CHAPTER 2

LITERATURE REVIEW

2.1 AUTISM SPECTRUM DISORDER (ASD)

The term ‘autism spectrum disorder’ (ASD) refers to a class of neurodevelopmental disorders characterized by qualitative impairments in the development of social and communication skills, often accompanied by stereotyped and restricted patterns of interests and behavior,with onset of impairment before 3 years of age [based on the criteria for pervasive developmental disorders in the Diagnostic and Statistical Manual of Mental Disorders, fourth edition.[1] ASDs include the diagnostic categories of autism, pervasive developmental disorder not otherwise specified (PDD-NOS), and Asperger’s syndrome.[2, 3]

The range of these disorders varies from severely impaired individuals with autism to other individuals who have abnormalities of social interaction but normal intelligence,Asperger’s syndrome. The ways in which autism is exhibited can differ greatly. Additionally,autism can be found in association with other disorders such as mental retardation and certain medical condition. The degree of autism can range from mild to severe. Mildly affected individuals may appear very close to normal. Severely afflicted individuals may have an extreme intellectual disability and unable to function in almost any setting.[4] The DSM-IV

criteria for Autistic Disorder are presented in Table 1 and are described below for each neurobehavioral domain.

Table 1. Diagnostic Criteria for Autistic Disorder [1]

A. A total of six (or more) items from (1), (2), and (3), with two from (1), and at least one each from (2) and (3):

(1) qualitative impairment in social interaction, manifest by at least two of the following:
   a) marked impairment in the use of multiple nonverbal behaviors, such as eye-to-eye gaze, facial expression, body postures, and gestures, to regulate social interaction;
   b) failure to develop peer relationships appropriate to developmental level;
   c) a lack of spontaneous seeking to share enjoyment, interests, or achievements with other people (e.g.,by lack of showing, bringing or pointing out objects of interest);
   d) lack of social or emotional reciprocity

(2) qualitative impairment in communication, as manifest by at least one of the following:
   a) delay in, or total lack of, the development of spoken language (not accompanied by an attempt to compensate through alternative modes of communication such as gesture or mime);
   b) in individuals with adequate speech, marked impairment in the ability to initiate or sustain a conversation with others;
   c) stereotyped and repetitive use of language, or idiosyncratic language;
   d) lack of varied, spontaneous make-believe, or social imitative play appropriate to developmental level.

(3) restrictive repetitive and stereotypic patterns of behavior, interests, and activities, as manifested by at least one of the following:
   a) encompassing preoccupation with one or more stereotyped and restricted patterns of interest that is abnormal either in intensity or focus;
   b) apparently inflexible adherence to specific nonfunctional routines or rituals;
   c) stereotyped and repetitive motor mannerisms (e.g., hand or finger flapping or twisting, or complex whole-body movements);
   d) persistent preoccupation with parts of objects.

B. Delays or abnormal functioning in at least one of the following areas, with onset prior to age 3 years:
   1) social interaction,
   2) language as used in social communication, or
   3) symbolic or imaginative play.

C. The disturbance is not better accounted for by Rett's Disorder or Childhood Disintegrative Disorder.

Diagnostic and Statistical Manual of Mental Disorders (*4th ed.*, pp. 70–71 Washington, DC:American Psychiatric Association, 1994.

2.1.1 Autistic Disorder

Autistic disorder is neurodevelopmental disorder with three major impairments : restriction of social interaction, communication (verbal and nonverbal) and behaviours. Diagnosis for autistic disorder according to DSM IV criteria with total of six (or more) items from (1), (2) and (3) with at least two from (1) and one each from (2) and (3).[4]

A.1. Qualitative Impairment in Social Interactions

It is important to understand that these criteria refer to a qualitative impairment in reciprocal social interactions, and not to the absolute lack of social behaviors. The impairment range from total lack of awareness of another person, to eye contact which is present but not used to modulate social interactions.[2]

A.1a. Marked Impairment in the Use of Multiple Nonverbal Behaviors, Such As Eye-to-Eye Gaze, Facial Expression, Body Posture, and Gestures to Regulate Social Interaction.

As infants, some children with autism do not lift up their arms or change posture in anticipation of being held. They may not or may cuddle or stiffen when held, and often do not look or smile when making a social approach. Some children do make eye contact, often only in brief glances, but the eye contact is usually not used to direct attention to objects or events of interest. Other children make inappropriate eye contact, by turning someone else's head to gaze into their eyes. Autistic children often ignore a familiar or unfamiliar person because of a lack of social interest. Some children do make social approaches, although their conversational turn-taking or modulation of eye contact is often grossly impaired.[2]

A.1b. Failure to Develop Peer Relationships Appropriate to Developmental Level.

Younger children may demonstrate lack of interest, or even apparent lack of awareness of peers or other children. Some children with autism have no age-appropriate friends, and often older children may be teased or bullied. A child may want "friends" but usually does not understand the concept of the reciprocity and sharing of interests and ideas in friendship. Verbal children may have one "friend" but the relationship may be very limited or may focus only on a similar circumscribed interest, such as a particular computer game. Often, children gravitate to adults or to older peers, in which case they play the role of a follower, or to much younger peers, where they become the director. Demands on social reciprocity are much less compared to interactions with age-appropriate peers.[2]

A.1c. A Lack of Spontaneous Seeking to Share Enjoyment, Interests, or Achievements with Other People (e.g., by a Lack of Showing, Bringing, or Pointing Out Objects of Interest).

Some children with autism do not reciprocate in lap play, but rather either hold the parent's arms as the parent performs the game in a mechanical fashion, or insist that the parent watch the child perform the game.Characteristic give-and-take in lap play that is seen in typically developing children by the end of the first year is often missing. They often do not point things out or use eye contact to share the pleasure of seeing something with another person, which is called joint attention.[2]

A.1d. Lack of Social or Emotional Reciprocity.

Some children with autism show no interest in other children or adults, and tend to play alone by themselves away from others. Others play with adults nearby, or sit on the outskirts of other children's play and either engage in parallel play or simply watch the other children. Some children involve other children in designated, often repetitive play, but often only as "assistants" without heeding any suggestions from the other children. Some tend to serve in the passive role in other children's play, for example as the baby in a game of "house," and simply follow others' directions. Other children may seek out one specific child with whom there is a limited solitary interest that dominates the entire relationship.[2]

A.2. Qualitative Impairment in Communication

The communication impairments seen in the autistic spectrum are more complex than presumed by simple speech delay and have similarities with the deficits seen in children with developmental language disorders or specific language impairments.[5] Expressive language function in autistic spectrum ranges from complete mutism to verbal fluency, although fluency is often accompanied by many semantic (word meaning) and verbal pragmatic (use of language to communicate) errors. Young autistic children, even if verbal, almost universally have comprehension deficits, in particular deficits in understanding higher order complex questions. Deficits in pragmatics, the use of language to communicate effectively, are also almost universally present. Some children with autism do not respond to their names when called by a parent or other favored caretaker, and often they are initially presumed to be severely hearing-impaired.[3]

A.2a. Delay in, or Total Lack of, the Development of Spoken Language (Not

Accompanied by an Attempt to Compensate Through Alternative Modes of Communication Such As Gesture or Mime).

In early infancy,some children with autism do not babble or use any other communicative vocalizations, and are described as very quiet babies. Some children have absolutely no spoken language when speech should be developing, and also fail to compensate with facial expressions or gestures. A typically developing infant or toddler may pull his mother over to a desired object, but then will clearly point to the object while looking at the mother's face. In contrast, a characteristic behavior of many children with autism is to use another person's hand to indicate the desired object, often called "hand over hand pointing." Some children even throw another's arm up towards the desired object that is out of reach, without any communicative pointing, gesturing, or vocalizations. Other "independent" children make no demands or requests of the parents, but rather climb at a young age and acquire the desired object for themselves.[2, 4]

A.2b. In Individuals with Adequate Speech, Marked Impairment in the Ability to Initiate or Sustain a Conversation with Others.

Some children with autism speak relatively fluently, but are unable to engage in a conversation. In a conversation, Partner A makes a statement in turn on the given topic that is directed at Partner B, who then makes another statement directed back at Partner A, which is continued over more than one cycle of turn-taking. Questions may be included, but they are obviously not the dominant sentence structure used in conversation. A hallmark of verbally fluent autistic children is their inability to initiate or sustain a conversation on a topic of mutual

interest, although they may be able to respond relatively well, or ask a myriad of questions, or talk “at” another person in a monologue or soliloquy about their favorite topic. [2, 4]

A.2c. Stereotyped and Repetitive Use of Language, or Idiosyncratic Language.

A hallmark of autistic speech is immediate or delayed echolalia. Immediate echolalia refers to immediate repetition of words or phrases spoken by another,the children are simply repeating exactly what was heard without formulating their own language. It is important to realize that immediate echolalia is a very crucial aspect of normal language development in infants under the age of 2 years. It becomes pathologic when it is still present as the sole and predominant expressive language after the age of about 24 months, and can often be present throughout the preschool or school-age years in children with autism. It is imperative to differentiate speech that consists predominantly of immediate echolalia from the more classic picture of immediate echolalia progressing rapidly to spontaneous phrase speech in typically developing toddlers. Delayed echolalia or scripts refer to the use of ritualized phrases that have been memorized (e.g., from videos, television, commercials, or prior overheard conversations). Many older children with autism incorporate the scripts in appropriate conversational context, which can give much of their speech a “rehearsed” and often more fluent quality relative to the rest of their spoken language. Children also show difficulties with pronouns or other words that change in meaning with context, and often reverse pronouns or refer to themselves in the third person or by name. Others may use literal idiosyncratic phrases or neologisms. Verbal children with

autism may speak in very detailed and grammatically correct phrases, which are nonetheless repetitive, concrete, and pedantic. These children typically answer factual questions correctly and appropriately, but when asked a question that requires understanding concepts or concept formation, they give details that are often only tangentially related to the actual question.[2, 3]

A.2d. Lack of Varied, Spontaneous Make-Believe, or Social Imitative Play Appropriate to Developmental Level.

Some children with autism do not use miniature objects, animals, or dolls appropriately in pretend play. Others use the miniatures in a repetitive mechanical fashion without evidence of flexible representational play. Some highly verbal children may invent a fantasy world which becomes the sole focus of repetitive play.[4] A classic example of the lack of appropriate play is the verbal autistic preschooler who "plays" by repeatedly reciting a soliloquy from 'Beauty and the Beast' while manipulating dollhouse characters precisely according to the script. When given the same miniature figures and dollhouse, but instructed to play something other than 'Beauty and the Beast', this same child is incapable of creating any other play scenario.[2]

A.3. Restricted, Repetitive, and Stereotypic Patterns of Behaviors, Interests, and Activities

Again, this category of stereotyped behaviors and interests, like the previous ones, encompasses qualitative deficits in several behaviors.

A.3a. Encompassing Preoccupation with One or More Stereotypic and Restricted

Patterns of Interest That Is Abnormal in Intensity or Focus.

Some verbal children with autism ask the same question repeatedly,regardless of what reply is given, or engage in highly repetitive perseverative play. Others are preoccupied with unusual special interests. For example, many children are fascinated with dinosaurs, but children with autism may not only amass exhaustive facts about every conceivable type of dinosaur, but also about which museums house which particular fossils, and so forth; these children will often repeatedly "share" their knowledge with others regardless of the others' interest or suggestions to the contrary.[2]

A.3b. Apparently Inflexible Adherence to Specific Nonfunctional Routines or Rituals.

Many children with autism are so preoccupied with "sameness" in their home and school environments or with routines,changed can lead to a tantrum or other emotional disturbance. Some may, for example, insist that all home furnishings remain in the same position, or particular colour of all clothing. Others may eat only from a specific plate when sitting in a specific chair in a specific room, which may not necessarily be the kitchen or dining room. Some children may insist on being naked while in the home, but insist on wearing shoes to the dinner table. This inflexibility may also extend to familiar routines, for example, taking only a certain route to school, or entering the grocery store only by one specific door, or never stopping or turning around once the car starts moving.[2]

A.3c. Stereotyped and Repetitive Motor Mannerisms (e.g., Hand or Finger Flapping or Twisting or Complex Whole-Body Movements).

Some children will have obvious stereotypic motor movements, such as hand clapping or arm flapping whenever excited or upset, which is pathologic if it occurs after the age of about 2 years. Running aimlessly, rocking, spinning,bruxism, toe-walking, or other odd postures are commonly seen in children with autism. Others may simply repetitively tap the back of their hand in a less obtrusive manner. It has been noted that, in higher function-ing youngsters, the stereotypic movements may become "miniaturized" as they get older into more socially acceptable behaviors, such as pill-rolling. It is also important to realize that not all children with autism have repetitive motor movements.[6]

A.3d. Persistent Preoccupation with Parts of Objects.

Many children demonstrate the classic behavior of lining up their toys, videotapes, or other favored objects, but others may simply collect "things" for no apparent purpose. Many engage in repetitive actions, such as opening and closing doors, drawers, or flip-top trash cans, or turning light switches off and on. Others are fascinated and repetitively flick strings, elastic bands, measuring tapes, or electric cords. Younger children with autism are often particularly fascinated with water, and they especially enjoy transferring water repetitively from one vessel into another. Some may taste or smell items. Others love spinning objects, and may either spend long periods spinning the wheels of a toy car or watching ceiling fans, or spinning themselves until they fall from dizziness. Some children will often look at objects out of the corner of their eyes.[2]

2.1.2 Asperger Disorder

Asperger, a pediatrician, described four children with "autistic psychopathy," who had presumably milder autistic behaviors and normal IQ. The diagnostic term was included for the first time in DSM-IV [1] , and the criteria for the qualitative impairments in social interaction, and restrictive and repetitive patterns of behaviors and activities are identical to those for Autistic Disorder. In contrast to the Autistic Disorder criteria which include deficits in verbal and nonverbal communication and play, Asperger criteria currently state that there is no evidence of "clinically significant" language delay, such that the child used single words by age 2 years, and communicative phrases by age 3 years.[1] Normal or near-normal Intelligence Quotient (IQ) is also the rule, including self-help skills, "adaptive behavior (other than in social interaction), and curiosity about the environment in childhood." The language in Asperger's is clearly not typical or normal. Individuals with Asperger disorder usually have pedantic and poorly modulated speech, poor nonverbal pragmatic or communication skills, and intense preoccupations with circumscribed topics such as the weather or railway timetables.[7] Their speech is often concrete and literal, and their answers often "miss the point." Some clinicians have mislabeled individuals with this speech pattern as having a Semantic-Pragmatic Language Disorder rather than Asperger's or autism.[8] However, this diagnosis of a language disorder is not an appropriate substitution for the diagnosis of autism, as it does not account for the social deficits and restrictive, repetitive interests. Socially, individuals with Asperger disorder are usually unable to developed friendships. Because of their naive, inappropriate one-sided social interactions, they are also often ridiculed by their

peers. Often they cease their attempts because of the cruel ridicule, and remain extremely socially isolated. Yet, they honestly desire success in interpersonal relationships, and are often quite puzzled when they do not succeed.[9] They often have both fine and gross motor deficits, including clumsy and uncoordinated movements and odd postures.[10] Recent retrospective review of the original four Asperger cases reported that these children actually meet current DSM-IV[1] criteria for Autistic Disorder.[11] As DSM-IV is now written, if criteria for Autistic Disorder are met, this precludes a diagnosis of Asperger disorder.[4]

2.1.3 Atypical Autism/ PDD Not Otherwise Specified (PDD-NOS)

These diagnoses are used when clinically significant autistic symptomatology is present, including deficits in reciprocal social interactions, verbal or nonverbal communication, or stereotyped behavior, interests, and activities, but full criteria are not met for an alternative specific diagnosis under the autistic spectrum or pervasive developmental disorder (PDD) umbrella; for example, in a child who does not meet the required total of 6 of the possible 12 criteria for the diagnosis of Autistic Disorder, or who had symptom onset after age 36 months. Also, children whose symptoms are atypical or not as severe would be coded under this diagnosis.[1] Atypical autism/ PDD-NOS is not a distinct clinical entity with a specific definition, although individuals given this diagnosis are traditionally thought to have milder symptoms. PDD-NOS is a diagnosis by exclusion of the other autistic spectrum disorders.[12] It is often used as a "default" or "wastebasket" diagnosis when either insufficient or unreliable information is

available, or when the practitioner is hesitant to use the term “autism.” Screening and diagnostic procedures for atypical autism/PDDNOS is the same as for the other autistic spectrum disorders,as is management.[2]

2.1.4 The Diagnosis of Autism Spectrum Disorder

In evaluating a child, clinicians rely on behavioral characteristics to make a diagnosis. Some of the characteristic behaviors of ASD may be apparent in the first few months of a child’s life, or they may appear at any time during the early years. For the diagnosis, problems in at least one of the areas of communication, socialization, or restricted behavior must be present before the age of 3. The diagnosis requires a two-stage process. The first stage involves developmental screening during ‘well child” check-ups; the second stage entails a comprehensive evaluation by a multidisciplinary team.[2]

Comprehensive Diagnostic Evaluation

The second stage of diagnosis must be comprehensive in order to accurately rule in or rule out an ASD or other developmental problem. This evaluation may be done by a multidisciplinary team that includes a psychologist, a neurologist, a psychiatrist, a speech therapist, or other professionals who diagnose children with ASD. Because ASD’s are complex disorders and may involve other neurological or genetic problems, a comprehensive evaluation should entail neurologic and genetic assessment, along with in-depth cognitive and language testing.[3] In addition, measures developed specifically for diagnosing autism are often used. These include the Autism Diagnosis Interview-Revised (ADI-R)[13] and

the Autism Diagnostic Observation Schedule (ADOS-G).[14] The ADI-R is a structured interview that contains over 100 items and is conducted with a caregiver. It consists of four main factors—the child's communication, social interaction, repetitive behaviors, and age-of-onset symptoms. The ADOS-G is an observational measure used to "press" for socio-communicative behaviors that are often delayed, abnormal, or absent in children with ASD.Another instrument often used by professionals is the Childhood Autism Rating Scale (CARS).[15] It aids in evaluating the child's body movements, adaptation to change, listening response, verbal communication, and relationship to people. It is suitable for use with children over 2 years of age. The examiner observes the child and also obtains relevant information from the parents. The child's behavior is rated on a scale based on deviation from the typical behavior of children of the same age.[2]

2.1.5 Causes and Treatment of Autism Spectrum Disorder

2.1.5.1 Biological Basis of ASD

Genetic

The researchers at the Institute of Psychiatry in London found that in identical twin there was 60 % chance of the second twin would develop autism. In contrast to non-identical twins,there was no increase at all in the chance of the second twin to develop autism. However, it also suggest that environmental factors also involved due to the fact that not every identical twin of an autistic child also develops autism.[16]

Research is now being conducted all over the world to determine specific

genes that increase the likelihood of someone developing autism. A group known as The International Molecular Genetic Study of Autism Consortium has pinpointed four chromosomes (number 2,7,16 and 17) which play critical roles in autism. The previous studies had also been identified the evidence for involvement of chromosomes 2 and 7. Chromosome 2 plays an important role in early brain development and chromosome 7 is known to be associated with many language disorders.[17, 18]

About 25% of ASD cases are associated with genetic abnormalities including the Fragile X syndrome, Angelman syndrome, tuberous sclerosis, and metabolic disorders such as phenylketonuria syndrome.[4] Genetic predisposition to environmental toxicity is consistent with differential representation of alleles of detoxification genes in ASD subjects versus controls.These genes include methylene tetrahydrofolate reductase (MTHFR), reduced folate carrier (RFC), transcobalamin II (TCN2), catechol O methyl transferase(COMT) and glutathione sulfotransferase GSTM1.[19, 20] Skew in allele frequencies for paraoxonase (an organophosphate detoxifying enzyme, PON1)[21] and glyoxalase I (GLO1)[22] in ASD is suggestive of chemical exposure,while bias of ferroportin (SLC40A1/SLC11A3) and metal-activated transcription factor (MTF1) alleles is consistent with heavy metal involvement.[23]

Parental Age

The published research found that older parents, both mothers and fathers, are more likely to have a child with an autism spectrum disorder (ASD). The study results suggest that mothers aged 35 or older have a 30% greater chance of

having an autistic child compared to mothers aged 25 to 29, while fathers older than 40 had a 40% higher risk than those aged 25 to 29. In addition, the study noted that firstborn children were the most likely to be affected by ASD.While firstborn offspring of 2 older parents being 3 times more likely to develop autism than third or later-born offspring of mothers aged 20–34 and fathers aged less than 40.[24]

ASD are elevated in the children of older mothers [25, 26], though other studies have queried this finding.[27, 28] As we previously discussed that chemical agents are accumulated in maternal fatty tissue.The age-effect could relate to this accumulated toxic exposure. The possibility of ASD in offspring might result from accumulated toxicity over more than one generation.[29]

Early brain damage

More children with autism than in the general population have suffered brain damage in pregnancy,during delivery or in the postnatal period.Gestational exposure to anticonvulsant medication [30], tobacco [31], alcohol [32] or cocaine [33] , as well as thalidomide [34], predisposes to ASD development in the child. In animal model, respiratory infection during pregnancy (but without fetal infection) was associated with later behavioral deficits in progeny reminiscent of autism.[35]

Children who suffer certain infections, such as rubella in pregnancy or herpes virus infection in the first few years of life,appear to be at much increased risk for the development of autism.[4] Some rare ASD cases have been attributed to congenital infection with rubella [36] or Cytomegalovirus.[37] Early postnatal oxygen deprivation in rats causes later autism-like behavior.[38] A small group of children

injured by perinatal hypoxia (and with selective limbic damage) developed severe infantile autism.[39]

The temporal lobes, the brain stem and the cerebellum are affected in many ASD cases and these areas are important for the development of social and communicative interaction. The brain stem is crucial for incoming sensory stimuli. Abnormalities are also seen in cerebellum of autistic brain. The cerebellum is involved in coordinating motor movements and postural control. It also has functions in the subserving of social interaction.[4] Limbic/temporal lobe damage has been linked to ASD development.[40, 41] Hauser et al suggested that dysfunction of the medial temporal lobe is a major factor in the pathogenesis of infantile autism.[42] Bilateral hippocampal dysfunction in early life is associated with a profound failure of cognitive capacities, including language learning and the acquisition of complex social and adaptive skills.These deficits correspond to the cognitive deficits of severe infantile autism.[39] Abnormalities of the medial temporal lobes,encompassing the limbic hippocampal formation and amygdala, were argued to underlie the cognitive,perceptual and language impairments of ASD.[43] The amygdale appears to have important role in the coordination of social interaction.[4]

The limbic brain (hippocampus, amygdala and functionally related brain regions) is vulnerable to toxic insult. The damage of the hippocampus can caused by specific agents include bacterial toxins [44], vitamin B1 deficiency [45], excess homocysteine [46], hepatic encephalopathy [47], copper deficiency [48], glucocorticoid

excess or deficiency [49], ethanol exposure [50], oxygen deprivation [51] and irradiation.[52]

Gluten and Casein

Gluten is a protein found in grains such as wheat, rye, oats and barley. Casein is a milk protein found in the milk of animals such as cow,sheep and goat. Autistic people often have gut problems including frequent gut dysbiosis. As a result, digestion is impaired resulting in the incomplete digestion of gluten and casein. It end up as peptides (protein building blocks) with a chemical structure that resembles of the opiates. These peptides can pass easily through the blood-brain barrier and interfere with the functioning of neurotransmitters such as Serotonin and dopamine, just as the opiate drugs, resulting neurological and psychological symptoms. Several published research showed that gluteomorphin and caseomorphin (the offending peptides) have been detected in the urine of autistic children.[53, 54]

Yeast/Candida

Researchers at the Centers for Disease Control found that people suffering from ASD (and other environmental illnesses) have elevated levels of certain organic acids in their urine. They found that tartaric acid level, substance is primarily produced by intestinal yeast as well as citramalic acid and 3-oxoglutaric acid, in autistic children higher than the normal value.They also got evidence that Nystatin treatment for 2 year old boy with autism was improved his eye contact and decrease his tartaric acid level.[55]

Another study found that a large percentage of autistic children have significant immune dysfunction and this may include a genetic weakness that impairs the body's ability to kill yeast as well as deficiencies of Immunoglobulin G (IgG) and Immunoglobulin A(IgA). IgA antibodies are responsible for killing pathogens in the gastrointestinal tract.Recent research has strongly suggested that D-arabinitol may be a candidate for definitive marker to detect the presence of increased growth of yeast in the intestines. D-arabinitol is a 'sugar alcohol' produced by yeast when they feed on a sugar called arabinose [56, 57].

Evidence for the role of yeast in ASD is the fact that the increase in these illnesses has paralleled the increase in the use of antibiotics which tend to wipe out beneficial bacteria in the gut allowing yeast such as Candida to proliferate [58].

Immune Dysfunction

Study has reported significant changes for immune responses in children with autism. These changes demonstrate dysregulation of the immune system (deficiency in some components of the immune system and excesses in others). Certain genes in the major histocompatibility complex (that regulates immune responses) are involved in autism.[59] Jyonouchi H et al, 2001 found excessive innate immune responses in ASD patients that indicated by TNF-alpha production. Peripheral blood mononuclear cells (PBMCs) of ASD patients produced TNF-alpha, IL-1beta,and/or IL-6 higher than control with lipopolysaccharide (LPS) as a stimulant (for innate immunity). Meanwhile, without stimuli ASD PBMCs produced higher levels of proinflammatory/counter-regulatory cytokines than control. With stimulants of phytohemagglutinin (PHA),

tetanus, IL-12p70, and IL-18, PBMCs of ASD patients produced high value of TNFalpha depending on stimulants. [60]

Study conducted by Vargas DL et al, 2005 have shown that certain cells in the brains of many autistic children are abnormally active. Microglia and astroglia are cells that produce pro- and anti-inflammatory cytokines that support nerve conduction and normal neuronal development. In autistic children, neuroglial cells produce an unbalanced cytokines. This can lead to inflammatory changes in the brain, which in turn can affect development and behavior.[61]

Autistic children exhibited abnormal cytokine responses to a variety of agents. The immune cells from autistic children responded in markedly different patterns than cells from control subjects when challenged with tetanus toxoid, bacterial antigens, plant allergens, and a preparation of the mumps, measles, and rubella vaccine (MMR). In response to bacteria, there is lower level of cytokines, which function as mediators of the immune response, carrying messages between B, T and other immune cells, in autism group. Immune system responses to PHA, in contrast, produced more varied cytokine levels: Higher levels of certain cytokines and lower levels of others. These findings hypothesized that children with underlying immune dysfunction can respond atypically to environmental stimuli.[62]

Recent study has demonstrated that the immune cells of autistic children are more highly activated than non-autistic children. In autism, the levels of both activating and suppressing cytokines are higher than normal, indicating a possible hypersensitivity to environmental exposures. In the other hand, levels of

interleukin-10 (IL-10), a critical inhibitory cytokine, were actually lower than expected in the same study subjects. This indicates a failure in the immune system's regulatory or balancing function.[63]

Heavy Metal Toxicity

Another finding in autistic children is a higher level of heavy metals than normal. Mercury is a known neurotoxin and could be especially harmful to the developing brains of young children. One source of mercury exposure in early life is through vaccinations. Burbacher et al found that ethylmercury (the form found in thimerosal in vaccines) had a higher affinity for the brain than other forms [64]. Mercury also disrupts biochemistry and can result in dysfunction of multiple enzyme systems and damage to cell membranes and many proteins involved in all bodily functions. There is evidence of a heavy metal mobilization deficiency in affected children. Hair is a significant export route for mercury, but mercury was largely absent from first baby hair of children who diagnosed with ASD. Levels of mercury varied inversely with severity of the disorder. The children with the least hair mercury were subsequently most severely affected [65]. Early-life (gestation/lactation) exposure to mercury has been expressed as an algorithm of maternal dental amalgams and sea-food consumption [65].

Mercury (Hg) is known to increase oxidative stress. Hg coupled with decreased levels of antioxidants (low glutathione and antioxidant enzymes), leading to the increased production of free radicals [66]. This can cause an increase in lipid peroxidation, protein oxidation, and DNA oxidation. Those are leading to increase oxidative stress. Increased oxidative stress results in impaired neuronal

development, decreased prostaglandin production, increased inflammatory response, altered immune response,impaired energy production, increased excitotoxicity, cell death, decreased synaptic efficiency and impaired serotonin receptor function. These combined effects lead directly to the pathogenesis and clinical presentation of ASD [66].

Mercury is not the only heavy metal that can cause health problems and vaccinations are not the only source of exposure to mercury. Other possible sources of heavy metal exposure are contaminated food and water supplies. Reduced metal levels in hair of ASD children have been confirmed. Children may be especially at risk if they are unable to mobilize organometals [67].

Metals, like lead and tin, are deposited in bone over decades. During pregnancy and lactation, turnover of maternal bone supplies the unborn child and infant with mineral [68]. About 5-10% of maternal skeletal mineral is lost during lactation [69]. If maternal heavy metal burden is significant, there will be toxic exposure of the unborn or breastfed child [70, 71].

Chemical exposure

Study has been conducted to determine the role of environmental chemicals in the development of autism spectrum disorder. Chronic exposure of toxic agents (xenobiotic agents) to a developing central nervous system may be the best model for defining the physiological and behavioral problem found in children with ASD. They was found that all of the children had liver detoxification profiles outside the normal range, indicating an increased toxic load on the liver. The results showed that 16 of the 18 children had levels of chemicals

exceeding the maximum safe limit for adults. In the 2 children where high levels of chemicals weren't detected directly, they were found to have raised D-glucaric acid in their urine which is an indicator of a high level of toxins being metabolized by the liver [72].

In a report published 2004, 'toxic contaminants' are linked to increases in the prevalence of attention deficit hyperactivity disorder, autism, and associated neurodevelopment al and behavioral problems in developed countries. The author also suggests that exposure of the foetus to chemicals while still in the womb could lead to development of these disorder [73].

Chemical exposure is also widespread with high levels in human organs. These agents accumulate predominantly in fatty tissues [74]. Maternal lipids, like metals and minerals, are passed to the child during gestation and lactation. Mobilization of accumulated lipid-soluble residues is likely to contribute to early-life exposure [75].

Oxidative stress

There is evidence supporting the role of oxidative stress in autism [76] that affect brain development during gestation or possibly after gestation, contributing to expression of autism [77]. Studies reported low plasma total GSH (tGSH) levels, elevated levels of oxidized GSH (GSSG) and low ratios of tGSH to GSSG in autism [19, 78]. Levels of transferrin (iron-binding protein) and ceruloplasmin (copper-binding protein), major antioxidant proteins in the serum, are significantly reduced in autistic children as compared to their developmentally normal non-autistic siblings [66].

Oxidative stress is defined as a persistent imbalance between antioxidants and pro-oxidants often resulting in irreversible cellular damages, occurs when cellular antioxidant defense mechanisms fail to counterbalance and control endogenous reactive oxygen and nitrogen species (ROS/RNS) generated from normal oxidative metabolism or from pro-oxidant environmental exposures.Recent reviews support the hypothesis that oxidative stress contributed to the pathology of autism [66, 79]. Higher plasma and red blood cell levels of pro-oxidant nitric oxide have been reported in autistic children [80, 81]. Several reports have documented that in autistic children levels of antioxidant enzymes, glutathione peroxidase and superoxide dismutase (SOD1), are lower relative to controls. Since these increased level the biomarkers of oxidative stress were documented in autism, it is possible that oxidative stress contributes to the cause or a consequence of having autism [81].

Under normal conditions, a dynamic equilibrium exists between the production of reactive oxygen species (ROS) and the antioxidant capacity of the cell [82]. ROS includes superoxide, hydroxyl, peroxyl, alkoxy, and nitric oxide (NO) free radicals. Some endogenous enzymes such as xanthine oxidase (XO), NO synthase, and monoamine oxidase (MAO) can directly produce ROS [82]. Normally, the ROS within the cells are neutralized by antioxidant defense mechanisms. The primary enzymes involved in direct elimination of ROS are superoxide dismutase (SOD), catalase, and glutathione peroxidase (GPx), whereas glutathione reductase and glucose-6-phosphate dehydrogenase are secondary antioxidant enzymes, which help in maintaining a steady concentration of glutathione (GSH) and

nicotinamide adenine dinucleotide phosphate (NADPH) necessary for optimal functioning of the primary antioxidant enzymes [83].

Glutathione S-transferases (GSTs), an important class of antioxidant enzymes originating from a group of genes, play important role to catalyze conjugation of Glutathione (GSH) to toxic electrophiles for protecting cells against reactive oxygen metabolites. These enzymes detoxify a broad range of substances including carcinogens, environmental toxins, and drugs.[84]

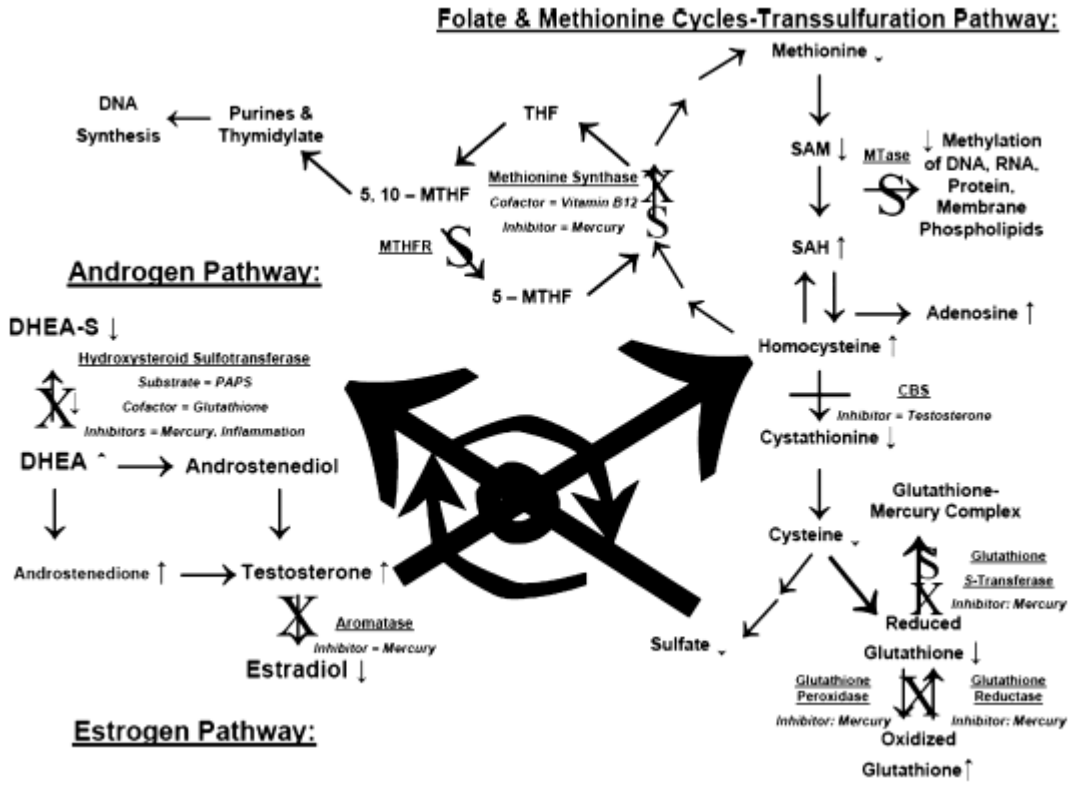


Figure 1. Biochemical abnormalities found in autistic disorders[85]
CBS,cystathioneβ-synthase;DHEA-S,dehydroepiandrosterone sulphate; DHEA,dehydroepiandrosterone;PAPS, 3'-phosphoadenylylsulphate; MTase, methyl transferases;MTHFR, methylenetetrahydrofolate reductase;SAH,S-adenosylhomocysteine;SAM,S-adenosylmethionine;THF, tetrahydrofolate;5-MTHF, 5-methyltetrahydrofolate; 5,10-MTHF, 5,10-methyltetrahydrofolate; SAHH, SAH hydrolase; X, Hg inhibition; S, genetic susceptibility sites associated with autistic disorders; ____ , androgen inhibition.

2.1.5. Treatment

2.1.5.1 Dietary

Dietary interventions are based on the idea that food allergies cause symptoms of autism, and an insufficiency of a specific vitamin or mineral may cause some autistic symptoms. A diet that some parents have found was helpful to their autistic child is a gluten-free, casein-free diet.[86, 87] Gluten is a casein-like substance that is found in the seeds of various cereal plants—wheat, oat, rye, and

barley. Casein is the principal protein in milk. Since gluten and milk are found in many of the foods we eat, following a gluten-free, casein-free diet is difficult.[86]

A supplement that some parents feel is beneficial for an autistic child is Vitamin B6, taken with magnesium (which makes the vitamin effective).[88, 89] The result of research studies is mixed; some children respond positively, some negatively, some not at all or very little.[90]

In the search for treatment for autism, there has been discussion in the last few years about the use of secretin, a substance approved by the Food and Drug Administration (FDA) for a single dose normally given to aid in diagnosis of a gastrointestinal problem. Anecdotal reports have shown improvement in autism symptoms, including sleep patterns, eye contact, language skills, and alertness. Several clinical trials conducted in the last few years have found no significant improvements in symptoms between patients who received secretin and those who received a placebo.[91]

2.1.5.2 Medications

Medications are often used to treat behavioral problems, such as aggression, self-injurious behavior, and severe tantrums, that keep the person with ASD from functioning more effectively at home or school. These usually have some effect on undesireable symptoms and also on behaviour and academic functioning in school.[4]

Anxiety and depression.

The selective serotonin reuptake inhibitors (SSRI's) are the medications most often prescribed for symptoms of anxiety, depression, and/or obsessive-compulsive disorder (OCD). SSRI's that have been approved for OCD are fluoxetine, fluvoxamine, sertraline and clomipramine.[92] Buspirone is serotonin partial agonist that is used for the treatment of anxiety, If patients do not respond to buspirone,we can try fluoxetine, sertraline, fluvoxamine, or paroxetine.[93] Treatment with these medications can be associated with decreased frequency of repetitive, ritualistic behavior and improvements in eye contact and social contacts.[4]

Self-destructive and aggressive behaviours

Drugs such as haloperidol or pimozide are needed to decrease hyperactivity or self-destructive behavior and often have some positive effects on socialization and learning.[4] Individuals who frequently exhibit aggressive behaviors and do not respond to behavioral interventions, risperidone or haloperidol may be the drug of choice. Patients who fail to respond to risperidone or haloperidol treatment,may be considered to treat with trazodone, carbamazepine, valproate, lithium, or propranolol.[93] Atypical neuroleptics such as olanzapine and clozapine, may useful when medication for aggression and other severe behaviour problems is required.[4]

Unusual Sleeping Patterns

Tsai found that about 30% of children with autism have moderate to severe sleep problems.[94] Melatonin may be considered as the first choice. Some

autistic children may respond to antihistamines, such as diphenhydramine and hydroxyzine, or clonidine. Imipramine, trazodone, or zolpidem may be considered in more severe cases.[93]

Enuresis

Some children with autism tend no respond to toilet training before age 7. In the treatment of enuresis, imipramine and desmopressin may be considered in children more than 7 years old who fail to respond to nonmedical intervention.[93]

Seizures.

Carbamazepine and valproic acid are effective anticonvulsants drugs with lowest frequency of major side-effects. Benzodiazepines such as clonazepam and nitrazepam are sometimes very effective to reduce seizure but may contribute to increase withdrawal and bizarre behaviours/ritualism in autism. If possible, their use should be restricted, except in emergency situations. Diazepam may be very effective during acute seizure, but should not be used for prophylaxis. The level of the medication in the blood should be monitored carefully and adjusted so that the least amount possible is used to be effective.[4]

Inattention and hyperactivity.

Clonidine, guanfacine, or imipramine may be considered in low or middle functioning autism with or without other neurological disorders, such as seizure. If patients do not respond to clonidine, guanfacine, or imipramine,we may consider naltrexone.[93] Central stimulants have beneficial effects on severe hyperactivity in autism,at least in individuals with IQ above 50. Side effects are generally mild, but reduction of appetite can sometimes be severe.[4] In high

functioning autism without other neurological disorders, stimulants, such as methylphenidate or dextroamphetamine, may be tried first.[93] Study reported that methylphenidate causes significant decrease in hyperactivity and impulsivity, but also have side-effect worsening of stereotypic movements.[95] This negative effect of stimulants occurs mostly in mentally retarded children with IQ below 45 or mental ages below 4.5 years.[96] Guanfacine, clonidine, or naltrexone may be considered in patients who do not respond to stimulants or in those who have other neurological disorders.[93]

2.1.5.4 Treatment of Heavy metal Toxicity

Supplemental minerals are needed such as calcium, magnesium, zinc, selenium, chromium, iron, molybdenum, and manganese, as well as sulfur and sulfhydryl compounds like glutathione and cysteine. Dietary sources of sulfur include garlic, onions, eggs, cruciferous vegetables (e.g., broccoli, brussels sprouts, cauliflower), and green leafy vegetables (e.g., kale, spinach, dandelion, endive).[97]

Nutritional agents that help with heavy metal toxicity include vitamin C [98], alginate, glutathione, methylsulfonylmethane (MSM), and minerals such as selenium and zinc. Amino acids and amino acid complexes, such as cysteine, methionine, seleno-methionine, S-adenosyl methionine (SAM), and alpha lipoic acid, all contain sulfhydryl groups and help chelate heavy metals out of the body.[99]

A chelating agent is used in a detoxification program to help pull heavy metal ions out of the body. A chelating agent is a substance that can form several bonds to a metal ion. EDTA is a chelation agent used to remove lead, cadmium, aluminium, and other metals from the body that given in an intravenous drip. It is important to supplement with multi minerals because beneficial minerals can be removed with chelation and to monitor renal function since EDTA is excreted through the kidneys.[100]

Deferoxamine is a chelating agent used for iron overload. DMPS is a chelating agent, used parenterally, for arsenic, mercury, and lead removal.It was found to be the most efficient chelation method for mercury removal from the kidneys.[101] DMSA is a water-soluble, oral form of chelation, effective for mercury, lead, cadmium, and arsenic. DMSA effectively removes inorganic and organic mercury from the blood, liver, brain, spleen, lungs, large intestine, muscles, bone and excreted in the urine as a cysteine-DMSA complex. The affinity of DMSA for metals is in this order: $Cd^{++} > Pb^{++} > Fe^{++} > Hg^{++} > Zn^{++} > Ni^{++}$.[99]

One study, using EDTA and DMSA, showed a decrease in tissue burden and an increase in urinary output of lead. The results of the combined therapy was better than each therapy alone. In addition, no increased burden of tissue metal toxicity was observed in the brain.[100]

2.1.5.5 Prevention of Heavy Metal Exposure

Heavy metals are common throughout the environment hence avoidance of exposure is important. Industries are urged to reduce or replace the heavy metals in their processes wherever possible. Exposure to environmental sources of lead, including lead-based paints, plumbing fixtures, vehicle exhaust, and contaminated soil [102], sources of mercury such as contaminated fish, industrial and agricultural wastes, cadmium : industrial waste, insecticides, old galvanized pipes as well as sources of arsenic : insecticides and industrial processes, some drinking water should be reduced or eliminated.[103]

Organic foods are recommended to avoid exposure to pesticides and chemicals. Detoxification diets include plenty of high-fiber foods, including oat bran and psyllium seeds, to help cleanse the digestive tract. Apples, pears, and legumes are high in pectins, which are believed to have chelating effects on heavy metals. Foods high in antioxidants are recommended, such as fruits, vegetables, and fresh juices. Foods that may contain heavy metals are avoided, including many fish and shellfish, as weel as foods that may stress the immune system, such as processed foods, fried foods, sugar, fat, alcohol, caffeine , meat, and dairy products Factory-farmed chicken and eggs are avoided as well, because chickens are often fed fish meal.[97] Study in 2001 reported that eating tofu may reduce lead levels in the blood. Tofu is rich in calcium, which may help reduce the blood's ability to absorb and retain lead.[104]

2.2 GSTM1 and GSTT1 GENE

Inter-individual and inter-ethnic differences play an important role in determining chemical exposure risk and detoxification. Identification of the common genetic variants is relevant as their recognition may provide opportunities for screening and targeted reduction of modifiable environmental risk factors.[105]

Glutathione S-transferases (GSTs) are an important class of of antioxidant enzymes, originating from a group of genes,that catalyze conjugation of Glutathione (GSH) to toxic electrophiles for protecting cells against reactive oxygen metabolites.[84] GSTs are phase II enzymes that conjugate GSH to activated toxins,xenobiotics and metabolites including products of Phase I enzymes such as cytochrome P450 oxidases. Due to such detoxifying action, the GSTs detoxify a broad range of substances including carcinogens such as aflatoxin B_1 , 2-amino-1-methyl - 6-phenylimidazo[4,5-*b*]pyridine (PhIP), benzo[*a*]pyrene, 7,12-dimethylbenz[*a*]anthracene, 5-methylchrysene, and 4-nitroquinoline-*N*-oxide; heavy metals [106] ; pesticides such as alachlor, atrazine, dichlorodiphenyltrichloroethane (DDT), lindane, and methyl parathion; the oxidative-damage products acrolein, base propenals, cholesterol α-oxide, fatty acid hydroperoxides, and 4-hydroxynonenal**;** the anticancer drugs 1,3-*bis*(2-chloroethyl)-1-nitrosourea (BCNU), chlorambucil, cyclophosphamide, melphalan, thiotepa and fosfomycin.[107]

At least seven distinct classes of soluble GSTs, that are highly expressed in the mammalian liver, have already been identified[108] which are alpha, mu, pi,

sigma, theta, kappa, and zeta. GSTM1 and GSTT1 are known to be highly polymorphic in different population. This genetic variation may change an individual's susceptibility to carcinogens and toxins, as well as affect the toxicity and efficacy of certain drugs.[107]

GSTM1 is located on chromosome 1p13.3 and is a homologous recombination involving left and right 4.2-kb repeats, resulting in a 16-kb deletion containing the entire GSTM1 gene. GSTT1 is located at 22q11.2 and, like GSTM1, is a deletion produced by a homologous recombination event involving left and right 403-bp repeats, resulting in a 54-kb deletion containing the entire GSTT1 gene [109].

Homozygous deletions of these genes, referred to as GSTM1 null and GSTT1 null, respectively, result in lack of enzyme activity. Subjects with at least one functional allele for GSTM1 and GSTT1 are grouped into the positive conjugator types and are designated as GSTM1-positive and GSTT1-positive, respectively [110]. The deleted genotypes that lead to the inactive form of the enzymes are named as ''GSTM1-null'' and ''GSTT1-null''. Associations of GSTM1 and/or GSTT1-null genotypes autism[20] have been reported. Variability in the distribution of the null genotype of GSTM1 and GSTT1, due to total or partial gene deletion resulting in the lack of the active enzyme [111].

2.2.1 Prevalence of GSTM1 and GSTT1 gene variants

Case-control studies in the United States have reported the GSTM1 deletion genotype varying from 23%-41% for those of African descent; 32%-53%

for those of Asian descent, 40%-53% for those of Hispanic descent, and 35%-62% for those of European descent [112, 113]. In the South American, studies have reported frequencies of 21% for Chileans[114] and 55% for Caucasian Brazilians, 33% for black Brazilians, and 20% for Amazonian Brazilians [115]. Several population studies have reported the deletion polymorphism among U.S. Caucasians as ranging from 48%-57%[116-118] and 46% among the French [119]. A large cross-sectional study conducted among Italians reported a frequency of 53%[120]; two studies conducted in Hungary and the Slovak Republic measured frequencies of 44% and 50% respectively [121, 122]. Groups such as Pacific Islanders and Malasians have a reported frequency of 62%-100%. Frequency for Japanese is ranging from 48%-50% and 35%-63% for Chinese [123]. A population-based study conducted among Chinese reported a frequency of 51% for the GSTM1 deletion genotype [124]. Two Korean case-control studies found frequencies of 53% and 56% for the GSTM1 deletion genotype [125]. GSTM1 null frequency of Indian population is 30,4–35,4%,lower than other Asian and Caucasian population (49–53,8%) [126].

Studies in the United States reported that GSTT1 deletion polymorphism is less common than the GSTM1 deletion polymorphism. The Frequency among those of European ancestry is 15%-31%, African descendent ranging from 22%-29% while those of Hispanic origin ranging from 10%-12% [112, 113, 116, 118]. European studies have reported that the GSTT1 deletion genotype was present among 21% of Italians and 28% of Slovakians [120, 122]. One South American study found that 11% of Amazonian Brazilians and 19% of both Caucasian and black

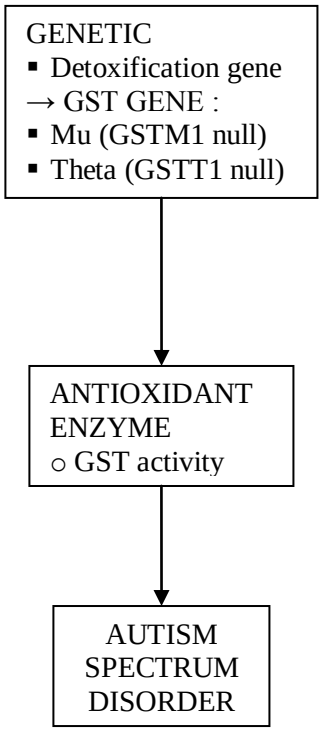
Brazilians had the deletion genotype [115]. Asians have the highest reported GSTT1 deletion genotype. One study reported 58% of Chinese and 38% of Malaysians have the GSTT1 null genotype[127]; two case-control studies repoerted 42% and 46% among Koreans [128, 129]. However,population-based study conducted among Chinese found a prevalence of 46% for the GSTT1 deletion genotype [124].

2.2.2 Autism and the GSTM1 gene

The vast majority of cases of autism are unrelated to known teratogens but the phenotypic expression of autism may be affected by the interaction of environmental factors with multiple gene loci. There is evidence supporting a role for oxidative stress in autism [76]. Oxidative stress could interact with common functional polymorphic variants of genes that protect against oxidative stress and could thus affect brain development during gestation or possibly after gestation, contributing to expression of autism. Glutathione (GSH) is the most important endogenous antioxidant due to its ability to bind electrophilic substrates through its free sulfhydryl group[77] and is the most abundant non-protein thiol,occurring in millimolar concentrations in human tissues. Low plasma total GSH (tGSH) levels, elevated levelsof oxidized GSH (GSSG) and low ratios of tGSH to GSSG have been reported in autism [130]. Some genetic polymorphisms of GSTs are known to affect enzyme function [131]. It is possible that a functional GST polymorphism potentiated by reduced levels of GSH (one of the substrates of GSTs) could contribute to the pathogenesis of autism [20].

Case-parent trios study along with controls, gives evidence of a heightened risk for autism with GSTM1*0 homozygotes. Absence of the GSTM1 gene in GSTM1*0 homozygotes interacting with other genetic and environmental risk factors could lead to failure of individuals with autism to detoxify important compounds, including agents or products of oxidative stress [20]. This is consistent with the hypothesis of a gene-environment interaction that alters the expression of autism because GSTs are detoxification enzymes that conjugate absorbed xenobiotics [85]. These findings could lead to the mechanism of action of select environmental chemicals and identification of an exogenous or endogenous moiety interacting with GSTs to contribute to the phenotypic presentation of autism [20].

2.4 CONCEPTUAL FRAME



2.5 HYPOTHESIS

1. GSTM1 null gene is risk factor of ASD.
2. GSTT1 null gene is risk factor of ASD.
3. Low erythrocyte GST activity is risk factor of ASD.
4. Enzyme activity of Glutathione s-transferase in ASD is lower than normal control.
5. There is association between GSTM1 and GSTT1 null genes with phenotype expression of ASD.
6. There is association between GSTM1 and GSTT1 null genes with Glutathione s-transferase activity in ASD.
7. There is association between Glutathione s-transferase activity with phenotype expression of ASD.