesis (C) – the neocortex of prenatal mouse that has been exposed to maternal antibodies, where the RG cells move into the SVZ much sooner than they should (Taken from Martínez-Cerdeño et al., 2016)

A study has been done that has focused solely on specific antigens of maternal antibodies. What they have found was that these antibodies bind to radial glial cells during development. Radial glial cells are primary cortical precursor cells during cortical development that generate neurons and astrocytes. During neurogenesis, redial glial cells generate cortical neurons. They are in the ventricular lumen and throughout the developmental stage move into the subventricular zone of the embryonic neocortex. (Figure 1) When maternal antibodies associate with the radial glial cells, they are released into the SVZ much earlier. This may influence the neuronal migration, which could mean that new cells would arrive prematurely into cortical gray matter and disrupt developing networks. In dorsal neocortex abnormal patterns of proliferation were observed, where the excitatory projection neurons are produced. Same was reported in ganglionic eminence, production site of interneurons. (Martínez-Cerdeño et al., 2016) This abnormal neurogenesis may potentially lead to the altered connectome present in patients with ASD.

Identified Proteins

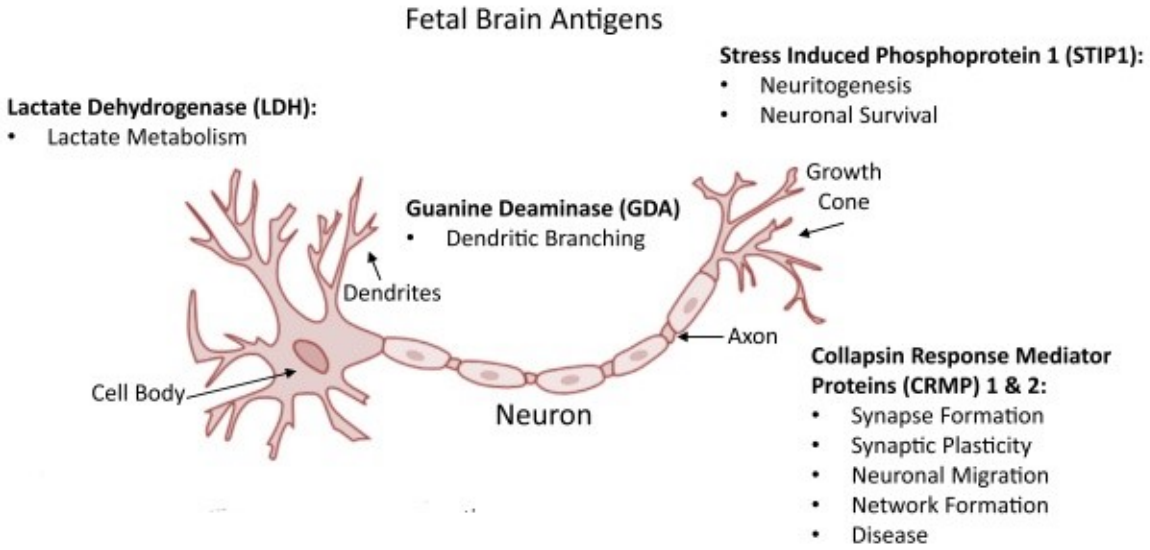

Figure 2- Specific proteins identified by maternal antibodies and their functions. (Taken from: Edmiston et al. 2016)

Thanks to spectrometry peptide sequencing, the proteins corresponding to the identified bands 37-,39-, and 73-kDa were identified as: cypin, stress-induced phosphoprotein 1 (STIP1), collapsin response mediator proteins 1 & 2 (CRMP1 & 2), lactate dehydrogenase A & B (LHD), and Y-box-binding protein (YBX-1) (Braunschweig et al., 2013). Reactivity to a combination of specific antigens was shown in 23% mothers of children with ASD as opposed to 1% in control group.

Mice injected with a series of peptide epitope sequences of LDH, STIP1 and CRMP1 have demonstrated stereotypical self-grooming behaviour, avoiding social interactions with failure at reciprocating juvenile play with peers. (M. Bauman et al., 2018) Another study focusing solely on plasma samples that contained antibodies specific for 37-kDa (LDH) and 73-kDa (STIP1 and CRMP1) has demonstrated an enlargement of brain in size and weight along with higher cortical neuron volume in mice exposed to the sample with reactive antibodies compared to control group (Camacho et al., 2014).

Cypin

Cypin, also known as guanine deaminase, is involved in purine catabolism, where it catalyses hydrolytic deamination of guanine into xanthine (Hitchings et al., 1944). Apart from metabolic reactions it has been shown that cypin is an important protein in neurogenesis. It promotes microtubule assembly and regulates dendrite patterning in hippocampal neurons (Akum et al., 2004). This has been shown by knock-out model in which cypin mRNA was prevented from maturation using mutated U1 small nuclear RNA (snRNA). The neurons that had this mutant snRNA indicated small or non-existent levels of cypin and fewer dendrites than normal.

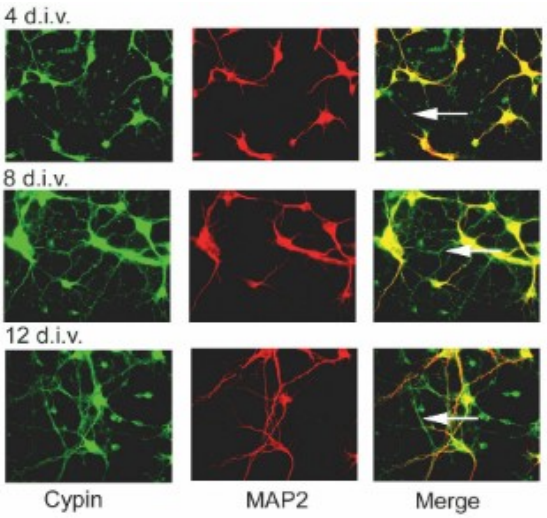

Figure 3- Expression of cypin in developing hippocampal neurons. Cypin is expressed as early as 4 days and continues to be until the neurons mature. It is also expressed in dendrites (MAP2) and axons (white arrows in Merge). (Taken from: Akum et al. 2004)

Another animal study has shown that single substitution mutations that have disrupted the enzymatic activity in rats, have rendered cypin unable to promote dendrite branching in hippocampal neurons. (Fernández et al., 2007)

Cypin is also known to interact with a postsynaptic density 95 (PSD-95/SAP-90) protein. PSD-95 is a membrane associated guanylate kinase, playing an important role in the structural organisation of glutamate receptors and their associated signalling networks at the excitatory synapses. Cypin is also located in axons and several nerve terminals and increases dendrite branching by negatively regulating PSD-95 and promoting microtubule polymerization (Firestein et al., 1999) Another protein interacting with cypin is snapin, binding to CRMP homology domain of cypin, which has been identified to be the domain responsible for promoting microtubule assembly (Akum et al., 2004) and competes with tubulin heterodimer that binds to cypin (Chen et al., 2005). If cypin expression is inhibited, there is a decrease in dendrite number of hippocampal neurons. (Chen et al., 2007)

CRMP1/2

The expression pattern of CRMP1 is like that of CRMP2 in the embryonic and postnatal cerebral cortex (Wang & Strittmatter 1996). Recent findings suggest that they both mediate Sema3A signalling, which regulates dendritic spine maturation, however through distinct signalling pathways (Makihara et al., 2016). Both have been shown to be involved in axonal guidance and synergic control of dendritic projection. (Yamashita et al., 2012)

A study done on CRMP2 gene-deficient ($crmp2^{-/}$) mice has shown that these mice had irregular development of dendritic spines in cortical neurons and density of dendritic spines in the cortical layer V pyramidal neurons was reduced. This protein is also responsible for the formation and maturation of dendritic spines along with the process of dendritic spine plasticity. (Zhang et al., 2018)

CRMP2 has also been shown to have a synergistic relationship with CRMP4 in regulating dendritic development and having an important role in proper bifurcation of apical dendrite of CA1 pyramidal neurons. (Niisato et al., 2013) By suppressing CRMP2 expression an accumulation of multipolar cells and suppressed neurite outgrowth can be seen. (Sun et al.,

2010) This suggests that CRMP2 is needed for transition from multipolar to bipolar in directional neuronal migration as well as neurite outgrowth.

Knock-out CRMP1 mice had shown hyperactivity along with impaired emotional and behavioural responses. (Yamashita et al., 2013) This protein has also been associated with regulating of neuronal migration in the cerebral cortex through Reelin signalling (Yamashita et al., 2006).

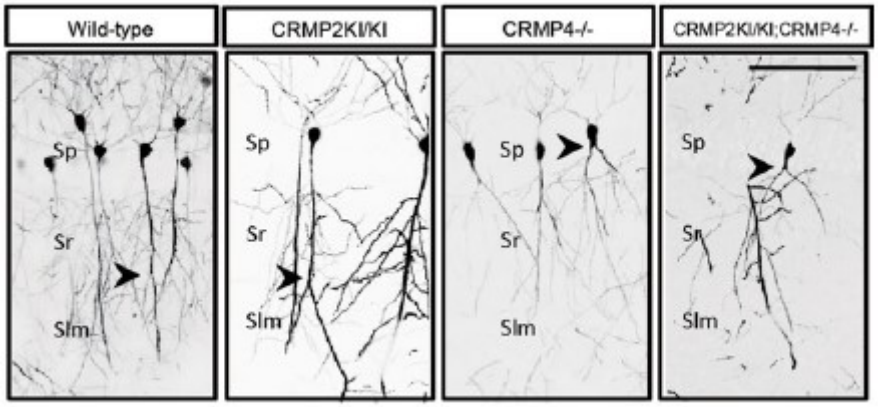

Figure 4 – This image shows the morphology of CA1 pyramidal neurons. It is evident that proper phosphorylation of CRMP2 is vital for proper bifurcation of these neurons and that together with CRMP4 they regulate dendritic development. (Taken from Niisato et al. 2013)

STIP1

STIP1 or also known as stress-inducible phosphoprotein is a co-chaperone of heat shock proteins hsp70 and hsp90 in intracellular environment (Chen et al., 1996; Picard, 2002). Extracellular STIP1 works as a ligand to prion protein and together they have an effect on neuroprotection and produce signals that rescue cells from apoptosis (Chiarini et al., 2002; Zanata et al., 2002). Together they also have an effect on both neuritogenesis and survival of hippocampal neurons (Lopes et al., 2005), astrocyte development (Arantes et al., 2009) and memory formation (Coitinho et al., 2007).

An animal study has shown that an increase in STIP1 shows no major phenotype but mice that had reduced STIP1 levels presented attentional deficits and hyperactivity (Beraldo et al., 2015). In other tasks however they have shown normal performance.

A study done by Ariza and his colleagues has demonstrated that administrating antibodies of mothers of children with ASD resulted in decrease of the number of spines in infragranular layers of frontal and occipital cortex (Ariza et al., 2017). The findings of these studies correlate with functions of STIP1 that have been previously identified. This study was however limited to the fact that the antibody administration was done only once, resulting in changes in layer V in frontal cortex and layer VI in the occipital lobe. Since human foetus has the antibodies throughout the whole time, the changes may be present in all layers.

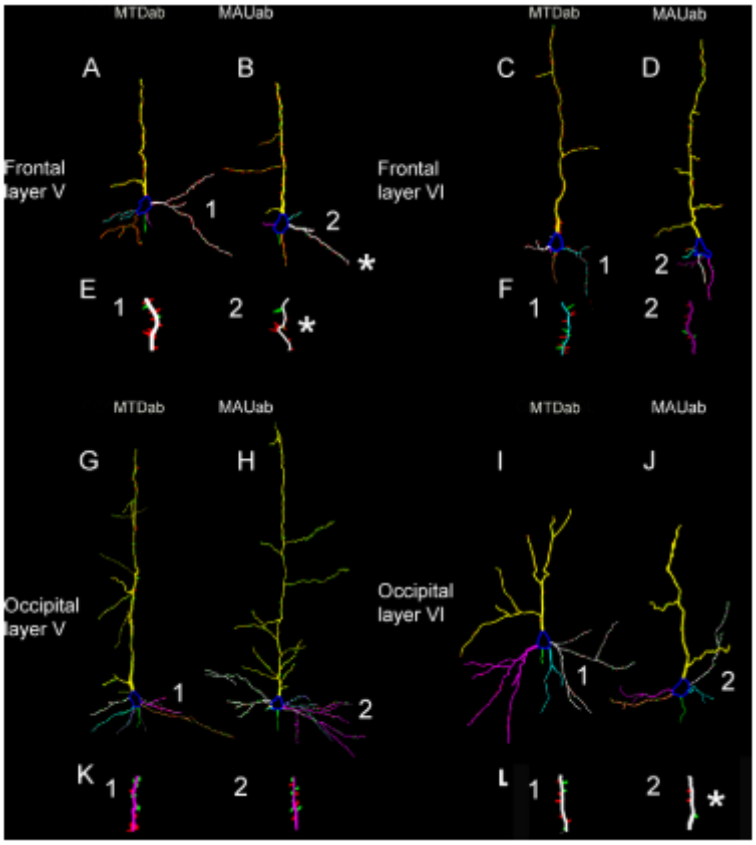

Figure 5- Reconstruction of representative pyramidal neurons in infragranular layers of the cortex of mice treated with normal IgG antibodies and with maternal autoimmune antibodies. A-F: Frontal cortex, G-L: Occipital cortex. Apical dendrites are in yellow, axonal initial segments in green and basal dendrites in other colors. Asterics show basal dendrites with singificantly less mature spines. (Taken from: Akum et al., 2017)

LDH

The enzyme lactate dehydrogenase can be found in the foetal brains of rodents, in mitochondria, where it functions in cellular metabolism (Hashimoto et al., 2008). Mitochondrial dysfunction has been linked to ASD by several studies (Frye et al., 2011; Goh et al., 2014; Weissman et al., 2008). A significant increase in LDH and pyruvate has been reported in children with ASD compared to a control group in the study (Khemakhem et al., 2017). This fits into reported abnormal energy metabolism in Saudi ASD patients, which have shown increased plasmatic levels of lactate and lactate oxidase (El-Ansary et al., 2010). This has also been shown in an animal study, where lower pH associated with higher lactate levels has been reported (Hagihara et al., 2018).

Even though LDH doesn´t have a part in neurogenesis, it is an important factor of cellular metabolism and therefore can contribute to pathological connectome in ASD by contributing to mitochondrial dysfunction.

Networks (connectome)

Connectivity is a major organizing principle of the nervous system and conceptualizing the brain as a network (the 'connectome') represents fundamental and innovative framework for understanding clinical brain disorders (Bullmore et al., 2009). Healthy brain is characterized by high efficiency of information transfer between brain regions at low connection cost in such a way that it optimizes balance between local functional segregation and global integration. This type of connectivity yields high complexity dynamics and maintains high processing power. Within human connectome, specific regions are characterized by very high connectivity and large information flow with other areas and therefore they are defined as network hubs.

In human whole-brain network, hub regions play central role in the global integration of information processing and they are located mainly within parietal and (pre-)frontal lobes. Due to their central role within the human brain network and functional significance, they, however, represent brain's Achilles heel. Hub damage will have a disproportionate impact

on the network's global efficiency of information processing; and thus be more likely to result to clinical symptoms such as impairment of cognitive functions, that depend on integrative network processes (Honey et al., 2008) Hub damage was demonstrated in integrative disorders such as Alzheimer's disease and schizophrenia and recently it has been postulated that damage of hubs may represent common mechanism crucial for development of any type of brain disorder (Crossley et al., 2014).

Connectivity in ASD has been discussed for a long time. Some people have argued that there is a surfeit of connectivity (Rubenstein et al., 2003), while other studies have shown examples of underconnectivity (Jakab et al., 2013). It seems as though both sides of the argument are right, high-local connectivity with a lot of cross-talk has emerged from abnormal neuronal and synaptic development and thereby causing hyper-arousal and potentially causing a weak central coherence – decreased long-range connectivity (Belmonte et al., 2003).

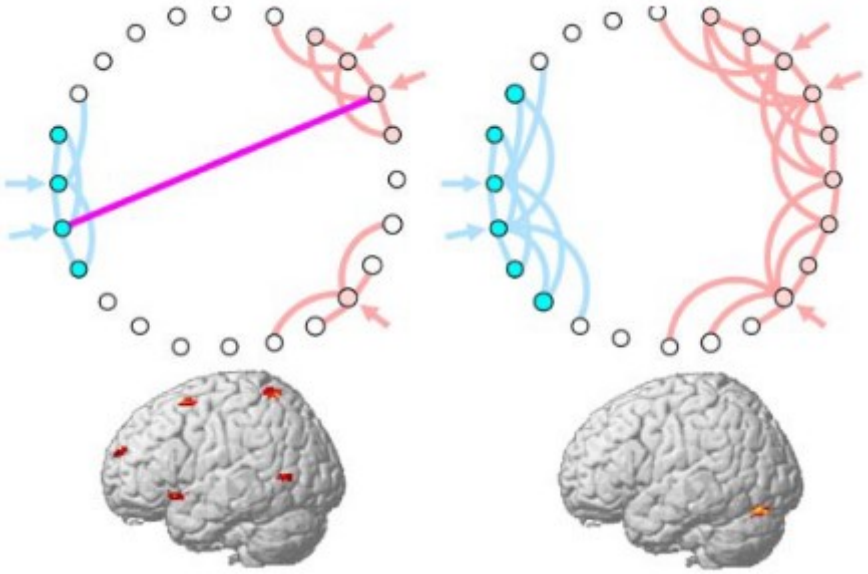

Figure 6 - Network on the left is of a healthy brain, a combination of strong-local connectivity along with selective long-range one. Inputs (two arrows) are easily differentiated from noise (single arrow) and can be easily linked across regions. The network on the left is of an ASD patient, local-connections are too strong and not efficiently developed and therefore a long-distance connection could´t be established. (Taken from: Belmonte 2004)

Decreased functional connectivity has also been reported between praecuneus and medial prefrontal cortex and anterior cingulate cortex. These are part of default mode network, which is active during resting state. Correlation of the magnitude of connectivity and the severity of social and communication deficits has been observed as well (Assaf et al., 2010). Hong et al. have also observed atypical connectome hierarchy, suggesting the inability of the brain in ASD patients to switching from hubs into long-range network communication. Diminished segregation of areas responsible for facial recognition and language as well as self-referential process has been reported. (Hong et al., 2019)

All these processes tend to be highly impaired in ASD patients, suggesting that faulty differentiation of neurons and their reduced branching may be one of the reasons behind this.

Hippocampus and amygdala

Abnormal structure of the hippocampus and amygdala have been implicated with ASD for a long time, as abnormal pattern of hippocampal development (Schumann, 2004) and its enlargement along with amygdala in young children and adolescents (Groen et al., 2010; Schumann et al., 2009). However, some studies report an enlargement of hippocampus but not of amygdala. (Maier et al., 2015) Reduced complexity of dendritic branching of CA1 and CA4 neurons was found in the hippocampus of autistic brains, CA4 neurons were smaller in children with ASD (Raymond et al., 1995).

The role of CA2 neurons has also been shown in a study done by Hitti et al. by developing a genetically engineered mouse which has been shown to have these neurons altered. This alternation has caused loss of social memory, indicating that these neurons have a role in sociocoginitive memory processing which has also one of the areas ASD patients differ to controls. (Hitti et al., 2014) Alternation of the shape of hippocampus in young children has been observed, consistent with inward deformation of subiculum. (Dager et al., 2007)

In memory retrieval, reduced whole brain connectivity between important hubs as hippocampus and fronto-parietal control region has been observed. This correlates with the reduced success of memory retrieval in ASD patients (Cooper et al., 2017).

Dendrite and spine modifications

Proper dendrite morphology is crucial for normal function of the nervous system. They are the most important elements of neurons that receive information. (Vetter, Roth, & Häusser, 2001) They form during development with at first slow dendrite growth, rapid period of dendritic extension and then a period of stabilization of dendritic arbour (Williams et al., 2004). Various dendrite number abnormalities have been reported in patients with ASD (Hutsler et al., 2010; Mukaetova-Ladinska et al., 2004), which correlates with the fact that cypin and CRMP have functions that modify it and therefore they can be associated with this pathological observation.

Reduced dendritic complexity has been observed in mutant mice strain C58/J, which has autistic-like behaviour. The number, size of dendritic processes and lengths of dendrites is lower in C58/J than in WT mice. These differences were reported in the prefrontal cortex and hippocampal pyramidal neurons, in different layers. (Barón-Mendoza et al., 2019)

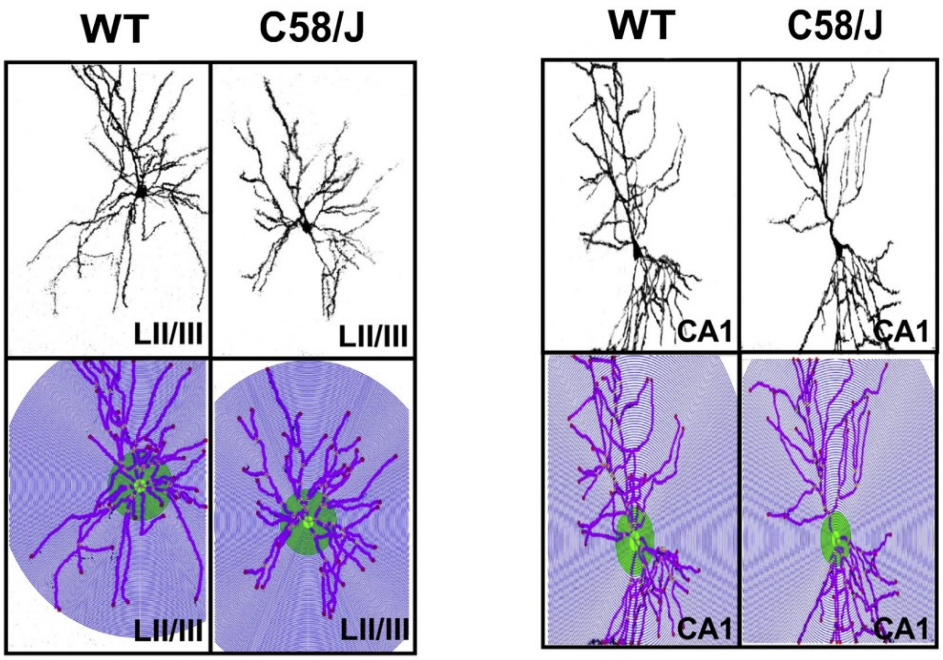

Figure 7 – On the right is ramification pattern of pyramidal neurons in PFC, on the right in the hippocampus. C58/J is a mutant strain of mice with autistic-like behaviour. What can be observed is that the dendrites in this strain are shorter in the PFC and less branched in the hippocampus. (Taken from Barón-Mendoza et al., 2019)

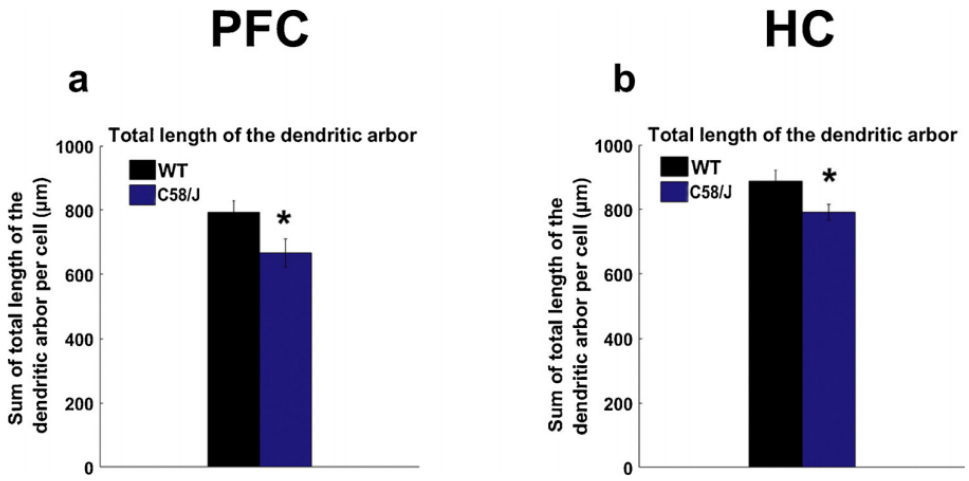

Figure 8 – These graph show the total length of dendritic arbor in both PFC and HC. The lengths in the autistic-like mutant mice are significantly lower than in WT. (Taken from Barón-Mendoza et al., 2019)

Protein	Function	Possible pathology
Cypin	-Dendrite patterning (Akum et al, 2004) -Ligand of PSD-95 at excitatory synapses (Firestein 1999)	-abnormalities in dendrite number (Hutsler et al., 2010; Mukaetova-Ladinska et al., 2004)
CRMP 1/2	-Dendritic spine maturation (Makihara et al. 2016) -proper bifurcation of apical dendrite of CA1 pyramidal neurons (Niisato et al., 2013) -Regulation of neuronal migration (Yamashita et al.,2006)	-abnormalities in dendrite number (Hutsler et al., 2010; Mukaetova-Ladinska et al., 2004) -Reduced complexity of dendritic branching of CA1 in the hippocampus (Raymond et al., 1995) -Premature migration of RG cells from VZ to VZP (Martínez-Cerdeño et al., 2016)
STIP1	-survival of hippocampal neurons (Lopes et al.,2003)	-abnormal pattern of hippocampal development (Schumann, 2004)
LDH	-cellular metabolism (Hashimoto et al., 2008)	-mitochondrial dysfunction in ASD (Frye et al., 2011; Goh et al., 2014; Weissman et al., 2008)

Table 2- summary of functions of proteins that have been associated with MAU ASD and possible pathologies in the ASD connectome

Conclusion

The goal main of this thesis was review current literature about the role of maternal autoantibodies in the pathogenesis of ASD. The literature demonstrates close relationship between the presence of inflammation and auto-antibodies against the foetal brain during the pregnancy and ASD. The evidence suggest that pathological antibodies often target proteins that physiologically play a crucial role in brain development. It involves proteins that are involved in the regulation of proliferation of neuronal precursors, neuronal migration, growth of axons and dendrites. The perturbation of these process can lead to the structural and morphological abnormalities not only at the cellular level, but it can affect neuronal circuit organization at the microscopic, mesoscopic or even at the whole brain level. Modern imaging techniques especially diffuse tensor imaging have demonstrated substantial alteration of the connectivity in children with ASD which affects both local (regional) circuitry and the whole-brain connectome. It seems plausible that interference with axonal growth during brain development and widespread presence of auto-antibodies can substantially impact not only local axonal growth but also long-range axonal connections. The magnetic resonance imaging techniques are being continuously developed and they provide better spatial resolution. Therefore, future experimental work in ASD models which will combine approaches to connectivity at multiple spatial scales can address these yet unresolved questions about the causal role of early exposure to maternal antibodies in the alteration of the whole brain connectome in ASD.

Specific peptides to which maternal antibodies bind have been now identified for LDH, Cypin, CRMP1/2. (Edmiston et al., 2018) This brings a possibility to recognize these antibodies circulating maternal plasma even before pregnancy – to assess the risks. Given the heterogeneity of ASD subtypes, I believe it´s important to be able to be as specific as possible when it comes to its aetiologies. It has been shown that different possible causes have been associated with various behavioural changes, so I believe working on non-human primate animal models with specific proteins would be the best move forward, to assess their exact mechanism of altering brain development.

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