Autism: An Unsolved Mystery?

A project submitted
by
Sheikh Sadia Islam
ID: 14146011
Session: Spring 2014
to
The Department of Pharmacy
in partial fulfillment of requirement of
Bachelor of Pharmacy (Hons.)

Dhaka, Bangladesh
September, 2018

Dedicated to my beloved family and respected teachers who supported and guided me through thick and thin and assisted me to achieve my goals.

Certificate Statement

This is to certify that the project titled “Autism: An Unsolved Mystery?” is submitted for the partial fulfillment of the requirement fordegree of Bachelor of Pharmacy from Department of Pharmacy, BRAC Universitycomprises of my own work under the supervision of **Dr. Sharmind Neelotpol**, AssociateProfessor of Department of Pharmacy, BRAC University and appropriate credit is givenwhen I have used the language, ideas and writing of another.

Signed

_____

Countersigned by the supervisor

_____

Acknowledgement

Firstly, I am grateful to almighty Allah for making me able to choose this field and study Pharmacy. Without His blessings, I would not be able to continue this project paper and submit it for passing my Bachelor degree in Pharmacy.

Foremost, I would like to express my deepest and sincere gratitude to my project supervisor Dr. Sharmind Neelotpol, Associate Professor, Department of Pharmacy, BRAC University. Without her helpful guidance, sincere encouragement, motivational directions and mentoring capacity I would not be able to carry out my project work.

After that, I would like to sincerely forward my regards and gratitude to Dr. Eva Rahman Kabir, Professor and Chairperson, Department of Pharmacy, BRAC University for her contribution and support to the students and department.

Moreover, I give my gratitude to the Department of Pharmacy, which has enriched me with all the knowledge and technical support. It has also given me the opportunity to run the research in partial fulfillment of the requirement for the degree of Bachelor of Pharmacy.

Lastly, I am also thankful to my family as well as my fellow mates and friends for their cooperation and support to complete my project work.

Abstract

Around the world, the rate of autism has consistently been rising. There are a few ecological factors working along with genetic susceptibilities that are contributing to this ascent. Being an undefined disease, autism shows some stereotype behaviors, for example: lining up toys, spinning objects, or opening and closing drawers or doors and no or less eye contact etc. that differs from person to person. The causes behind these stereotypical behaviors of autistic children are still an unsolved case to the researchers. Therefore, the aim of this study is to identify the probable etiologies of autism. By reviewing related articles, it have been found that some possible causes of autism are neurodevelopmental and environmental responsive genes for example NAGLU, FLAP, CHL1 etc. Some genetic syndrome like fragile X syndrome, tuberous sclerosis, rett syndrome etc. might also be the underlying causes of autism. Besides these causes, maternally derived autoantibodies, maternal infection, maternal antidepressant uses during pregnancy, maternal polycystic ovary syndrome, heavy metal and trace elements exposure, epigenetics etc. can also possibly cause autism. However, the researchers could confidently determine none of these causes to be responsible for autism and therefore the case has been unsolved so far.

Table of Contents

List of Tables

List of Figures

List of Acronyms

AD	Alzheimer's Disease
ADDM	Autism and Developmental Disabilities Monitoring
ADI-R	Autism Diagnostic Interview- Revised
ASD	Autism Spectrum Disorders
CARS	Childhood Autism Rating Scale
DSM	Diagnostic and Statistical Manual of Mental Disorders
GFAP	Glial Fibrillary Acidic Protein
GST	Glutathione-S-Transferase
GWAS	Genome-Wide Association Studies
MAR	Maternal Antibody Related
PCOS	Polycystic Ovary Syndrome
RRB	Restricted and Repetitive Behaviors
RSM	Repetitive Sensory-Motor
TSC	Tuberous Sclerosis Complex

1. Introduction

"Table Talk" is a story about a twelve-year-old boy, which was narrated by Martin Luther king. The boy may have had characteristics of restricted social exchange and imagination. Luther portrayed the boy as a soulless mass of flesh possessed by the devil. If he was living in the modern era, Luther would have best described the young man as having "Autism Spectrum Disorders". The federal definition of ASD as per the United States legal code is as follows: "A child is classified as having autism when the child has a developmental disability that significantly affects verbal and nonverbal communication and social interaction, that is generally evident before age three, and that adversely affects educational performance." (Walter, 2011).

ASD is defined by constant deficit in social communication and social dealings, restricted and repetitive patterns of behavior, interests, or activities (Janet et al., 2016; Tao et al., 2016; Benvenuto et al., 2009). Different organizations and authors have defined autism from their own perspective, though the basic knowledge about autism is almost same to all of them. However, WHO defines ASD as a range of conditions characterized by some degree of impaired social behavior, communication and language, and a narrow range of interests and activities that are both unique to the individual and carried out repetitively (WHO, 2016). Similarly, John Simpson, a young man with Asperger Syndrome living in the United Kingdom expressed his view about of autism at the Birmingham conference, in May 2007. He created his own more positive triad for the autism spectrum which is: "The need for predictability, the need for motivation and an uneven cognitive profile" **(**Autism Awareness Centre, 2017*).*

In the later part of twentieth century, Eugen Bleuler utilized the term autism to portray some schizophrenic symptomatology. Later, autism was recognized as a different issue from schizophrenia in the 1960s.Autism is still considered as an unexplained disorder. Regrettably there has been a radical increment of its frequency in the recent couple of decades **(**Zaky, 2017).Instances of autism were least observed from 1976 to 1979. The figure below (Figure 1.1) shows the frequency rate (per 100,000 children) of autism:

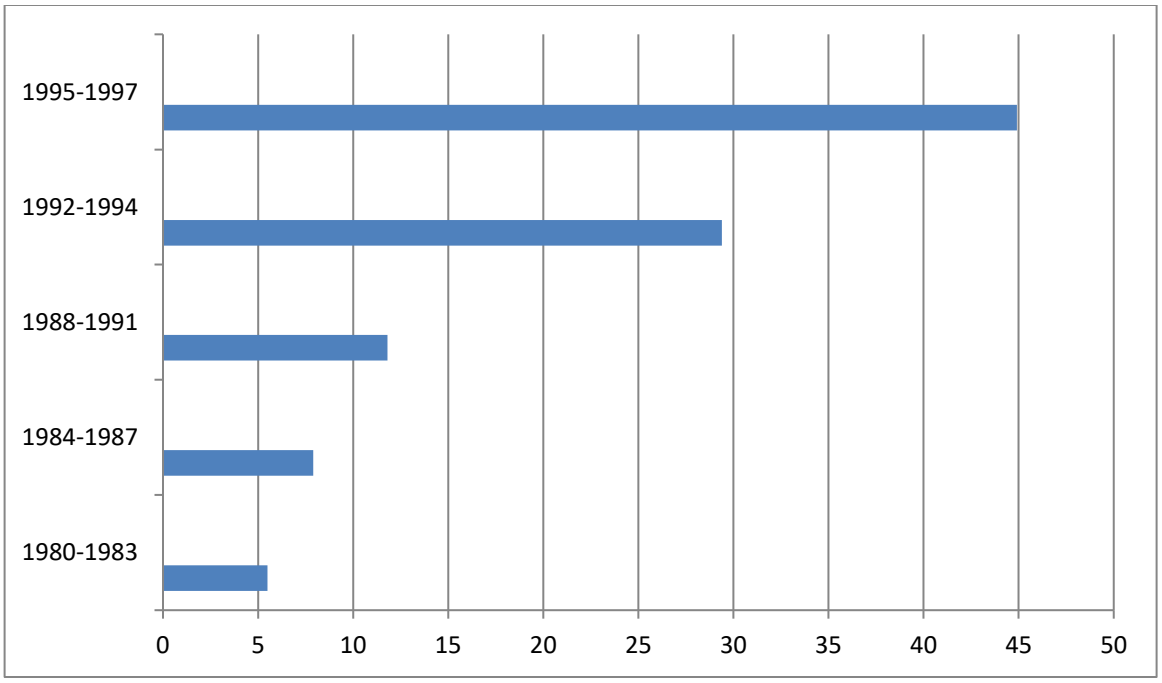

Figure 1.1: The frequency rate of autism from 1983 to 1997

The 8-fold increment in frequency influenced for the most fraction of children conceived after 1987**(**Gustavo**,** 2007).In recent two decades, prevalence of ASD has been expanding severely all over the world. The rate of diagnosis of ASD, multiplies from 4.5 for every 10,000 children in 1943 to 110 for every 10,000in 2009 in western nations **(**Bappaditya et al., 2017).

There are some misconceptions among the common people regarding autism. It is thought that autistic people never make eye contact, preferably isolate themselves from the society and are insensitive and indifferent, thus making it difficult to develop any social relationship. However, the fact says otherwise. In fact, the autistic people do express themselves, but in their own way. They might do eye contact and also try to interact with people, but they have a lack of social abilities to respond effectively. Additionally, they are thought to express their emotional feelings in a manner dissimilar with ordinary people (Walter, 2011).

The onset of autism is usually spotted during the first 3 years of life (Kolvin, 1971). However, there have been case reports of late-onset of autism in eleven, fourteen and

thirty one year old people who had been infected by herpes encephalitis already. Consequently, we can say that autism is not necessarily a developmental disorder in fetal stage (Helen, 2011).Nevertheless, ASD is generally apparent at an early age, typically by the age of one. Symptoms can become noticeable for children who are few months old to children who are three(Walter, 2011). The onset, or time when the disorder is recognized, is usually prior to the third year of the child's life. An autistic person can be of any race, culture, society, economic group or gender, although males are diagnosed more frequently than females. A person with ASD may suffer in conjunction with other conditions like: deafness, attention deficit disorder (ADD), Down syndrome, cognitive disabilities, blindness, cerebral palsy, epilepsy, etc. Due to the unpredictable nature of ASD, experts say there are no two children with autism that shows the same behavior (Walter, 2011).

ASD is a heterogeneous condition with no single pathognomonic highlight or particular diagnostic test. It is now analyzed based on behavioral criteria since its fundamental disease mechanisms remain inadequately clarified. As a result, there are no blood-based analytic tests to identify, or affirmed drugs to treat ASD's usual symptoms (Ozge et al., 2018).Diagnosis of ASD is now performed by testing the patient, who show variation from the acceptable level of behavioral capacities, dialect and intellectual capacities. So far there are two universal frameworks for diagnosing ASD. In 2013 the changes in Diagnostic Statistical Manual (DSM-5) by the American Psychiatric Association incorporated the induction of ASD. There it is portrayed as a single diagnosis and the other analytic sub-groupings were eliminated, for example Autism, Asperger's disorder, and atypical Autism. DSM-5 suggests the utilization of a scope of specifiers featuring the significance of a person's potency and weakness. These significant specifiers may be utilized to depict current symptoms for each of the ASD domain. It also acknowledges that seriousness may differ with time, which is more natural. Specifiers additionally incorporate regardless of whether there is brain disability, speech impedance, other related issue or comorbidities e.g. medicinal, hereditary, mental or behavioral (Kirsty et al., 2013).

Autistic children are often found to have difficulties with social communication, restricted and repetitive behaviors, regression or a phase of stasis, which mainly affect language, eating practice, losing eye contact and difficulties with learning etc. (Kirsty and Ann, 2013).Children with ASD also exhibit perseveration patterns such as rocking back and forth and nail biting. They also show some stereotyped behaviors such as lining up all their toys in a certain way according to size, shape and color. Although not a specific diagnostic criteria, a majority of individuals with ASD also exhibit difficulties with initiating, performing, mirroring, inhibiting and planning motor actions (Breanna et al., 2017; Gustavo, 2007). Notwithstanding the core side effects of ASD, related conditions and practices have also been announced later on (Yongshou et al., 2018). The symptoms of autism also incorporated variations from the norm in tactile preparing (hypo sensitivity or on the other hand extreme sensitivity), gastrointestinal (GI) side effects or even self-damaging practices (Yongshou et al., 2018).Another study (Kolvin, 1971)reveals the criteria by Potter, which were quite similar to the above criteria, such as:

1. A lack of interest in the environment.
2. Disorder of thought, symbolization and attention, perseveration, incoherence and attenuation, occasionally to the extent of mutism.
3. Difficulty in creating emotional bond.
4. Reduction, inflexibility and alteration of affect.
5. Alterations of behavior, with either an increase of motility leading to incessant activity or a diminution of motility leading to complete immobility or bizarre behavior, with a tendency to perseveration or stereotypy (Kolvin, 1971).

Other symptoms include trouble in blending with others, might not have any desire to snuggle or be nestled, almost no eye to eye connection, inert to ordinary instructing techniques, managed odd play, twists objects, unsuitable connections to objects, evident hypersensitivity or hyposensitivity to distress, uneven motor skills etc. (Autism Awareness Centre, 2017).

An autistic child may have delayed response to learning, or may be amazingly superior at other skills. One could show severe physical and cognitive impairments, while another child could have extraordinary memory in science, art, math or memory, but may be deficient in their social skills. Other traits include having the inability to control sentiment, reactions, and behaviors. They may be more sensitive, or they could display a blank look, appearing to be emotionless (Walter, 2011). In the ASD population, it is a common tendency to be sensitive or unusual in reacting to sensory stimulation through taste, touch, smell, hearing or site. Fifteen percent of people with autism undergo synesthesia compared with 1% of the general population. The exceptional and extraordinary phenomena of synesthesia may be distinct as one sensory input being answered to by a different sense. For instance, "seeing" a sound or "hearing" a color. This outstanding ability of autistic savants was described by Luria in 1920.Luria described a young man named Kim Peek with a memory that had "no distinct limits". The man could translate sounds into colorful visual images. Once the sounds were pictured in his mind, the young man could repeat those long series forward or backward with the same ease. In recent times, defect of a corpus callosum and left brain abnormalities was given as a probable explanation for these notable abilities of the well-known autistic savant, Kim Peek (Joseph et al., 2008).

Despite of the fact that autism has been recognized as a different disease apart from other psychological disorders in life since 1960s, its exact cause is still obscure. However, what is known these days without question is that it occurs due to the connection between nature and support. Genetic vulnerability occurs as polygenes prompts numerous organic confusions. Additionally, it supports the negative neuro-pathological impact of numerous ecological risks. However, parenting style (e.g. icebox mother) once guaranteed to be the underlying cause behind the incident of ASD was turned out to be invalid **(**Zaky, 2017). An autistic person with a typical insight remainder can be additionally analyzed as having Asperger disorder or autism (Jiao et al., 2011). Accordingly, it is imperative that the optometrist knows about the idea of autism—especially its history, etiology, treatment, and its connection to vision. Before Kanner, the abnormal behaviors were analyzed as youth schizophrenia (Joseph et al., 2008).

Aim

Therefore, the aim of this study is to explore possible causes of Autism Spectrum Disorders.

Objectives

The research questions addressed in this study are:

1. Does family history have any effect on autism?
2. Do mothers' lifestyles affect autism?
3. Do heavy metals correlate with the onset of autism?
4. Do antipsychotic drugs significantly affect autism?

2. Research Methodology

The aim of the study is addressed by reviewing relevant literatures. The literature search was performed using the Web of knowledge, Pubmed database, NCBI resources, Springer-link, Elsevier, WHO, Bio-Med Centre etc. using the keywords: “Autism”, “Symptoms”, “Etiology of Autism”, “Reasons behind Autism”, “Causes of Autism”, “History of Autism”, “Lead and Autism”, “Heavy metals and Autism”, “Genetics and Autism” and “Parents’ lifestyle and Autism” etc. The review paper discusses the possible etiology of autism.

3. Etiology of Autism

Autism spectrum disorder is a neurodevelopmental disorder of mysterious etiology (Anthony, 2003). Recently the prevalence of ASD has been increased noticeably. A nearly fourfold increase in ASD between 1997–1999 and 2006–2008 was exposed by the Centers for Disease Control and Prevention (CDC) and National Health Interview Survey (NHIS). In USA a 78% increase in ASD prevalence between 2002 and 2008 was disclosed by CDC's Autism and Developmental Disabilities Monitoring (ADDM) Network. Approximately 1in 54 boys and 1 in 252 girls living in the ADDM Network population were recognized as having ASDs. An increase in probable ASD prevalence of 23% was also revealed when the data of 2008 were compared with the data of 2006. The data verify that the projected prevalence of ASDs identified in the ADDM network surveillance populations continues to increase (Yassa, 2014). Figure 3.1 shows the puzzling etiology of ASD.

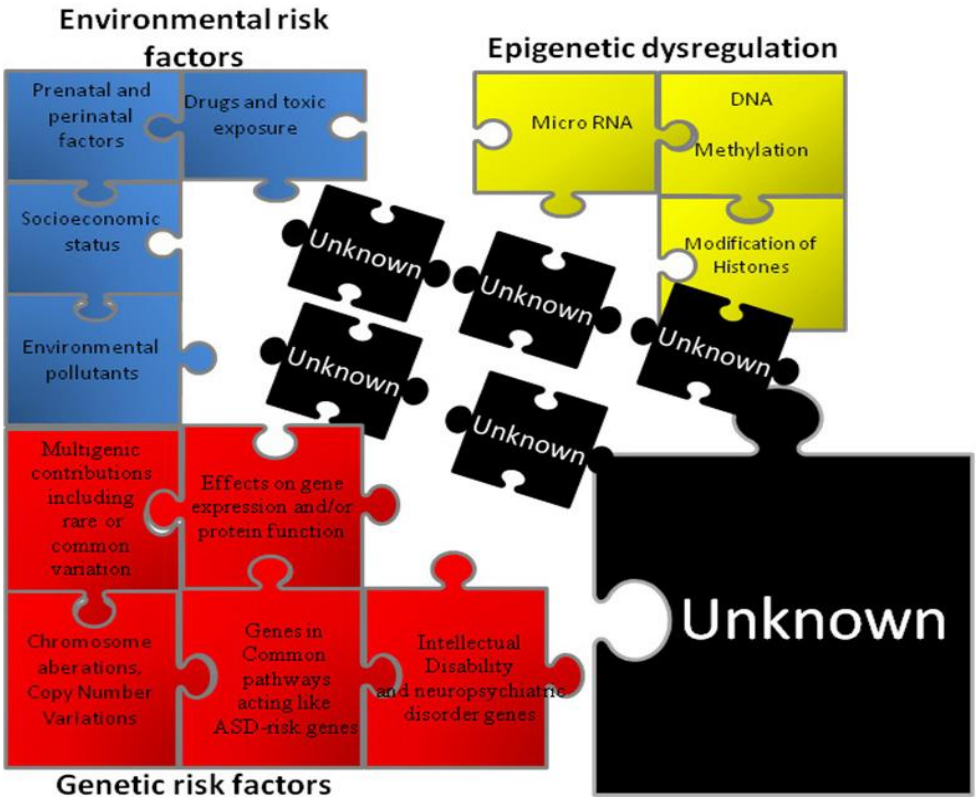

Figure 3.1: The puzzling etiology of ASD (Altevogt et al., 2008).

ASD is a result of prenatal disruption of neurogenesis. It generates an overabundance of cortical neurons and disrupts neuronal circuit formation and function of frontal, temporal and amygdala social and language networks in large-scale. This was theorized after the discovery of enlarged brain volumes in frontal and temporal cortex in many ASD children. The evidences from magnetic resonance imaging (MRI), revealed increased cortical surface area or thickness during premature growth, increased volume of frontal axon tracts, accelerated and atypical head circumference growth during early life, and greater brain weight in ASD.A subset of ASD patients has small brain size, sometimes associated with extremely rare chromosomal defects. A study was conducted on 8,310 toddlers to adults from 44 MRI and 27 head circumference studies. The studies showed significant brain and head circumference overgrowth in ASD compared to controls across ages. The most pronounced brain and head size increases occur at early ages. Researchers assumed that prenatal processes, such as abnormal cell proliferation, might be the cause of early-age brain growth defects in ASD, including both ASD overgrowth and undergrowth. Mechanisms regulating cerebral cell proliferation are well defined, and in humans, this fetal stage occurs in the 1st and 2nd trimesters (Courchesne et al., 2018). However, ASD is assumed to be involved with environmental factors as well as genetic vulnerability (Matthew et al., 2018).

3.1. Environmental Factors

Environmental factors are directly associated to the risk factors for autism.

3.1.1. Aluminium exposure

The continuous exposure of human to the environmental pollutant aluminium has uncertainly been connected to ASD. Researchers have measured the content of aluminium from the brain tissue of autistic people by using transversely heated graphite furnace atomic absorption spectrometry. They also used Aluminium selective fluorescence microscopy to identify aluminium in brain tissue. In the brain tissue of autistic patients the aluminium content remained consistently high. The mean (standard deviation) aluminium content across all 5 individuals for each lobe were 3.82(5.42), 2.30(2.00), 2.79(4.05) and 3.82(5.17) μg/g dry wt. for the occipital, frontal, temporal and parietal lobes respectively. These are the highest values for aluminium in human brain

tissue recorded so far. Five individuals were confirmed to have ASD by autism diagnostic interview-revised (ADI-R). Out of 5 there were 4 males and 1 female, aged 15–50 years old. The percentage of aluminium in these tissues was measured by an established and fully validated method. They studied over a serial brain sections from 10 individuals (3 females and 7 males) who died with a diagnosis of ASD and recorded the presence of aluminium in these tissues (Matthew et al., 2018).

Aluminum (Al) is frequently used as vaccine adjuvant nowadays. It is an established neurotoxin as well as a really powerful immune stimulator. Therefore, Al has the potential to induce neuroimmune disorders (Bjørklund et al., 2018). Another study (Tomljenovic, 2011) shows that: (i) children having the highest ASD prevalence usually have the maximum exposure to Aluminium from vaccines; (ii) the expanding disclosure to Al adjuvants extensively show a relationship with the increase in ASD prevalence in the United States over the last two decades; and (iii) a noteworthy association exists between the total Al administered to playgroup children and the current prevalence of ASD in seven Western countries, particularly at 3–4 months of age. The data from the following table shows the fundamental correlation between Al in vaccines and ASD (Tomljenovic, 2011).

Table: 3.1 Estimated total Al exposure from vaccines (μg/kg bw) per vaccination (Tomljenovic et al, 2011).

	ASD prevalence/10,000	Birth	1 month	2 month	3 month
UK	157	73.5	62.5	109-245	55.7-184
US	110	73.5	0	109-245	0
Canada	65	73.5	0	84-220	0
Australia	62.5	73.5	0	84-220	0
Sweden	53.4	0	0	0	32.1–160.4
Iceland	12.4	0	0	0	32.1–160.4
Finland	12.2	0	0	0	32.1–160.4

Table 3.1 Demonstrates aluminium exposure from vaccines per kg of body weight (bw) in children from seven Western countries for example, UK, US, Canada, Australia, Sweden etc. It was observed that children from UK, US, Australia and Canada have higher disclosure to Al from vaccines than do children from Scandinavian countries where autism prevalence is lower (Tomljenovic et al., 2011).

Aluminium is toxic to living cells and its involvement with inflammatory cells in the vasculature, meninges and central nervous system is uncertain to be mild. In spite of being greatly loaded with aluminium, microglia remains potentially viable for some time. This microglia will predictably be compromised and dysfunctional microglia is considered to be involved in the etiology of ASD. In addition the suggestion from the data that aluminium entry into the brain via immune cells circulating in the blood and lymph is expedited in ASD might begin to explain the earlier posed question of why there was so much aluminium in the brain of a 15 year old boy with an ASD. Therefore,

it can be presumed that presence of aluminium over the safety range might be causing prevalence of ASD (Matthew et al., 2018).

Some sources of aluminium are airborne dust, antiperspirants, immunizations, allergy injections, food, water and antacids for the general population, but food is the single largest contributor of aluminium intake (Yang et.al, 2014).

3.1.2. Fluoride exposure

Fluoride exposure in fetuses, newborns, and small children is also frequent. It usually results from the synthetic fluoridation of water and a remarkable increase in the amount of industrial fluoride based products exposed into the atmosphere. Researchers portrayed noteworthy altered behavior in rats which were exposed neonatally to fluoride in drinking water. The results showed that with prenatal exposure, the males were more affected and females were affected if they were exposed to flouride after adulthood (Mullenix et al., 1995). Fluoride interferes with a number of glycolytic enzymes as well. As a result, it causes a noteworthy suppression of production of cellular energy (Strunecka et al., 2002).

Studies have established that other effects might be the result of a synergistic action of fluoride plus Al3+ that are, with the aluminofluoride complex (ALFx). This complex acts as analogue of phosphate group while activating of G proteins (Strunecka et al., 2002). The AlFx activate G protein and trigger a series of biochemical reactions as well as various modifications in the brain showing different pathological consequences (Strunecka et al., 2007). Fluoride form complex with any pre-existing Al3+ within body fluids and thus generate the AlFx. This could lead to increased intracellular level of Ca2+, G protein regulated systems' acceleration, and sustained activation of receptor functions. It is also shown that AlFx effects learning and behavior. It also provokes a failure of cerebrovascular integrity in investigational animals. Within the brain during fetal development, the accumulation of AlFx could also cause microglial reaction. The reactions could also be expected from the high frequency of reactive oxygen species

(ROS) and lipid peroxidation products (LPP). That could be a major source of priming of microglia and excitotoxicity (Blaylock et al, 2009).

Some symptoms of ASD suggest abnormalities in melatonin physiology and dysfunctions of the pineal gland, such as the hazards with sleeping and the early onset of puberty. Researchers stated that the accumulation of flouride in the pineal gland and excretion of fluoride in higher doses results in less amount of melatonin metabolite in the urine and reach puberty rapidly. In ASD the nocturnal production of melatonin is reported to be reduced. Melatonin is accountable for the regulation of several stages of life, including development as well as aging. However, the production of melatonin by the pineal is regulated by glutamate receptors. The inhibition of melatonin release might be caused by the glutamate activity. Melatonin has been demonstrated to show dominant neutralizing effects that raise the concentration of enzymes which are antioxidant in the brain. Later on it was discovered that children with the ability of the lowest melatonin production had the most neurobehavioral difficulties. The long-term fluoride load has several health effects with a conspicuous resemblance to the ASD. These include hypocalcemia, hypomagnesemia, hypothyroidism, sleep pattern disturbance, and deficiency in IQ (Blaylock et al., 2009).

Fluoride is present virtually in all foods and drinks for example, artificially fluoridated water, naturally fluoridated water, fluoridated salt, fluoridated milk as well as in therapeutic agents (Buzalaf et al., 2011).

3.1.3. Phthalates

Phthalates have been identified to be the other environmental agents that possibly might be associated with the causes of autism. Phthalates are high production synthetic chemicals with widespread exposure to human. They are frequently used in plastics as well as other products consumed by human. Phthalates leak into the atmosphere and expose humans through intake of foods, inhalation etc. There was a significant relationship, found in the comparison of the concentration of phthalates and the prevalence of autism (Wolska et al., 2014). Moreover, Polychlorinated biphenyls (PCBs)

might also be considered as risk factors of autism since exposures to PCBs in prenatal stages have been identified to influence cognitive function. The size of the splenium of the corpus callous is also affected by Prenatal PCB exposure. Usually, the smaller the splenium the larger was the association among PCBs and errors commission. PCBs have become pervasive in both the environments, to which they can be released during such processes as follows:

1. The burning of coal in power stations,
2. The burning of coal and wood for household and community heating,
3. Fortuitous events like fires (e.g. in buildings) etc (Wolska et al., 2014).

In addition, environmental contaminants including PCBs, herbicides, perchlorates, mercury, and COAderivatives (such as resorcinol, phthalates, and anthracenes interfere with thyroid function. The environmental contaminants alone, or in addition to insufficient dietary iodine intake, can affect maternal thyroid function during pregnancy. These outcomes can result in low tri-iodothyronine (T3) levels in the fetal brain during the period of neuronal cell migration (i.e., weeks 8–12 of pregnancy) and may produce morphological brain changes leading to autism (Ratajczak, 2011).

3.1.4. Heavy Metals and Trace Elements Exposure

Studies show that exposure to heavy metals, such as mercury, lead, chromium, cadmium and arsenic during growth have correlation with the causes of autism (Fido et al., 2005; Aisha et al., 2015). In children with autism, the problem usually appears due to decreased excretion rather than high exposure. The half-life of lead, mercury, and other toxic metals in the blood is weeks to months, so those metals quickly leave the blood and build up in tissue and/or bone (Adams et al., 2013). The heavy metals are thought to be as reproductive as well as developmental toxins. They could result in fetal developmental damage, birth defect, neurological deficiency, developmental hindrance, learning disabilities as well as behavioral difficulties (Blaurock-Busch et al., 2011).Another study claimed that environmental toxicants might affect cellular mechanisms in a way that alter brain growth in the fetus. For instance, the redox state—the balance between reducing agents and oxidizing agents in a cell—in developing

neuronal cells may play a role in the cause of autism. Besides, researchers highlighted the immune system and possibilities of having role of immunologic susceptibilities in the development of autism (Altevogt et al., 2008).

3.1.4.1. Lead (Pb) and Mercury (Hg)

In Egypt a study was conducted to find out the correlation between exposure to lead and/or mercury as heavy metals and autistic symptoms (Yassa, 2014).The aim of the study was whether the heavy metals with chelating agents can prevalence the autistic symptoms. From 45 children with autism from Upper Egypt, blood and hair samples were obtained. The patients were chosen from two to ten years. Also, 45 children served as controls in the same age range, after taken an informed consent. They were given a set of question to fill to review the possible etiologies of autism. The samples were investigated strictly for lead and mercury by using atomic absorption and ICP -MS method. Data from the two groups were evaluated, then follow up of the autistic children after treatment with chelating agents were done. The results showed major difference between the groups, the level of mercury and lead in those autistic children were high. In addition, there was decline in the autistic symptoms with the decrease in the lead and mercury level in blood (Yassa, 2014). Lead intoxication can consequence in dysfunction of certain cellular signaling processing, the action potentials in certain nerve cells and various proteins and enzymes (Yorbik et al., 2010).This can be clarified by the deficiencies in methylation of these toxins. That further results in retaining these toxins in their bodies for long period, which provokes the toxic effects as well as emergence of the autistic symptoms. Researchers discovered the decrease in the capability of methylation, which triggers the autistic symptoms. They also addressed that there was metallothionein disorder in those children with autism and suggested that it is an inborn error of metallothionein role; this can clarify the high levels of heavy metals in those children. Additionally, these children with autistic symptoms exposed facts of abnormal thiol metabolism. It also makes clear the significant modification in deposition of heavy metals. So, lead and mercury might be thought as one of the main reasons of autism (Yassa, 2014).

In the environment mercury is released from either natural or anthropogenic sources. Natural sources include topsoil enriched in mercury, volcanoes, geothermal sources, and the re-emission of vegetation, land or water surfaces. Coal combustion, Hg mining, gold smelting, nonferrous smelting, iron steel production, domestic wastes, and cement production are main anthropogenic sources of mercury (Zhao et al., 2018). The table (Table 3.2) describes human tissue mercury levels associated with ASD symptoms severity.

Table: 3.2 Human tissue mercury levels are associated with ASD symptom severity (Kern et al., 2016).

Body Tissue	N Age	Findings
Hair	ASD 18 1–6 y	Increasing hair mercury concentrations significantly correlated with increased ASD severity
Whole blood and RBC	ASD 51 Controls 40 3–15 y	Found a strong association in the degree of severity of autism for all the severity scales with mercury (whole blood and RBC)
Red Blood Cells (RBC)	ASD 30 Controls 30 3–12 y	Levels of mercury GST, and vitamin E were correlated with severity of social and cognitive impairment measures

GST = glutathione-s-transferase, RBC= Red blood cell

In Arab another study (Blaurock-Busch et al., 2011) shows that there was significant difference in the amount of heavy metals present in the hair, nails and urine samples between the control groups and the subjects. The research was performed on 25

participants (22 boys and 3 girls) between the age of 3 and 9 years. They were diagnosed with Autism spectrum disorder (ASD). A control group of 25 age-matched and sex-matched children was selected. They had no history of psychiatric, medical disorders or developmental delay. Compared to the control group, hair and urine mercury and Lead levels were higher in the autistic group, which point toward that past and immediate exposure of mercury and Lead is greater in the autistic group. Consumption of Seafood could be the cause (Blaurock-Busch et al., 2011).

Lead is hardly present in tap water rather; it is present primarily in household plumbing systems containing lead in pipes, solder, fittings or the service connections to residences. Soil is one of the sources of lead to human and most important pathway of its human exposure. Motor vehicle ejections are one source of lead in the atmosphere specifically to the ambient air. Heaviest effluence of ambient air occurs near the highway. Another source of lead contamination of air is incineration of lead containing waste and lead in electric wires as well as recovery processes (Getaneh et al., 2014).

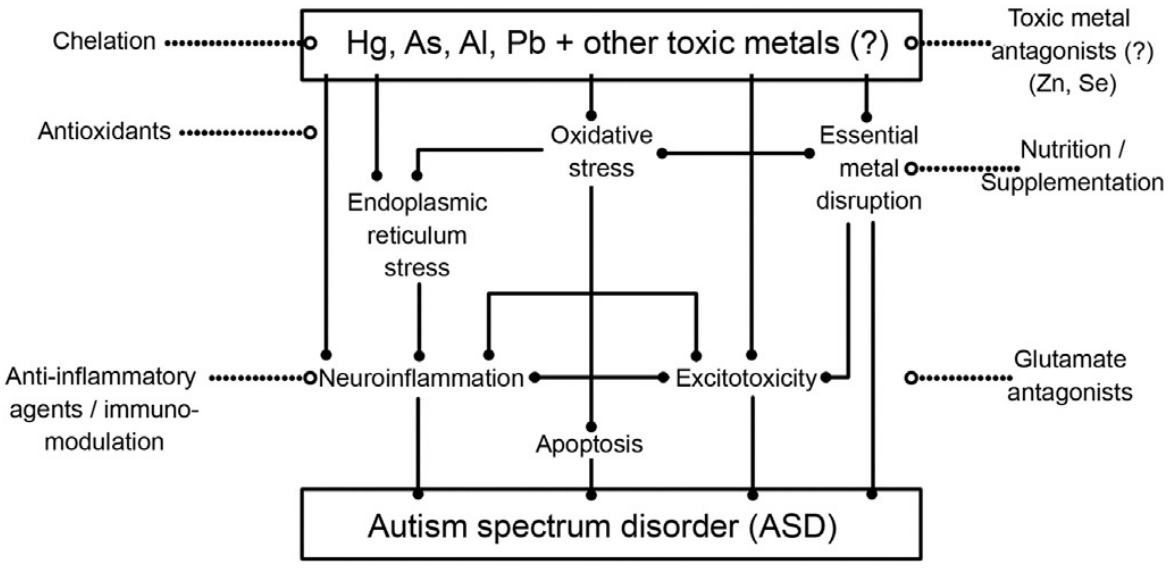

Figure 3.2: The mechanisms of the patho-genetic role of toxic trace metals in autism spectrum disorder (Bjørklund et al., 2018).

Figure 3.2 illustrates the mechanisms of lead, mercury and others heavy metals, that are causing ASD through oxidative stress, essential metal disruption, neuroinflamation and excitotoxicity (Bjørklund et al., 2018).

Researchers (Janet et al., 2016; Currenti, 2010) provide the information about production of numerous auto-antibodies which react with definite brain proteins and brain tissues in autistic children. These auto-antibodies can also act to alter the role of the particular brain tissue. Additionally, studies showed that anti-brain antibodies are incorporated with cognitive and behavioral profiles in autistic children more severely. Moreover, recent studies have found that certain brain auto-antibodies correlate with mercury levels in children with ASD. They particularly examine the relationship between mercury in human tissue and symptom severity in ASD. In the studies they examined blood and nails. The results provided evidence that the higher the mercury levels, the worse the autism symptoms. Other studies, however, have found that the higher the hair mercury concentrations, the worse the autism symptoms (Janet et al., 2016; Currenti, 2010).

The neurotoxic effect of Mercury is well known. Immune system is also affected by mercury. Mast cells are occupied with sensitive responses, inflammation as well as innate and obtained immunity. Individuals with autism have a 10-fold larger number of hyperactive mast cells in most tissues. Mercury excites vascular endothelial growth factor and interleukin (IL)-6 releases from mast cells. The blood–brain barrier could be disrupted by these mediators and cause brain inflammation (Kempuraj, et al., 2010). Therefore, these studies provide strong evidences that long term exposure to heavy metals like lead and mercury may influence tissue concentration of heavy metals and trace elements, consequentlycausative to the genesis of ASD (Blaurock-Busch et al., 2011).

3.1.4.2. Cadmium

Heavy metals interrupt the purpose of enzyme. They also disrupt cellular signaling processes and generate oxidative stress which leads to apoptosis. They could play either a fundamental or a regulating role in the etiology of autism. Cadmium (Cd) is an extremely toxic metal whose intoxication mainly arises from smoking and also from environmental pollution due to unintentionally discarded batteries, water, food, and air contamination. It is stated that Cd has adverse effects on cells, including cell cycle progression, differentiation, proliferation, apoptotic pathways as well as DNA repair and replication. A study conducted in Turkey included3–12 years old thirty autistic children of (24 boys and 6 girls), who were admitted to Gülhane Military Medical Academy, Child and Adolescent Psychiatric Department. Twenty mentally and physically healthy children (13 boys and 7girls) that had similar socio-demographic characteristics were included as a normal control group in the study. This study has verified a significant decrease in urinary excretion rate of Cd which may indicate its deposition within the body. It has been recommended that autistic children may have insufficient heavy-metal-detoxifying mechanisms, which may result in body burden of heavy metals. This theory has been supported indirectly by various studies reporting decreased levels of heavy metal in the hair of children with autism. The study also suggested that autism may be associated with significant alterations of some rare element concentrations, including Cd, Pb, and Cr within body fluids (Yorbik et al., 2010).

3.1.4.3. Arsenic

Arsenic is an established, detrimental neurotoxicant. Several studies suggest environmental Arsenic exposure in children is related to the risk of subsequent ASD diagnoses but the findings vary greatly from one case report to another (Bjørklund et al, 2018).A study in Arab showed that the concentration of arsenic was significantly higher in the hair of the autistic children, though the urinary excretion of arsenic was significantly indifferent. There is strong evidence that high amount of arsenic in water have been associated with advanced fetal and infant mortality, growth interference, reduced intellectual ability and attention discrepancy disorders. This may point towards

that the long term exposure to even minute amounts of arsenic may trigger the genesis of autism (Blaurock-Busch et al., 2011).

In USA researchers hypothesized about having a correlation between industrial arsenic expulsion and the prevalence of autism. They used data from the Autism and Developmental Disabilities Monitoring (ADDM) Network. This is a multi-state community health supervision system for ASD and other developmental disabilities recognized by the CDC. They utilized data from 2000, 2002, 2004, 2006, and 2008 to measure ASD prevalence among 8-year-old children. A slightly increased occurrence of autism in children in the closest distance to arsenic and air pollutants releasing industrial facilities was observed. Prior studies have demonstrated that concentrations of particulate matter and air pollutants are higher in areas closer to industrial facilities. In those areas, exposure to arsenic and ambient air pollutants have been shown to trigger oxidative stress and inflammation in humans, which may have a contribution to the pathogenesis of neurodevelopment and ASD (Dickerson et al, 2015). The following table (Table 3.3) illustrates the possible impacts of toxic metals in patients with autism spectrum disorders.

Table: 3.3 Possible impact of toxic metals in patients with autism spectrum disorder (Bjørklund et al., 2018).

Toxic metal	Possible mechanisms of action
Lead (Pb)	Cognitive and neurobehavioral deficits in later life, alter neurochemistry, and elevate the affected persons'risk of being arrested for a crime in adulthood; stimulation of autoimmunity and neuroinflammation; induction of the serum anti-ribosomal P antibodies production
Mercury (Hg)	Impairment of GST; restricted effect on detoxification orsequestration of Hg; higher antineuronal antibodies;immune, sensory, neurological, and motor dysfunctions.
Aluminum (Al)	Interaction of Al with numerous glycolytic enzymes, and induction of a great suppression of cellular energy synthesis and elevate the potential neurotoxic activities in children, polymorphisms in the glutathione-Stransferase(GST) genes; direct binding of Al3+ ion by oxygen-based ligands; activation of microglia to cause neuroinflamation.
Arsenic (As)	Modification of brain morphology, degeneration of gliosis, neuronal, up-regulation of Bax and Bak expression, as well as depression of Mcl-1 in the cerebral cortex; impaired neurite growth due to suppression of AMP-activated protein kinase (AMPK) activation; inhibition of β-catenin signaling pathway.

3.1.4.4. Chromium

Chromium (Cr) is a transitional trace element with many industrial uses. It is utilized in stainless steel and pressure treated wood. As an essential trace element Cr (III) plays an important role in metabolism of glucose and cholesterol. On the contrary, it is accounted that both Cr (III) and Cr (VI) have perilous effects on growth of embryonic mice, such as neuronal tube defects, distortions, and deaths. Recent study has showed significant increase in urinary excretion rate of Cr in autistic children compared to healthy controls. Extremely cytotoxic Cr (VI) could direct to formation of reactive oxygen species and related products, resulting in oxidative stress, in lipid peroxidation, oxidative distortion of proteins, genomic DNA deformation and loss of membrane integrity, cellular dysfunction, and ultimately causing cell death. It is said (Yorbik et al., 2010) that oxidative stress may possess a role in the pathogenesis of autism owing to the finding that antioxidant enzyme activities are significantly lower in autistic children. Consequent studies were in agreement with increased oxidative stress in autism. A variety of studies have stated that Cr (VI) is responsible for reducing potency of different substances for examples, glutathione (GSH), lipoic acid, ascorbate, NAD(P)H, cysteine, fructose etc. and thus disrupts the functions of these substances resulting in altered psychological pattern and develop autism (Yorbik et al., 2010).

3.2. Genetic factors

There are unquestionable evidences of a genetic element in autism. At present a genetic cause of autism can be acknowledged in 20% to 25% of autistic children. The recognized genetic causes include cytogenetically visible chromosomal abnormalities (~5%), copy number variants (CNVs) (10-20%) and single gene disorders in which neurologic findings are associated with ASD (~5%) (Ivanov et al., 2015). With identical (monozygotic) twins, if one is autistic, the possibility of the other twin having some characteristics of autism is 90%. On the contrary, for fraternal (dizygotic) twins, the chance of other twin to have a form of autism is only 2–3%. The results fit best with models in which variants of several genes contribute to the outcome. Relatives of autistic people may have some of its symptoms but do not meet all the criteria for the disorder.

Another study showed 60% classic autism in monozygotic twins versus 0% in dizygotic twins. That shows the higher possibility of monozygotic genes as causative agent for autism. Reexamination for a more extensive autism phenotype expanded concordance amazingly from 60– 92% in monozygotic twins and 0– 10% in dizygotic sets. This proposes interaction between different genes cause "idiopathic" autism, yet epigenetic components and introduction to natural modifiers may add to variable articulation of autism related attributes. Data from whole genome screens in multiplex families suggest interactions of at least 10 genes in causation of autism (Helen, 2011).Some speakers emphasized mainly on the probable role of infections while discussing the potential mechanisms for how genes and environment could come together to cause autism (Altevogt et al., 2008).

3.2.1. Chromosome Aberration

Chromosome aberration might have role in autism in children. A study in Bulgaria stated that genetic changes in cells are constantly reported as one of the most frequent and assured causes of autism. Genetic irregularity in cells noticed with karyotype analysis is found in approximately 5% of autistic children and another 3-5% can be identified in situ hybridization (FISH) techniques using fluorescence. The most universally reported cytogenetic hazards found in persons with autism are the duplication of 15q11–q13 of the Prader-Willi/Angelman syndrome region (1-3%), deletions involving 7q, 22q13, 2q37, 18q, Xp and abnormal number of sex chromosomes in a cell (Ivanov et al, 2015).

3.2.2. Single Gene Disorders

Established genetic disorders can include autistic features in their clinical presentation, for instance fragile X syndrome, rett syndrome, tuberous sclerosis, phenyl ketonuria and neurofibromatosis, and. The frequently causing autism are single gene disorders are fragile X syndrome (around 3-5%) and tuberous sclerosis (TSC1/TSC2 - around 1%). However, a few percentage of children was noticed on the basis of an autism diagnosis, who have fragile X syndrome, not less than half of the children with fragile X syndrome had some autistic activities. The circumstances is not different with tuberous sclerosis

(TSC) - 25-50% of mentally handicapped individuals with TSC carry out diagnostic criteria of autism but only 1.1-1.3% of people were primarily diagnosed with ASD have TSC. Remarkably, the medical symptoms of ‘syndromic’ autism could be highly heterogeneous. Moreover, the presence of the same well distinguished mutation or genomic rearrangement can cause autism due to differences in genetic background and epigenetic influences (Ivanov et al, 2015). The following table (Table 3.4) illustrates some genetic syndromes associated with autism.

Table: 3.4 Some genetic syndromes associated with autism (Ivanov et.al. 2015).

Specific genetic disorder	Gene(s)/ chromo-some region involved	Autistic signs	Prevalence	Estimated rate (%) of autism in the disease	Estimated rate (%) in autism	Mental retarda-tion
Fragile X syndrome	**FMR1**	Poor eye contact, social anxiety, language impairment, stereotyped behaviors	1/3500 - 1/9000	18 - 33%	1-3%	Variable
Tuberous sclerosis	**TSC1, TSC2**	learning difficulties, behavioral problems	1 - 1.7/10000	25 - 60%	1 - 4% (8 - 14% if seizures are present)	Variable
Rett syndrome	**MECP2**	Stereotyped behaviors, language impairment, disturbance in social relatedness, loss of eye contact	1/8500 females	80 - 100%	< 5%	Severe
Untreated phenylke-tonuria	**PAH**	Self-injurious behavior, lack of social responsiveness	1/10000 - 1/15000	-	5.70%	Severe
Prader-Willi syndrome	**Del paternal allele at 15q11–q13**	Repetitive behaviour and social deficits	1/10000 - 1/30000	19 - 36.5%	1%-3%	Severe
Angelman syndrome	**Del/mut in maternal UBE3A**	**Severe speech impairment ste-reotyped behaviors, immutability**	**1/10000 - 1/12000**	**50 - 81%**	**≤ 1%**	**Severe**
Williams-Beuren syndrome	**7q11.23 del**	Social communication impairment, ranging from excessive talkativeness and overfriendliness to absence of verbal language and poor social relationships	1/7500 - 1/25000	7%	< 1%	Variable
Smith-Magenis syndrome	**17p11.2 del**	Self-injurious behavior, stereotyped behaviors (self-hugging), immutability	1/15000	93%	< 1%	Variable
Velocar-diofacial/ Di George syndrome	**22q11.2 del**	Speech delay, social skills dif-ficulties	1/4000 - 1/6000	20 - 31%	< 1%	Variable
Phelan-McDermid syndrome	**22q13.3 del**	Moderate to severe delays and often do not develop functional language.	unknown	50 - 70%	< 1%	Severe

3.2.3. Genome Wide Expression

Genome-wide expression patterns in biomaterials collected from ASD patients and controls is explained recently in a study. The studies found the involvement of different expression in genes in the neurodevelopment in ASD samples in comparison to the controls. The genes named NAGLU, FLAP/ALOX5AP, CHL1, and ROBO1are involved in neuronal segregation, development of brain and axon guidance were found down regulated. FGF12, MYT1L, and GAS7 were also found down regulated that are associated with neuronal delineation and outgrowth. Semaphorin 4C (SEMA4C) and glutamate dehydrogenase (GLUD1) was discovered upregulated that are occupied in development of neuron and in glutamatergic neurotransmission. It is also found that the immune system incorporation with different expression in the genes: pro-inflammatory cytokines, NK and CD8+ cell-related genes including receptor genes and genes involved in the cytotoxicity pathways, suggesting neural inflammation resulting in autism. These data obtained up to now propose that genes which are associated in the nervous system growth and in the immune system may function in the pathogenesis of autism. Analysis blood as well as lymphoblast cell lines are significant as a complement to SNP genotyping and CNV analyses in order to evaluate the significance of genetic variants as well as identify ASD biomarkers (Ivanov et al., 2015).

3.2.4. Contactin-associated protein (CNTNAP)

Cortical dysplasia-focal epilepsy syndrome was first described in 2006 in Amish children displaying cortical dysplasia, focal epilepsy, relative macrocephaly, diminished deep-tendon reflexes, language regression, MR and ASD. The disorder is recessive and caused by mutations in the *CNTNAP2* gene, which codes for contactin-associated protein-like 2 (CASPR2) that is involved in localization of voltage-gated potassium channels (K (v) 1.1) at the juxtaparanodes of the nodes of Ranvier. Recent studies (Buxbaum, 2009) assessed this gene in ASDs. First, following up on a linkage result of a language-related autism QTL, it was suggested that common variants of *CNTNAP2* may increase risk for ASDs in male-only families and it was shown that *CNTNAP2* is expressed in language- and cognition-related circuits. This finding was also observed in

a related study using overlapping AGRE families. Finally, rare variants of the *CNTNAP2*, and mainly the I869T variant, also show some association with ASD (Buxbaum, 2009).

3.2.5. Epigenetic Causes

Epigenetic mechanisms, for instance DNA methylation, alteration of histone proteins and miRNA direct chromatin structure and/or gene expression without altering DNA sequence. Epigenetic abnormalities are connected with several neurodevelopmental diseases which includes Autism Spectrum Disorders. Regulation of neuronal configuration and function through epigenetic mechanisms is thought to be vital in the development of the nervous system. Regulated gene expression during methylation of DNA has been observed in a number of genes associated with ASD. In comparison of autistic children to the healthy control twins, Hyper-methylation of precise CpG sites in the promoter regions of BCL-2 and RORA that directs to down regulation was identified. Another gene is associated with ASD and has an epigenetic regulation and that isSHANK3.Fluctuation in the modifications of the histone proteins could be another epigenetic mechanism related to ASD. SMCX gene encodes histone 3 lysine 4 (H3K4) me3 - specific demethylase. It is associated with ASD and cognitive dysfunction. Another aspect could be epigenetic control of gene expression through miRNA. Some researchers have scrutinized the expression profile of miRNA in ASD samples compared to healthy controls. They all have noticed individually expressed miRNA and their predicted target genes in the ASD samples suggesting that miRNA may trigger the causes of this disorder. The function of epigenetics in autism has come into sight recently, and predicts a growing area of research (Ivanov et al., 2015).

3.2.6. Endogenous Biochemicals

Alpha-fetoprotein in Human body is a pregnancy-associated protein with an uncertain physiological role. Human alpha-fetoprotein binds retinoids and restrains estrogen-dependent cancer cell proliferation. Retinoic acid (a retinol metabolite) and estradiol (an estrogen) can both initiate cellular gene transcription. In a study in USA researchers showed that alpha-fetoprotein facilitates critical gestational periods. It prevents retinoic

acid and maternal estradiol from inappropriately stimulating gene expression in developing brain regions which are sensitive to these chemicals. Prenatal/maternal factors linked to increased autism risk include valproic acid, cytomegalovirus, thalidomide, depression, alcohol, rubella, schizophrenia, obsessive–compulsive disorder, autoimmune disease, stress, allergic reaction, and hypothyroidism. These risk factors may initiate expression of genes which are sensitive to retinoic acid and/or estradiol. This can be happened by direct promotion or by reducing production of alpha-fetoprotein. They proposed that autism is not a genetic disorder, but is rather an epigenetic disruption in brain development. This disruption can be caused by gestational exposure to chemicals and/or conditions which either inhibit alpha-fetoprotein production or directly promote retinoic acid-sensitive or estradiol sensitive gene expression. This causation model leads to potential chemical explanations for autistic brain morphology and the disparities among high-functioning and low-functioning autisms including mental retardation, physical malformation and sex ratio.

Additionally, researchers said that during prenatal sexual differentiation, overexposure to FA could be assumed to be risk factors of autistic-like symptoms in rats. In fact in males it was uttered more rigorously. Behavioral irregularities include reduced pain sensation, more anxiety, as well as less social interaction. Alternatively, hepatoma cells' production of HAFP at the mRNA level is shut off by the type 2 herpes simplex virus. However, prenatal herpes exposure has not been recognized to be in correlation with autism risk factors. Then again during the birth process congenital herpes is most often acquired rather than during the periods when fetal brain is developed. However, Herpes belongs to the RCH (toxoplasmosis–rubella–cytomegalovirus–herpes) family of infections, while rubella as well as cytomegalo virus has both been caught up as risk factors for autism in prenatal stage. If one virus in a disease family shuts down production of hepatoma of HAFP and resulting in generation of the causes of autism, then considerably it is rational to suppose that other viruses in that disease family might show the same effects (King, 2011).

3.2.7. Genome-Wide Association Studies (GWAS)

The region on chromosome 5p14.1 between CDH9and CDH10 has been implicated by genome-wide association studies (GWAS) to be the first potential universal genetic risk factor for children with autism. However, for all ASD cases, the independent replicated GWAS has recurrently not been attained. Phenotype and genetic heterogeneity between patients are assumed to significantly reduce the power of overall genome-wide case-control studies in ASD. It can be a probable justification for the deficiency in replication as well as much of the 'missing heritability' in these complex disabilities. In large-scale genetic research of ASD, a number of strives have been made to trim down heterogeneity. A core symptom of ASD is restricted and repetitive behaviors (RRB). It is shown in the prior studies that RRB might have a fundamental genetic component and might be influenced by genes which are not dependent of those related with the social as well as communication difficulties. Furthermore, Autism Diagnostic Interview-Revised (ADI-R) provides broadly-accepted quantitative measures for RRB, making it a promising sub-phenotype for related studies. RRB comprises heterogeneous groups of behaviors. Throughout the last decade research has utilized factor-analysis to examine the structure of RRB using different subsets of ADI-R items and sub populations of ASD. The symptoms severity varies among those. Surprisingly, in spite of their different methodologies, many of these analyses come together on a two-factor solution for RRB comprising 'repetitive sensory-motor' (RSM) and 'insistence on sameness' (IS). The RSM subcategory measures motor mannerisms, sensory seeking behaviors, and the repetitive use of objects, whereas the IS subcategory measures compulsions, rituals and complications. IS and RSM were found to be differentially related to other ASD variables.

Particularly, extreme connections were observed among RSM with IQ, other adaptive behaviors, and delay in saying first words and phrases. It suggests that, compared to IS, RSM may be connected with ASD severity more. Nevertheless, it is revealed that the IS subcategory is thought to be under stronger additive genetic control than the RSM subcategory. However, Major familial aggregation of the IS subcategory has been

continually reported. On the other hand no significant concordance for familial aggregation for RSM has been declared. Behavioral subcategories are of particular interest to researchers exploring the genetic components that cause ASD symptoms. In a recent genome wide linkage analysis, RSM and IS subcategories were allied to various chromosomal regions. Those were only moderately overlapped regions that were documented using ASD diagnosis as the phenotype formerly (Tao et al., 2016).

Recent studies showed that, many genetic variants linked to ASD have a high degree of pleiotropy (i.e., where one gene affects more than one phenotype). Rationally some genetic variants have a role in both RSM as well as IS and were detected with higher association magnitude using multivariate association model. It should be referred to that a partial trisomy of 8p has been diagnosed in a 6-year-old female with autism. This region is also incorporated in a large (6.14 Mbp) chromosome replication noted in an autistic patient. The patient presented atypical behaviors, including repetitive behaviors, tendency to self-injury and bad temper. This section of chromosome includes a dosage sensitive gene that contributes to RRB phenotypes. In addition, based on mRNA expression data from two public databases, top SNP, rs2322600 and several proxy SNPs correlate with expression ofBNIPL3. This gene is located almost 890 kilo base pairs upstream (rs2322600: P-value = 9.17E-06). Long-range regulation of mRNA expression by genetic elements locate das far away as 1 Mbp, might be thought as a contender for RRB. BNIPL3 has not previously been reported to be associated with ASD, but it encodes a mitochondrial outer membrane protein that is essential for mitochondrial clearance and has been thought to have a role in hypoxia-induced autophagy. Recent research has shown that autistic children and adolescents have high dendritic spine density in the brain and this excess is due to a defect in dendritic spine “pruning” a process essential for normal brain development. The same study also demonstrated that the abnormal spine pruning is caused by a defect in autophagy in neurons. Mitochondria localize in both pre- and postsynaptic department (axon terminals and dendritic spines). However, mitophagy is crucial for brain development and dendritic spine pruning.PTK2B has been widely studied in a large meta-analysis of AD GWAS since it was recognized as a novel Alzheimer’s disease (AD) contender gene. PTK2B kinase

controls the integrity of focal adhesions, which are major sections for integrating signals for cell growth, natural cell death as well as neuronmigration, cellular functions crucial for normal development of brain. Since several neuronal cell-adhesion genes have been detected in rare ASD cases and a GWA study has shown that neuronal cell-adhesion molecules may be collectively connected with ASDs, it is reasonable that PTK2B might contribute to autism through its roles in regulation of the integrity of focal adhesions (Tao et al., 2016).

3.3. The Role of Immune Activation in the ASD Pathogenesis

The immune activation also can trigger the symptoms of autism.

3.3.1. Immune Alterations

The efficiency of the immune system can also be considered to be a chief determinative issue in the causation of ASD. Based on different studies of the effects of activation of chronic microglia as well as level of prominent inflammatory cytokine in adults, it can be expected to have similar effects on brain development in the postnatal stage. In autism the abnormalities in immunity are common, generally as immune over activation or dysregulation (Blaylock et al., 2009). A recent review showed the immunological irregularities reported in autism. The abnormalities contains irregular reactivity of lymphocytes, a shift in Th1/Th2 balance, increased release of TNF- and IL-1ß with mononuclear stimulation, presence of various anti-brain antibodies, and specific antibodies to neurotransmitter receptors (Xie et al., 2017). The immune changes within the brain itself are mainly concerned with ASD and related behavior disorders.

In another study, researchers examined the brains of 11 autistic patients of age ranges from three to forty five years, dying of non-infectious diseases. Control brains for the study were selected from age-matched people who died due to same reasons. Immunochemistry, cytokine protein arrays as well as enzyme-linked immune-sorbant assays in the brains and CSF of the patients and controls were performed. The greatest amount of damage was seen in the cerebellum, with huge loss of both Purkinje cells and granule cells. Prominently researchers observed extensive activation of microglia as well

as astrocytes all over the brains of autistic people. The maximum action was found in the cerebella, anterior cingulate gyrus and less activity was found in the medial frontal gyrus. A study shows the analysis of level of cytokine were scrutinized including both pro-inflammatory and anti-inflammatory cytokines, found a predominance of inflammatory cytokine activity. Most evident was elevations in macrophage chemo-attractant protein-1. It plays a role in innate immune reactions which includes monocytes, macrophage, and T-cell activation and trafficking into injured zones. IL-6 was considerably prominent in both the brain and CSF of autistics. This cytokine is known to play a vital role in neurodevelopment, however when chronically elevated, can disrupt brain development and function. It is important to keep in mind that in the older brains activation of the brain's innate immune system, particularly the microglia and the resident immune cells of the CNS are still being observed. The cause of the activation of chronic immunity within the brain could integrate a number of factors, such as vaccine adjuvants, mercury and Al3+ accretion within astrocytes as well as microglia, viral fragments or vaccine derived living viruses which ultimately results in autism (either measles virus or contaminant viruses) (Blaylock et al, 2009).

3.3.2. The Role of Microglia

Usually, microglia exists in a state of inactivation. During the period they constitutively convey growth factors only but do not transmit cytokines or excitatory amino acids. A range of insults, both innate and systemic, can hurriedly trigger the inactivated microglia, which results in their alteration into amoeboid forms. After that it can migrate through the brain's extracellular space. Researchers demonstrated extensive facts that activation of excessive or prolonged microglia can cause hazards for neurogenesis and neurodevelopment, which appears to involve both microglia-induced inflammatory cytokines and excitotoxicity. Consistently it occurs with the elevated glutamate and inflammatory cytokines in the blood, CSF, and in autistic brains. Researchers described four patients with rett´s syndrome who had drastically elevated CSF glutamate levels, assumed to be originated from microglia. There is also convincing evidence that chronic activation of microglia results in a predominant neurotoxic effect on the brain, with excitotoxic levels of glutamate secretion. This shows that chronic microglial activation

generates an atmosphere aggressive to developing neurons, dendrites and synapses. Excitotoxicity is evoked by inflammatory cytokine membrane receptors that interact with GluR. It is accepted that NMDA receptors are co-localized with TNF- receptors TNFR1 and TNFR2, allowing such crosstalk. TNFR1 is primarily neurotoxic and TNFR2 is neuroprotective. Neurons contain mostly TNFR1 subtype. The effect of chronic immune cytokine receptor activation on NMDA receptors determines the eventual impact on neurodevelopment, which can vary considerably in the brain (Blaylock et al., 2009).

Recent studies established that TNF- boosts the glutamate secretion from microglia by up-regulation of glutaminase enzyme. This enzyme is liable for the generation of glutamate from glutamine. Systemic activation of immunity can activate microglia of brain, which in turn excites microglial-induced glutamate release. Increased glutamate toxicity by this progression further causes danger to development of neuron. This is dependable with the symptoms observed in the ASD. in another study, levels of glial fibrillary acidic protein (GFAP) and ss-act in three areas of the brain, namely, area 9, area 40 and cerebellum was quantified, in age matched autistic and control postmortem specimens. Significant elevations in levels of GFAP in all three brain areas in autism were observed. This report confirms activation of microglia and astroglia in autism. These data point towards that a variety of autistic children have prominent levels of glutamate in blood and CSF and that they have precise immune diseases. These are characterized by chronic high levels of brain inflammatory cytokines and chemokines, both of which are thought to interfere with neurodevelopment significantly. In addition, it is found that inflammatory cytokines amplify the neurotoxic effects of glutamate considerably. Additionally, they found that higher levels of IL-6 increased the sensitivity of the NMDA receptors in developing cerebellar granule cell neurons, resulting in a noteworthy membrane depolarization and increased intracellular calcium levels. Neurotoxicity to the neurons was drastically elevated in granules cell neurons exposed to the higher levels of IL-6. However, excessive systemic immune stimulation through a combination of numerous and closely spaced vaccinations, immune and excitotoxic effects of the mercury additive thimerosal, systemic infections, food allergies, Candida

infections, and genetic factors all play a part. Activation of microglia is strongly linked to IL-1ß, which can enter the CNS following systemic immune activation, and evoking the brain inflammations even though other sources of systemic immune activation may play a vital role (Blaylock et al, 2009).

3.3.3. Autoimmune reactions versus brain

There is a serological relationship of measles virus and human herpesvirus-6 with brain auto antibodies in autism. For instance, measles-IgG-positive autistic sera were also positive for brain antigens, i.e., 90%were positive for anti-myelin basic protein and 73% for antineuron-axon filament protein. Human herpesvirus-6 antibody in autistic sera was similarly positive for brain antigens, i.e., 84% were positive for anti-myelin basic protein and 72%for anti-neuron-axon filament protein. Besides, in children with autism, neuron-specific antigens may cross-react with encephalitogenic proteins from milk, *Chlamydia pneumoniae* and *Streptococcus* group A. The antibodies may have been synthesized as a result of an alteration in the blood-brain barrier, allowing pre existing T-lymphocytes and central nervous system antigens access to immune competent cells, which may start a vicious cycle of brain inflammation (Ratajczak, 2011).

3.4. Vitamin D deficiency

In turkey, a study showed that vitamin D deficient children have a number of autistic symptoms that gradually vanish with vitamin D treatment in high-dose. Estrogen as well as testosterone has significant effects on calcitriol's metabolism, differences that might clarify the remarkable sex ratios in autism. Calcitriol usually down-regulates generation of inflammatory cytokines in the brain. Those cytokines are usually incorporated with autism. During pregnancy consumption of fish containing Vitamin D, reduces autistic symptoms in children. Autism and ADHD are frequent in areas of impaired ultraviolet B (UVB) penetration such as pole ward latitudes, urban areas, areas with high air pollution as well as areas of high precipitation. The dark-skinned persons are more vulnerable to autistic symptoms. Apparently severe maternal vitamin D deficiency is unusually regular in people with dark-skin. A recent research reported that simple

Gaussian distributions of the enzyme might clarify both the genetics and epidemiology of autism. The enzyme promotes neural calcitriol combined with extensive gestational and/or early childhood vitamin D deficiency. The effects of vitamin D on brain development and function, as a neuro-immunomodulatory agent, lead to behavioral, and neuro-psychiatric diseases have recently been reviewed. Deficiency of vitamin D is being related with numerous psychiatric conditions with a developmental basis, such as autism and schizophrenia. Deficiency in vitamin D in premature stage affects neuronal differentiation, axonal connectivity, dopamine ontogeny, as well as brain structure and function. Researchers reported a reduced amount of serum 25(OH) D in autistic children. The studies possibly can classify them as being "Vitamin D inadequate", which lends support to the hypothesis that autism is a vitamin D deficiency disorder. However, the rate of autism has been steadily rising globally. In the present work, serum 25(OH) D levels had significant negative correlations with ADOS ($P < 0.001$) which indicates the possible link between the extent of Vitamin D deficiency and the degree of the severity of autism. Vitamin D receptors and vitamin D metabolizing enzymes are present in CNS. Calcitriol, the active vitamin D, affects numerous neurotransmitters and neurotrophic factors, relevant for mental disorders. Besides, researches exposed that abnormalities in the brain can be leaded by the deficiency in vitamin D. It can also cause large lateral ventricles, poor tissue differentiation, and reduced expression of neurotropic factors. This shows a possible involvement among autism with its distressing behavior and hypo vitaminosis D in children (Bener, 2014).

3.5. Intracellular pathogens

Autistic patients have been observed to have the measles virus; cytomegalovirus, human herpesvirus6, as well as the bacterium *Yersinia enterocolitica* live inside their monocytes. These intracellular pathogens promote lowered hematopoiesis, lowered immunity, and altered blood-brain barrier function often escorted by demyelination. The viruses may induce an immune response, which results in neuroinflammation, autoimmune reactions, and brain injury. Because the reactivity of the immune system is shifted from a balance of cell- and antibody-mediated activities to favor the latter, pathogens are highly proficient of hiding inside cells for prolong stages and then

occasionally inducing an immune response during replication cycles, resulting in a chronic pattern of inflammatory disease or autism (Ratajczak, 2011).

3.6. Imbalance in neural systems

Seizures are known to be associated with autism and have been often seen in autistic subjects in response to functions that need attention; quite a few people have proposed that autism might be caused by an imbalance between excitation and inhibition in key neural systems including the cortex. However, Three main types of defects have been revealed in autism: the brainstem and cerebellum, the limbic system (amygdale and hippocampus), and the cortex. Abnormal regulation of brain development in autism results in early overgrowth followed by abnormally slowed growth. The strongest evidence implicates the glutamatergic and GABAergic and serotonergic systems, with weaker evidence for catechol-aminergic, peptidergic, and cholinergic systems. The serotonergic system may be dysregulated in autism; serotonin levels are initially lower than normal but gradually increase to a greater extent than adult levels by 2–15 years of age (Ratajczak, 2011).

3.7. Parental roles

Parental lifestyle can have effects on the etiology of autism.

3.7.1. Maternal depression, antidepressant use during pregnancy

In a study, the information was obtained about the uses of antidepressant during pregnancy for 1679 cases of autism (743 with intellectual disability and 936 without) and 16845 control children who were born from 1995 onwards. The relations of maternal depression and autism have simply followed a parallel trend, though with wide confidence intervals, largely resulting from relations with autism without brain discrepancy. These evaluations alleviates on modification for maternal antidepressant use amid pregnancy. It demonstrates the aftereffects of examination utilizing the variables joining a maternal history of gloom and stimulant use amid pregnancy. Posterity of mothers with a background marked by sadness and detailed antidepressant use amid pregnancy appeared to have solid relationship with ASD. A background

marked by wretchedness without antidepressant use during pregnancy was not related with an increased danger of Autism spectrum disorder. Various Studies demonstrated the relationship between antidepressant utilizes and autism. Any antidepressant use during pregnancy in mothers of 1.3% cases was contrasted with 0.6% of controls equating with a right around two-fold increment in danger of autism. These affiliations too were generally seen because of relationship with a mental imbalance without scholarly handicap with any expanded chances for autism with scholarly inability. In another study it was investigated that the potential perplexing impact of some other psychiatric medications that did not bring about any detectable change to the outcomes: completely balanced chances proportion for any stimulant use amid pregnancy, autism with intellectual inability, and autism without scholarly disability. Comparative outcomes were apparent for maternal utilization of the two SSRIs and non-specific monoamine reuptake inhibitor antidepressants, in spite of the fact that numbers were little and certainty interims generally wide. The population fraction estimate suggested that, assuming an unconfounded causal association, 0.6% of cases of autism spectrum disorder could be prevented if antidepressant use during pregnancy was totally removed. However, a maternal history of depression was incorporated with a higher risk of autism in off spring (Ayesha et al., 2017), but there was no evidence of a correlation with paternal depression. The associations were mostly limited to children of mothers who reported using antidepressants at the first antenatal meeting. The prevalence in risk was assessed with SSRIs and with other monoamine reuptake inhibitor antidepressants. Each of these increased risks assumed to be confined to ASD without intellectual disability and still continued after adjustment for several confounding factors (Rai et al., 2013).

3.7.2. Maternal thyroid autoantibody

Immune system disturbance may add to hazard for autism. Thyroid peroxidase counter acting agent (TPOAb) is an autoantibody engaged with immune system thyroiditis. In a study it was shown to have a correlation in positive maternal serum TPO-Ab (TPO-Ab+) (characterized as N156 IU/ml) amid pregnancy with autism in children. The investigation was carried out on a settled case-control outline of the Finnish Prenatal Study of Autism (FiPS-An), a national birth companion that incorporates tentatively

drawn documented maternal serum examples from the whole pregnant populace of Finland starting in 1983. Instances of autism in children (ICD-10F84.0) conceived from 1987 to 2005 were discovered by performing linkages between national birth and inpatient/outpatient registries. All diagnosed cases in Finland over the birth years, and comparing subjects without ASD or severe/profound intellectual disability were matched 1:1 on date of birth, sex, birthplace, and residence in Finland. Maternal serum specimens' were assayed in 967 matched case–control pairs for TPO-Ab by a chemi-luminescent micro particle immunoassay blind to case/control status. Data were analyzed by conditional logistic regression for matched sets. The prevalence of maternal TPO-Ab+was significantly increased in pregnancies giving rise to autism cases (6.15%) compared to controls (3.54%). The odds of autism were increased by nearly 80% among offspring of mothers who were TPO-Ab+ during pregnancy, compared to mothers negative for this autoantibody. There was also a significant relationship between maternal TPO-Ab, defined as a continuous variable, and odds of autism. Amount of maternal thyroid hormones did not differ between groups, however, the maternal TPO-Ab+ may possibly serve as a marker for other factors that increase risk of autism in children. It may be that heritable factors associated with maternal TPO-Ab+ are related to autism, given associations noted above between parental autoimmune thyroidity and autism in probands. These findings provide the first biomarker-based evidence that a class of known maternal autoimmune disorders is related to autism in offspring (Brown et al., 2015).

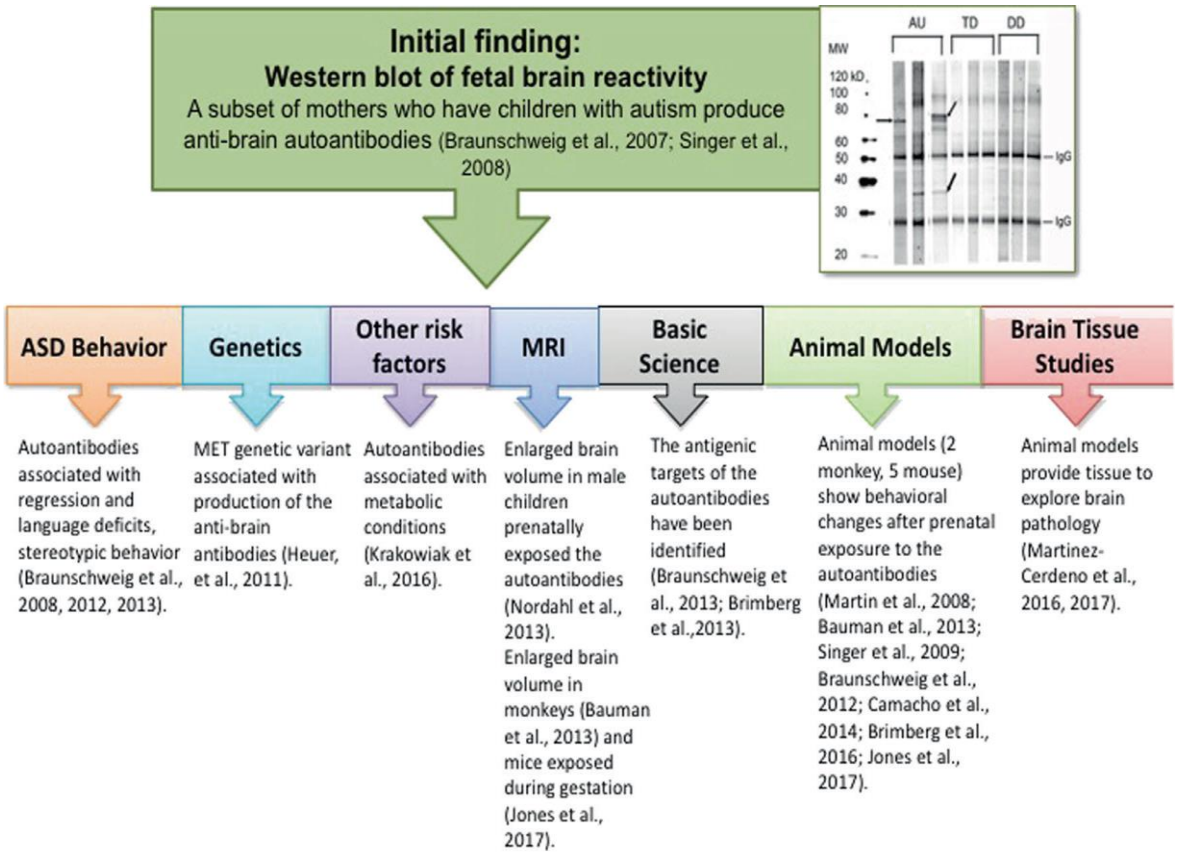

Figure 3.3 Overview of MAR ASD findings (Jones et al., 2018).

Figure 3.3 has shown a relationship between the presence of the ASD auto antibodies and behavioral deficits, the cMET allele polymorphism, metabolic conditions during pregnancy, enlarged brain volume in both humans and animal models, and changes in the way neurons develop following exposure (Jones et al, 2018)

3.7.3. Fetal brain-reactive maternal autoantibody

There observed a rising body of convincing proof supporting the connection among maternal autoantibodies affects to the fetal brain proteins and risk of ASD. The supermolecule autoantigens as well as coupled immune dominant epitope sequences targeted by these ASD-specific maternal autoantibodies were recently known, and it's the popularity of assorted mixtures of those proteins by maternal autoantibodies that confers the specificity of MAR ASD providing the chance of a biomarker for the

danger of having a baby with syndrome. Whereas it seems that specific patterns of autoantigen reactivity square measure related to distinctive behavioral phenotypes, human experimental and animal studies alike have systematically noted inflated repetitive stereotypes, inappropriate social approach behaviors, and alterations within the neurodevelopmental mechanical phenomenon of exposed offspring. Recent proof from multiple investigators has illustrated the harmful role that dysregulation of the maternal system throughout gestation will play within the demonstration of changes in neurodevelopment, leading to the event of neurobehavioral disorders like ASD. One potential etiologic pathway through that the maternal system will interfere with neurodevelopment is maternal autoantibodies that acknowledge proteins within the developing fetal brain. This mechanism of pathologic process is currently thought to steer to a subphenotype of ASD that has been termed maternal antibody related (MAR) ASD. So, the presence of brain-reactive maternal autoantibodies might be a risk issue for MAR ASD (Jones et al, 2018).

3.7.4. Maternal polycystic ovary syndrome and the risk of autism

Although several studies indicate the interaction of genetic and environmental factors within the etiology of syndrome spectrum disorder (ASD), the restricted understanding of the underlying mechanisms hampers the effective ways for investigation and prevention of the disorder. Recent studies support that prenatal androgenic hormone exposure contributes to the event of ASD. This might counsel that maternal polycystic ovary syndrome (PCOS), a condition related to excess androgens, would increase the chance of ASD within the offspring. A search was conducted over a matched case–control study nested at the full population of Sweden (children aged 4–17 who were born in Sweden from 1984 to 2007). The sample consisted of twenty-three 748 ASD cases and 208 796 controls, matched by birth month and year, sex and region of birth. PCOS and ASD were outlined from ICD codes through linkage to health-care registers. Maternal PCOS exaggerated the percentages of ASD within the offspring by fifty-nine and the percentage exaggerated among mothers with each PCOS and blubber, a condition common to PCOS that's associated with a lot

of severe hyperandrogenemia. Risk estimates failed to dissent between sexes. However, children of woman with PCOS seem to have the next risk of developing ASD (Kosidou et al., 2015).

3.7.5. Family history of autoimmune diseases

Various investigations were acquired to decide the current prove on the connection between family history of immune system ailments (ADs) and risk of autism in youngsters, as present proof recommends conflicting outcomes. Hazard gauges from singular investigations were pooled utilizing irregular impacts models. Sub-bunches examinations were directed by some investigation level components. A few examinations demonstrated that family history of all ADs joined was related with a 28% higher danger of autistic children. For some particular ADs, prove blend for danger of a mental imbalance in kids demonstrated a factually noteworthy relationship with family history of hypothyroidism, type 1 diabetes, rheumatoid joint pain, and psoriasis. The outcomes differed in a few subgroups. However, a general expanded danger of autism in kids with family history of ADs was recognized (Wu et al., 2015).

3.8. Meat consumption

A study in Poland exhibited a relationship between expanding meat utilization and autism predominance in three chosen nations, and in addition crosswise over seventeen nations. Given the current situation with learning, it is difficult to decide the correct specialist causing these relationships. There are a few conceivable situations which loan support to the affiliations appeared previously. What takes after is our humble proposition for investigating conceivable courses of occasions and procedures that may underlie the relationship impacts appeared in the above tables and figures. The familial human want for meat makes individuals uncritical shoppers. The scan for shoddy nourishment, especially shabby meat, drives makers to actualize arrangements that make huge dangers for human wellbeing, atmosphere and worldwide assets. The consciousness of ecological components is expanding. Coordinate connections amongst manifestations and particular elements have been recognized now and again. For

example, the mass meat generation framework itself postures dangers of transmitting irresistible specialists causing some neurological issue (Pisula, 2014).

4. Conclusion

Autism has been a burning topic throughout the years due to its undefined etiology and its prevalence over decades. But the underlined etiology of prevalence of autism is questionable. So, since Autism spectrum disorders has come to the light, scientists and researchers are working passionately to define the causes behind it. Various studies came up with strong evidences about the involvement of genetic and environmental factors. Some genetics variants have been seen to have effects on neurodevelopmental disorders which are associated with some symptoms of autism. Additionally, long term exposure to heavy metals and toxic trace elements has provided enough data to increase risk factor for autism. Maternal use of antidepressant has also shown to be incorporated with the fetal brain development adversely. But those studies could focus on some of the aspects of autism rather than the whole. A number of studies also indicated towards the parental life style and the family history of psychiatric diseases and others. But the results of those studies varied due to subjects or control size. However, further research is needed to explore the mechanism behind the possible causes which are thought to trigger the autism spectrum disorders.

5. Limitation of the study

Enough articles and journals of recent years were not available regarding autism. In addition, data in context of Bangladesh was not available with respect to autism.

6. Future research scope

This review further seeks to do advanced research in this field to lessen the hazards. Moreover, the development of effective treatment would be a field of research by minimizing the side effects.

References:

Adams J., Audhya, T., Mc Donough-Means S., Rubin R.; Quig, D.; Geis, E.; Gehn, E.; Loresto, M.; Mitchell, J.; Atwood S.; Barnhouse, S.; and Lee, W. (2013). Toxicological Status of Children with Autism vs. Neurotypical Children and the Association with Autism Severity. *Biol Trace Elem Res*; 151(2):171-180.

Aisha S. D. & Mohammad H. R. (2015). Autism spectrum disorder prevalence and proximity to industrial facilities releasing arsenic, lead or mercury *Science of the Total Environment*; 536:245–251.

Anthony R. T. (2003). Is fever suppression involved in the etiology of autism and neurodevelopmental disorders? *BMC Pediatrics;*3(9): 1-6.

Autism awareness centre inc. Autism 'Triad of Impairments' Redefined: Lessons from John Simpson. Retrieved from the online website: https://autismawarenesscentre.com/lessons-learned-from-john-simpson/

Autism awareness centre inc. Definition of Autism. Retrieved from the online website: https://autismawarenesscentre.com/definition-autism/.

Ayesha C.S. & Martin E. R. et al. (2017). Associations of Maternal Antidepressant Use During the First Trimester of Pregnancy with Preterm Birth, Small for Gestational Age, Autism Spectrum Disorder, and Attention-Deficit/Hyperactivity Disorder in Offspring. *JAMA*.; 317(15):1553-1562.

Bappaditya A and Santoshi H. **(**2017). Systematic Review on Prevalence for Autism Spectrum Disorder with Respect to Gender and Socio-Economic Status. J Ment Disord Treat, 3(1), 1000133.

Bener A., Khattab A.O. & Al-Dabbagh M.M. (2014). Is high prevalence of Vitamin D deficiency evidence for autism disorder in a highly endogamous population. *Pediatric neuro sciences;* 9 (3):227-233.

Benvenuto A., Moavero R., Alessandrelli R., Manzi B. & Curatolo P. (2009). Syndromic autism: causes and pathogenetic pathways. *World J Pediatr*; 5(3)*:*169-176.

Bjørklunda G. & Skalny V. A. (2018). Toxic metal(loid)-based pollutants and their possible role in autismspectrum disorder. *Environmental Research*; 166:234–250.

Blaurock-Busch, E.; Amin, O.; and Rabah, T. (2011). Heavy Metals and Trace Elements in Hair and Urine of a Sample of Arab Children with Autistic Spectrum Disorder. *A Journal of Clinical Medicine*; 6(4): 247-257.

Blaylock R.L. & Strunecka A. (2009). Immune-Glutamatergic Dysfunction as a Central Mechanism of the Autism Spectrum Disorders. Current Medicinal Chemistry; 16: 157-170.

Breanna E. S and Daisha L. C. (2017). Preliminary Evidence for Inflexibility of Motor Planning in Children with Autism Spectrum Disorder. *Autism Open Access, an open access journal*, 7(2), 1000208.

Brown A. S. & Surcel H.M. et al. (2015). Maternal thyroid autoantibody and elevated risk of autism in a national birth cohort. *Progress in Neuro-Psychopharmacology & Biological Psychiatry*; 57: 86–92.

Buxbaum J. D. (2009). Multiple rare variants in the etiology of autism spectrum disorders. *Dialogues Clin Neurosci;* 11:35-43.

Courchesne E. & Pramparo T. et al. (2017). The ASD Living Biology: from cell proliferation to clinical phenotype. *Molecular Psychiatry;*https://doi.org/10.1038/s41380-018-0056-y.

Courchesne E. & Pramparo T. et al. (2018). The ASD Living Biology: from cell proliferation to clinical phenotype. *Molecular Psychiatry*; https://doi.org/10.1038/s41380-018-0056-y.

Croen L. A. & Najja D. V. et al. (*2007).* Maternal and Paternal Age and Risk of Autism Spectrum Disorders. *Arch Pediatr Adolesc Med;* 161:334-340.

Currenti S. A. (2010). Understanding and Determining the Etiology of Autism. *Neurobiol*.; 30:161–171.

Eric L. & Ruth A. E. (2000). The Environment as an Etiologic Factor in Autism: A New Direction for Research. *Environmental Health Perspectives*; 108(3): 401-404.

Fido B. & AL-Saad S. (2005). Toxic trace elements in the hair of children with autism. *SAGE Publications and The National Autistic Society*; 9(3): 290–298.

Geier D. A, Kern J. K, Garver C. R, et al. (2009). Biomarkers of environmental toxicity and susceptibility in autism. *J Neurol Sci*.; 280:101–108.

Getaneh Z. et al. (2014). Exposure and Health Risk Assessment of Lead in Communities of Jimma Town, Southwestern Ethiopia. *Bull Environ Contam Toxicol.*, 93(2): 245-250.

Gustavo C. R. (2007). Autism: Transient *in utero* hypothyroxinemia related to maternal flavonoid ingestion during pregnancy and to other environmental antithyroid agents. *Journal of the Neurological Sciences*, 262(1–2), 15-26.

Helen V. R. (2011) Theoretical aspects of autism: Causes—A review, *Journal of Immunotoxicology*, 8:1, 68-79.

Janet K. K, David A. G, Lisa K. S, and Mark R. G.(2016). The relationship between mercury and autism: A comprehensive review and discussion. *Journal of Trace Elements in Medicine and Biology*, 37, 8-24.

Jiao Y, Chen R, Ke X, Cheng L, Chu K, Lu Z and Herskovits E. H. (2011). Predictive models for subtypes of autism spectrum disorder based on single-nucleotide polymorphisms and magnetic resonance imaging. *Advances in Medical Sciences*, 56, 334-342.

Jones K. L. & Water J. V. D. (2018). Maternal autoantibody related autism: mechanisms and pathways. *Molecular Psychiatry*; https://doi.org/10.1038/s41380-018-0099-0.

Joseph N. Trachtman, O. D. (2008). Background and history of autism in relation to vision care. *Optometry*, 79, 391-396.

Kempuraj D., Asadi, S., Zhang, B., Manola, A., Hogan, J., Peterson, E., and Theoharides, T.C. (2010). Mercury induces inflammatory mediator release from human mast cells. *J. Neuroinflammation*. 7:20.

Kern J. K. &Geier D. A. et al. (2016).The relationship between mercury and autism: A comprehensive review and discussion. *Journal of Trace Elements in Medicine and Biology*; 37: 8–24.

King C. R. (2011). A novel embryological theory of autism causation involving endogenous biochemicals capable of initiating cellular gene transcription: A possible link between twelve autism risk factors and the autism 'epidemic'. *Medical Hypotheses*; 76:653–660.

Kirsty Y**.** and Ann L. C. (2013).Diagnosing autism/autism spectrum disorders. *Paediatrics and Child Health*, 23(1), 5-10.

Kolvin I. (1971). Studies in the childhood psychoses. I. Diagnostic criteria and classification. *Br. J. Psychiatry*, 118, 381–384

Kosidou K. & Dalman C. et. al. (2015). Maternal polycystic ovary syndrome and the risk of autism spectrum disorders in the offspring: a population-based nationwide study in Sweden. *Molecular Psychiatry;* 1-8.

Matthew M, Dorcas U, Andrew K and Christopher E. (2018). Aluminium in brain tissue in autism. *Journal of Trace Elements in Medicine and Biology*, 46, 76–82.

Oztan O. & Jackson L. P. et al. (2018). Biomarker discovery for disease status and symptom severity in children with autism. Psychoneuroendocrinology: 89; 39–45.

Peter G. (2018). Evidence the U.S. autism epidemic initiated by acetaminophen (*Tylenol*) is aggravated by oral antibiotic amoxicillin/clavulanate (*Augmentin*) and now exponentially by herbicide glyphosate (*Roundup*). *Clinical Nutrition ESPEN*, 23, 171-183.

Pisula W. & Pisula E. (2014). Autism prevalence and meat consumption – A hypothesis that needs to be tested. *Medical Hypotheses*; 83:488–493.

Rai *D. &* Brian K Lee B. K. et al. (2013). Parental depression, maternal antidepressant uses during pregnancy, and risk of autism spectrum disorders: population based case-control study. *BMJ;* 346:2059.

Ratajczak H. V. (2011). Theoretical aspects of autism: Causes—A review, *Journal of Immunotoxicology*; 8(1):68-79.

Retrieved from the online website: http://www.who.int/mediacentre/factsheets/autism-spectrum-disorders/en/

Strathearn L. (2009). The elusive etiology of autism: nature and nurture? *Frontiers in Behavioral Neuroscience;* 3:1-3.

Strunecka, A.; Patocka, J.; Blaylock, R.L. & Chinoy, N.J. (2007). Fluoride interactions: From molecules to disease. *Curr. Signal Transd. Ther*.; 2:190-213.

Strunecka, A.; Strunecky O. & Patocka J. (2002). Fluoride plus aluminum: useful tools in laboratory investigations, but messengers of false information. *Physiol. Res.*, 51: 557-64.

Tao Y., Gao H., Ackerman B., Guo W., Saffen D. & Shugart Y.Y. (2016). Evidence for contribution of common genetic variants within chromosome 8p21.2-8p21.1 to restricted and repetitive behaviors in autism spectrum disorders. *BMC Genomics*; 17:163

Tomljenovic L. & Shaw C. A. (2011). Do aluminum vaccine adjuvants contribute to the rising prevalence of autism? *Journal of Inorganic Biochemistry*; 105:1489–1499

Walter A. (2011). The history and significance of the autism spectrum. *Theses and Dissertations*. 513.

Xie J. &Li Huang *L. (2017).* Immunological cytokine profiling identifies TNF-α as a key molecule dysregulated in autistic children. *Oncotarget*;8(47): 82390-82398.

Xu G. & Jing J. (2013). Maternal Diabetes and the Risk of Autism Spectrum Disorders in the Offspring: A Systematic Review and Meta-Analysis. *J Autism Dev Disord*; 44:766–775.

Yang Y.; Tian J. & Yang B. (2018). Targeting gut microbiome: A novel and potential therapy for autism. *Life Sciences*; 194: 111–119.

Yassa H. A. (2014). Autism: A form of Lead and Mercury Toxicity, *Environmental Toxicology and Pharmacology*

Yorbik Ö. & Kurt İ. & Haşimi A. &Yorbik Ö. (2010). Chromium, Cadmium, and Lead Levels in Urine of Children with Autism and Typically Developing Controls. *Biol Trace Elem Res*; 135:10–15.

Zaky E. A (2017) Autism Spectrum Disorder (ASD); The Past, The Present, and The Future. *J Child Adolesc Behav*, 5, 116.

Zhao L. and Xu L. et al. (2018). Characteristics and sources of mercury in precipitation collected at the urban, suburban and rural sites in a city of Southeast China. *Atmospheric Research,* 211: 21-29.