RESEARCH ARTICLE

A REVIEW ON APPROACHES TO DIAGNOSE THE AUTISM SPECTRUM DISORDER: THE NEED OF THE HOUR.

Kajal Singla¹, Abhinob Baruah¹, Shilpa S Chapadgaonkar¹ and *Nilanjan Das².

1. Department of Biotechnology, ManavRachna International University, Faridabad, India.
2. Accendere Knowledge Management Services Private Ltd., India.

Abstract

Autism spectrum disorder is a complex multigenic neurodevelopmental disorder with a prevalence of 1 in 68 children affecting male more than female (1 in 42 boys and 1 in 189 girls). In present study, focus has been put forth on several aspects of autism diagnosis. To that end association of certain biomarkers such as oxytocin, vasopressin, retinoic acid and 5-hydroxymethylcytosine has been discussed. As autism is also known to be caused by parental influence, prenatal stress, improper uterine environment, drugs taken during pregnancy and role of vertically transmitted diseases has also been given importance. Biological cause correlates autism with various other disorders such as schizophrenia, fragile X syndrome and mitochondrial disorders. Moreover, autism is known to be caused by more than 100 genes. Thus, a thorough emphasis has been given to highlight inter and intra-association of all the relevant diseases and biomarkers to give more insight regarding diagnostic measures of autism.

Abbreviations:

AD - Alzheimer's disease
ADHD - Attention deficit hyperactivity disorder
AMPAK - α-amino-3-hydroxy-5-methyl-4-isoazolepropionic acid receptor
ASD - Autism spectrum disorder
ALT - Alanine aminotransferase
AMPAK - Alpha-Amino-3-Hydroxy-5-Methyl-4-Isxazole Propionic Acid receptor
AST - Aspartate aminotransferase
AVP - Arginine vasopressin system
BDNF - Brain derived neurotrophic factor
cADPR - Cyclic ADP Ribose
CCL - Chemokine C-C motif ligand
CD - Creatin Deficiency
CD38- Cluster of differentiation 38
CDH9 - Cadherin
CDH10 - Cadherin 10
CHD8 - Chromodomain helicase DNA binding protein 8
CK - Creatine kinase

Corresponding Author:- Nilanjan Das.
Address:- Accendere Knowledge Management Services Private Ltd., India.
DLG - Disks large homolog
DSM - Diagnostic and Statistical Manual of Mental Disorders
DYRK1A - Dual- specificity tyrosine-(Y) - phosphorylation regulated kinase 1A
ETC- Electron transport chain
F2-IsoPs - F2t-Isoprostanes
FMR1 - Fragile X mental retardation
FXS - Fragile X syndrome
FXTAS - Fragile X-associated tremor/ataxia syndrome FXTAS
GABAA - Gamma-aminobutyric acid A receptor
GABAB - Gamma-aminobutyric acid B receptor
GABR - Gamma – aminobutyric acid type A receptor unit
GI - Galvanized iron
GluR - Glutamate receptor
GRIK1 - Glutamate Ionotropic Receptor Kainate Type Subunit 1
GRIN2B - Glutamate receptor, ionotropic, N- methyl D-aspartate 2B
GTP - Guanosine Tri-Phosphate
GWAS - Genome wide associated studies
HIV - Human immunodeficiency virus
HFA - High-functioning autism
ICSI - Intracytoplasmic sperm injection
ID - Intellectual disability
IFN- GAMMA- Interferon gamma
G Immunoglobulin - IgG
IgM - Immunoglobulin M
IL - Interleukin
IL-1RACP - Interleukin 1 receptor accessory protein
IL1RAPL1 - X-linked interleukin-1 receptor accessory protein-like 1
IVF- In vitro fertilization
KATNAL2 - Katanin p60 subunit A1 like 2
LTD - Long term depression
LCLs - Lymphoblastic cell lines
LTP - Long term potentiation
MAGUK - Membrane-associated guanylate kinase
MeCP2 - Encoding methyl CpG binding protein 2
MTHFR- Methylenetetrahydrofolatereductase
mTOR - Mechanistic target of rapamycin
NLGN - Neuroligin
NMDA - N-methyl-D-aspartate
NRXN1 - Neurexin 1
OXT - Oxytocin
OXTR - Human oxytocin receptor
PAX - Paired box
PICK1 - Protein interacting with C-linked kinase
PKU - Phenylketonuria
POGZ - Pogo transposable element with ZNF domain
PSD - Postsynaptic density protein
PTEN - Protein tyrosine phosphatase
RELN - Reelin
Rh- Rhesus factor
RhoA - Member A of the Ras homologue gene family
S100B - S100 calcium-binding protein B
SAH - S - adenosylhomocysteine
SAM - S - adenosylmethionine
SAP - Synapse-associated protein
SEMA5A - Semaphorin 5A
SHANK3 - SH3 and multiple ankyrin repeat domains 3
INTRODUCTION:
Autism is a complex multi-gene disorder having overlapping features with many other disorders [1]. The diagnosis of such a complex disorder at an early stage remains elusive even after decades of research. To brief up the entire scenario, focus has been given on two main aspects; one being the cause or risk factors for ASD and the other is the diagnostic criterion. Diagnosis of any disease can be made easier by identifying specific biomarkers, therefore a detailed explanation of various biomarkers and their role in ASD have been discussed. Various factors associated with the incidence of ASD which have been classified into three domains: chemical, environmental and biological influences. A summation of these two topics explains the overall complexity of the disorder and its diagnosis.

Diagnosis of any disorder at an early stage may lead to cure or up to a restricted level. Diagnosis of autism is of a greater challenge as it is known to be influenced by gene, environment and various enzymes and chemicals. The identification of specific biomarkers will help in identifying the disorder at its early stages. Biomarkers are biological characteristics observed in individual’s blood or tissues, which are indications of diseased or normal features. Different types of biomarkers are considered for the diagnostic purpose such as genetic, epigenetic, metabolic, oxidative stress, mitochondrial dysfunction, methylation, immune and maternal antibodies etc. Present study is intended to give a thorough overview of present scenario in autism diagnosis and to highlight the all possible aspects associated with autism suitable for futuristic precisely targeted biomarker research for autism diagnosis.

Diagnosis of Autism:-
The search for variations in the genetic makeup of an individual always has an edge over any other diagnostic method. Genetic biomarkers reveal any such change in the affected individual. Taking into account the higher concordance rate of ASD in monozygotic twins (92%) than in dizygotic twins (10%), literature supports a hereditary component in the susceptibility to ASDs [2], which implies gene has its role in ASD. More than 500 different genetic loci are known to play a role in ASD risk [3]. Genome wide associated studies (GWAS) were performed to identify de novo variations associated with ASD. Deletions at the Neurexin 1 (NRXN1) locus, duplications at 7q11.23, duplications at 15q11-13, and deletions and duplications at 16p11.2 were also found to be associated [3]. Earlier studies found rare, functional mutations in genes encoding for NRXN1, SHANK3 (SH3 and multiple ankyrin repeat domains 3 ), and SHANK2, all of which are proteins that affect the functioning of synapses and have been linked to other known genetic disorders [4]. Synapses are structures in brain that helps in transmission of signal between nerve cells. It has been found that inappropriate numbers and/or morphologies of synapses may lead to a too weakly or too strongly connected network. Such abnormal synaptic connectivity is frequently observed in mental retardation (MR), a condition present in 75% of individuals with ASD [5]. In addition, whole exome
Sequencing verified by several reports have found genetic mutations associated with autism including SNC2A, CHD8, DYRK1A, POGZ, GRIN2B, and KATNAL2 [6]. Common genetic variants on 5p14.1 that are associated with susceptibility to ASDs were also identified by a few other scientists. Among the common polymorphisms found, the methylenetetrahydrofolatereductase (MTHFR) polymorphism is one of the most widely studied genetic correlations with autism [7]. Many other GWAS were performed and a few other genes were identified which include: cadherin (CDH9), cadherin 10 (CDH10), semaphorin 5A (SEMA5A), and taste receptor, type 2, member 1 (TAS2R1), and are found on chromosome 5p14 [8]. Four other large, novel deletions on 2q22.1, 3p26.3, 4q12, and 14q23 that include new genes and regions linked to ASDs were also reported by a few other scientists. The presence of one or many such variant may cause ASD.

Even though concordant rates were observed in monozygotic twins, the severity of the disorder however varied which lead to the role of non-genetic epigenetic factors in ASD. Epigenetics is the study of heritable changes in gene activity that are not caused by changes in the DNA sequence; it also can be described as the study of stable, long-term alterations in the transcriptional potential of a cell that are not necessarily heritable. Epigenetic changes in ASD occur through methylation, histone modification [9], chromatin remodelling and transcriptional feedback loops [10]. Various studies have shown that DNA methylation differences can occur in many loci including AFF2, AUTS2, GABRB3, NLGN3, NRXN1, SLC6A4, UBE3A [9], the oxytocin receptor [11], MeCP2 (a cause for most cases of Rett syndrome) in the frontal cortex [12], and changed chromatin structure in prefrontal cortex neurons at hundreds of loci [13]. The severity of the autistic phenotype is related to DNA methylation at specific sites across the genome [10]. Environmental and physiological influences are important factors accounting for inter-individual DNA methylation differences, and these influences differ across the genome [14]. These epigenetic biomarkers explain the hereditary component of the disorder. There are no autism-defining, metabolic biomarkers, but examining the biomarkers of pathways associated with ASD points out to potentially treatable metabolic abnormalities and provides a baseline that can be tracked over time. Each child may have different metabolic pathologies related to SNPs, nutrient deficiencies, and toxic exposures. Examples of metabolic disorders that can lead to an autistic-like presentation include phenylketonuria (PKU) [15], disorders of purine metabolism [16], biotinidase deficiency [17], cerebral folate deficiency [18], creatine deficiency [19], and excess propionic acid (which is produced by Clostridium) [20, 21].

Oxidative stress can be detected by studying antioxidant status, antioxidant enzymes, lipid peroxidation, and protein/DNA oxidation, all of which have been found to be elevated in children with autism. Reduced levels of glutathione, glutathione peroxidase, methionine, and cysteine along with increased levels of oxidized glutathione are statistically different in ASD [22]. The level of antioxidants excreted in urine was found to be significantly lower than normal in autistic children. These findings were correlated with the severity of the ASD among individuals [23]. A list of biomarkers and their role have been discussed in brief: Plasma F2t-Isoprostanes (F2-IsopPs) are known to be the most sensitive indicator of redox dysfunction [24]. They are found in higher level in patients with ASD along with gastrointestinal dysfunction. Urine 8-OHdG is biomarker for oxidative damage to DNA. Decreased levels of major antioxidant serum proteins transferrin (iron-binding protein) and ceruloplasmin (copper binding protein) have also been observed in patients with ASD. Plasma 3-chlortyrosoine (3CT) and 3-Nitrotyrosine (3NT) levels were reportedly increased with age for those with ASD and mitochondrial dysfunction but not for those with ASD without mitochondrial dysfunction.

Mitochondrial dysfunction is marked by impaired energy production. Some children with ASD have been reported to have a spectrum of mitochondrial dysfunction of differing severity [25]. There is no reliable biomarker to identify all cases of mitochondrial dysfunction [26]. Mitochondrial dysfunction markers include lactate, pyruvate and lactate-to-pyruvate ratio, carnitine (free and total), quantitative plasma amino acids, ubiquinone, ammonia, CD, AST, ALT, and creatine kinase (CK) [25, 19]. Many studies of ASD reported elevations in lactate and pyruvate, or a decrease in carnitine, while others report abnormal alanine in ASD patients [25] or elevations in aspartate aminotransferase and serum CK [27].

The methylation pathway provides methyl groups for many functions, including the methylation of genes, which can result in the epigenetic changes of turning genes on and off. Impaired methylation may reflect the effects of toxic exposure on sulphur metabolism. Oxidative stress initiated by environmental factors in genetically vulnerable individuals can lead to impaired methylation and neurological deficits, both of which may contribute to the manifestation of autism [28]. A marker of methylation dysfunction is decreased SAM/SAH ratio in patients with ASD [29].
Immune biomarkers also play a major role in knowing the onset and severity of ASD. Cytokines are the immune biomarkers and increased level of plasma cytokine is known to exhibit regressive onset and severity of autistic and behavioural symptoms [30]. Altered pro-inflammatory cytokines, complement proteins, chemokines, adhesion molecules, and growth factors are also correlated with ASD. More specifically, altered TGF-beta, CCL2, and CCL5, IgM and IgG classes of immunoglobulin circulating levels are linked with a worsening of the behavioral scores [31]. An imbalance in Th1/Th2 is found as well, which may play a role in the pathogenesis of the autism [32]. Further research has shown that increase in $S100B$, a calcium binding protein produced primarily by astrocytes, is a biomarker that reflects neurological/brain damage in ASD and its severity [33].

It has been proposed that autoimmune autistic disorder is a major subset of autism [34], and autoimmunity may play a role in the pathogenesis of several disorders with these disorders [35]. Many auto-antibodies are known to be found in the nervous system of children with ASD [36, 37]. These can be measured as biomarkers in this subset of ASD patients. The anti ganglioside M1 antibodies [38], antineuronal antibodies [39], and serum anti-nuclear antibodies [39, 40] are associated with the severity of autism. Other auto-antibodies that are known to play a pathological role in autism include, anti neuron-axon filament protein (anti-NAFP) and glial fibrillary acidic protein (anti-GFAP) [41], antibodies to brain endothelial cells and nuclei [35], antibodies against myelin basic protein [42,43], and anti-myelin associated glycoprotein, an index for autoimmunity in the brain [44]. High levels of BDNF antibodies were found in ASD patients [45], and low BDNF levels may be involved in the pathophysiology of ASD [46].

There is always an understanding that when the mother is affected by bacteria or virus, the maternal antibodies cross the underdeveloped blood brain barrier of the fetus [47] leading to impaired fetal neurodevelopment and long-term neurodegeneration, neurobehavioral, and cognitive difficulties [48]. Such diseases are called vertically transmitted diseases.

Autism is a neuro-developmental polygenic disorder [1, 49] that affects people with an array of other disorders as well [1, 2, 50]. The actual cause of the disorder is abstruse. In the first half of the article, a focus has been given on identifying or categorizing the disorder with the help of biological markers. The second half will deal with the cause of such a disorder. For a better understanding, the causes have been classified in three main categories: 1) chemical causes 2) environmental causes and 3) other biological causes. Each of which has the influence of genes.

Knowing the influence of certain enzymes and chemicals in ASD is of absolute importance.

1. Chemical causes of Autism:-
1.1 GABA and mGluR5: Various reasons have been attributed to the cause of autism and one of which is the decrease in number of synapses with increasing age in autistic children [5]. Glutamate (an excitatory neurotransmitter) and GABA (gamma amino butyric acid), an inhibitory neurotransmitter are known to play a key role in development of synapses and neuroplasticity. Abnormalities in GABA-A receptor ion channels, GABA-B transmitter systems and G-protein coupled metabotropic glutamate receptor (mGLuR) have a link with ASD and its syndromic form. Studies have revealed the role of many signalling proteins that either directly or indirectly modulates mGLuRsignalling (such asneurexin, PTEN, mGLuR8, neuroligin 3 and 4). This also causes mGLuRdependant long term depression (LTD). GABA protein is a negative modulator of mGLuR pathway and stimulation of GABA-B has been responsible for decrease in synaptic release of glutamate [51]. However, genetic studies have a different approach towards this.

Genetic Understandings: Neurological and psychiatric disorders are often due to aberrant synapse formation, function, plasticity and malformed dendritic spine. Intellectual disorders are often genetically influenced and a lot of which are X-linked (e.g. alpha thalassemia X-linked intellectual disability syndrome). Two different mutations in the synaptic scaffold proteins have been reported; SHANK family and the MAGUK (Membrane-associated guanylate kinase) family of proteins. SHANK3 is located in chromosome 22 and codes for large postsynaptic scaffold protein SHANK3. It causes Phelan-McDermid syndrome or 22q13.3 deletion syndrome. SHANK genes cause alterations in synaptic morphology, signalling and behaviour characteristics.

Among the MAGUK family of proteins, postsynaptic density protein 95 (PSD-95) is coded by DLG4 (disks large homolog 4) gene in humans. DLG-3 codes for the synapse-associated protein 102 (SAP102) and is linked with ID. It causes premature STOP codons before PDZ domain. The truncated proteins bind to NMDA receptors and other proteins that regulate NMDA pathways.
Mutations in X-chromosome include genes such as IL1RAPL1, Oligophrenin-1 and TM4SF2 genes. The IL1RAPL1 gene (interleukin-1 receptor accessory protein-like 1 gene) has many mutations in patients with ID and ASD (non-syndromic). The IL1RAPL1 proteins belong to a new Toll/IL-1 receptor family and shares 52% homology with IL-1 receptor accessory protein (IL-1RaP). It is a post-synaptic protein and specifically binds to post synaptic density protein-95 (PSD-95). Thus alterations in these proteins due to mutations can cause ID and ASD. The Oligophrenin-1 gene mutations or deletions in the synaptic Rho GTPase-activating protein also cause several modifications. Oligophrenin-1 has relation with XLID (X-linked ID), indicating alterations in function of member A of the Rassh homologue gene family (RhoA), implicated in ID. It interacts with the postsynaptic adapter protein homer. Alterations in oligophrenin-1 expression result in various deficits of synaptic function and plasticity. The TM4SF2 gene codes for tetranspanin 7 (TSPAN 7) proteins, is an evolutionary conserved protein associated with numerous proteins in the so called tetranspan enriched micro-domains (TEMs) of the plasma membrane. Mutation in this gene causes premature stop codon TGA (glu218-to-ter) and a 2 bp deletion (564 delGT) resulting in premature stop codon in position 192 which is directly associated with ID. TSPAN7 is a synaptic protein that regulates the association of PICK1 (protein interacting with C-linked kinase) with AMPARs and controls AMPAR trafficking [52].

1.2 5-hydroxymethylcytosine: 5-hmC is considered as sixth base of DNA and plays many roles such as chromatin modification and down regulation of gene expression. It varies in concentration depending on the location of the cell and is present in all the cells [53-55]. Engrailed-2 gene expression plays an important role in histone lysine methylation and acts as a transcriptional repressor during the start of post natal period until perinatal period where it gets down regulated. Over expression of EN-2 was found in autistic cerebellum (from post mortem sample). This gene also plays a role in development of serotonin and other neurotransmitters. It is crucial for the Purkinje cell maturation. In individuals affected with autism, the down regulation of this gene is hindered. 5-mC (hypo methylation) is converted into 5-hydroxymethylcytosine in the presence of ten-eleven-translocase during TET mediated 5-mC demethylation. MeCP2 binds to 5-mC and 5-hmC in a similar fashion. The conversion of 5-mC to 5-hmC also occurs due to oxidative stress during postnatal period in cerebellum. The concentration of 5-hmC is associated with the over expression of EN-2. There is a significant increase in the levels of 5-hmC, DNA methyltransferases, TET dioxygenases in autistic individuals compared to control [54].

Concentration of 5-hmC is high in autistic individuals and MeCP2 binding is decreased in autistic individuals along with overexpression of EN-2. There are six PAX5 binding sites in the upstream of EN-2 gene where 5-hmC concentration is high and it activates the enhancer. MeCP2 binds only to oxidized bases. Here the 8-oxo-dG is responsible for oxidative stress in the bases leading to conversion of 5-mC to 5-hmC and reducing the MeCP2 binding. Therefore, it is clear that there is an overall inter-relation between one another compounds discussed above which ultimately leads to the disorder [54].

1.3 Oxytocin, Vasopressin and CD38: Oxytocin (OXT), a nonapeptide secreted by axons and dendrites of the oxytocinergic neurons located in the paraventricular and supraoptic nuclei of the hypothalamus [56-60] regulates social behaviors, increased gaze, inferring the emotions of others, generosity, trust and face recognition [61-66]. Moreover, OXT is critical in regulating positive social interactions for an individual’s mental and physical fitness.

CD38 is a type 2 transmembrane protein having varied functions such as control of leukemia malignancy, as a HIV marker in blood and is known to play a role in social behaviour [67]. It possesses ADP-riboseylcyclase activity that produces cyclic ADP-ribose (cADPR) from beta-NAD+ (vitamin-B3). cADPR is a potential intracellular messenger that helps in cell migration and a co-factor for the movement of Ca2+ through Ca2+ permeable membranes [68].

CD38 SNPs (R140W found in Asian population and rs3796863 found in American HFA and in Israel) are proposed to be a possible reason behind oxytocin disruption leading to autism [67, 68]. Polymorphism in V1Ar gene in human AVP system is also known to be associated with ASD [69]. Posterior pituitary peptides, including AVP, act centrally as regulators of learning and memory thus any polymorphism or change in their expression will lead to psychiatric problems [70].

1.4 Retinoic acid: Autism prevalence in males boosts the fact that elevated testosterone level in the foetus can be a prompting factor for ASD. Retinoic acid related orphan receptor alpha (RORA) is functionally relevant to ASD. It is dysregulated by environmental factors (gene-environment interactions) and is also hormone sensitive [71]. Thus it suggests that the endocrine disrupting molecules mimic the sex hormone and interfere with normal expression posing a greater risk of autism. RORA is a relevant candidate gene for ASD because its expression is reduced in
ASD especially in subtype associated with severe language impairment based on gene expression profiling of lymphoblastic cell lines (LCLs) [72]. Moreover, reduced expression of RORA increases methylation in RORA promoter in LCL individuals with ASD and can be reversed by exposure of the cell to a methylation inhibitor. It has been found that RORA protein is reduced in post-mortem cerebellum and frontal cortex of individuals with ASD, also dihydrotestosterone is known to decrease RORA expression while estradiol increases it [73]. RORA regulates transcription of aromatase which converts testosterone to estrogen. Level of aromatase is also less in post-mortem cerebellum and frontal cortex of autistic individuals [74]. RORA has sexual dimorphism. It helps in cerebellar development, Purkinje cell differentiation, and regulation of multiple genes at glutamatergic synapse. In addition, RORA is associated with protection of neurons against oxidative stress and inflammation and in regulation of circadian rhythm [75-79].

These are the enzymes and chemicals reported for the cause of autistic traits and hence the disorder. Beside this, environmental factors might contribute greatly to ASD either individually or in conjunction with genetic and/or chemical factors.

2. Environmental causes for Autism:-
Considering the environmental aspects, parents and their lifestyle mainly parental age, maternal stress, migration during pregnancy, maternal exposure to toxicants, uterine environment, vertically transmitted diseases during gestation etc. are the few stated imperative factors that might lead to the risk for autism [80-90].

2.1 Parental influence: Couples having children in late thirties might develop greater risk for autism for childrens. Prenatal stress of mothers, migration during pregnancy, exposure to pollution also largely contributes for the disorder. Gestational diabetes, gestational bleeding, multiple birth, being first born compared to being 3rd or after are the other condition when the offspring may be affected [80]. Maternal antibodies (including cytokines) can cross the blood brain barrier of the foetus and can cause neurological damages; this is due to infections to which the antibodies respond and along with infection it also causes damage to the foetus.

Elevated serum IFN-GAMMA, IL-4, IL-5 is common in mothers who give birth to autistic child. Foetal IL-4 exposure in late pregnancy causes abnormalities in hippocampal structure and morphology and decreased learning in adulthood [81].

2.2 Prenatal stress: Prenatal stress of mothers’ affects the child’s neurological development [82] though the actual genetic influence or correlation between the genes and prenatal stress and its role in ASD is unknown.

2.3 Uterine environment: Uterine environment is one of the proposed reasons for the risk of autism though the mechanism is still unknown. In vitro childbirth techniques IVF and ICSI too have an effect on the child’s growth and development. During perinatal period, prematurity, abnormal presentation in general and breech presentation in particular and planned caesarean sectioning too have an influence. In neonatal period, umbilical cord complications, low 5-min Apgar score, being small for gestational age, low birth weight (<1500g), foetal distress or meconium aspiration as well as birth injury or trauma, summer birth, feeding difficulties, neonatal anaemia, ABO or Rh incompatibility and hyper-bilirubinemia are found to be significantly associated with ASD [83]. Perinatal effects such as anaesthesia during pregnancy, assisted vaginal delivery, post-term birth and high birth weight or head circumference are reported to have an effect on foetus [83].

2.4 Drugs taken during pregnancy or before: In utero exposures, two known teratogenic medicines that are thalidomide and valproate have proven to affect the foetus with mental disabilities. Folic acid and vitamin D (calcitriol) consumption have known to prevent spina bifida. The enzyme 5, 10-methyltetrahydrofolate reductase (MTHFR) converts folate into a form where it can be used for methylation. Increase in the intake of folate results in mutation in MTHFR, C677T mutation which increases the risk of autism. Yet folic acid helps in proper methylation, the children with MTHFR mutation and doesn’t receive folic acid have a risk of developing autism [84]. Deficiency of Vitamin D leads to dysregulation of 36 proteins in the brain [85]. Calcitriol (activated vitamin D) can down regulate harmful cytokines in the brain, partially reverse brain damage and increase cellular level of antioxidant glutathione which removes free radicals and chelates heavy metals including mercury [86-89].
2.6 Infections during pregnancy: Maternal infections during pregnancy also increase the risk of autism. Maternal antibodies sometimes attack the foetus brain as a result of immune response or autoimmune disorders [90].

ASD has overlapping features with many other disorders thus the demarcation between ASD from the other is a matter of challenge amongst scientists. A mixture of genetic, chemical and environmental causes also leads to these reported disorders.

3. Biological causes for Autism:-

3.1 Schizophrenia: Several studies reported that individuals involved in violence and aggressive behaviour suffers from psychosis [91-94] in most cases and it has also found that their background is associated with factors such as sexual abuse, low income, use of drugs and alcoholism [91, 92, 95-97]. Affected individuals showed poor speech and reasoning skills which might be a neurocognitive deficit [98]. They exhibit reduced brain activity in right hypothalamus and right superior temporal gyrus region. Same misbehaviour patterns have also been observed in autistic children in some cases which might be associated with poor theory of mind [98]. These two disorders often overlap in their identity and common in males. Autism has a prevalence of 4.1:1 and schizophrenia with 1.4:1 for male. However, there are various ways of differentiating these two disorders with the age of onset and language impairment. Autism onset is during early childhood whereas that of schizophrenia is after onset of puberty. In autism there is severe and profound language impairment whereas in schizophrenia there is a wide array of neuropsychological disorders including language. It is hence clear that autism and schizophrenia has non-genetic causes as well [99].

3.2 Fragile X syndrome: FXS is known to be associated with ASD and is a monogenic disorder that is linked to silencing of FMR1 gene [100]. FXS is the most common genetic cause of ASD, accounting for 5% of cases [101,102]. There are 15-60% of reported cases of ASD in males with FXS. ASD in FXS is a selective disorder of core socialization skills that is relatively independent of general and verbal cognitive skills [100]. Among the genetic disorders associated with ASD, the behavioral phenotype has been best characterized in FXS [103]. Illustrating the central role of adaptive socialization in ASD in FXS is the fact that, whereas most males with FXS and ASD have impaired socialization skills, only those with severe autistic symptoms also show prominent social withdrawal [104]. It has also been observed that social avoidance becomes a prominent component of ASD in FXS after age 5 [100]. Studies however have demonstrated that ASD diagnosis and autistic behaviours are relatively stable over time in FXS [105-107]. A unique relationship between ASD and anxiety in FXS has been postulated, in part, because of the high prevalence of anxiety in individuals with the genetic disorder. Around 75% of young males with FXS are known to display excessive shyness and social anxiety and 50% have panic attacks [108], whereas there are reports that females with FXS also have excessive shyness, social anxiety, and avoidance personality [109]. Other studies emphasize that although individuals with FXS are interested in social interaction, they often display anxiety- and withdrawal-like behaviors in response to unfamiliar people and novel situations [110-111]. Although studies describe general features of individuals with FXS who meet DSM-IV criteria for different anxiety disorders, they are not specific regarding social anxiety and its delineation from the diagnosis of ASD. Many mouse models have also been developed to study the association between ASD and FXS. The relationship between FMR1 premutation (i.e., intermediate level expansion of the CGG polymorphism), usually a carrier status that is not associated with atypical methylation or gene silencing, and clinical manifestations has been one of the most controversial ones in the FXS literature [100]. At present, it is well accepted that two disorders, fragile X-associated tremor/ataxia syndrome (FXTAS) and primary ovarian insufficiency, are linked to FMR1 premutation [112].

3.3 Mitochondrial disorder: The mitochondria have two membranes, inner (contains the ETC) and the outer membrane. The mitochondrial genome codes for 13 of the ETC proteins [113]. The cell nucleus encodes other mitochondrial proteins (more than 1000) which mediates processes such as the regulation of ion homeostasis, stress responses, cell survival and signal transduction [114]. Neurons depend on mitochondrial energy supply as they have limited capacity to obtain energy through glycolysis when oxidative phosphorylation is compromised [115]. Studies reported that increased mitochondrial mass is required for neuronal differentiation and suggested that mitochondria plays active roles in synaptic plasticity [114]. Events in learning and memory processes such as long term potentiation (LTP), has been associated with changes in mitochondria [116, 117]. Mitochondria works as mediators of some of the effects of glutamate and brain derived neurotrophic factor (BDNF) on synaptic plasticity [118]. Studies reported that patients with psychiatric disorders exhibit mitochondrial abnormalities at the structural, molecular and functional levels. Mitochondrial disorders are associated with psychiatric complications, bipolar
disorder, depression, anxiety disorder etc. and are more common in ASD. ASD patients display peripheral markers of mitochondrial energy metabolism dysfunction, such as elevated lactate, pyruvate and alanine levels in blood, urine and/or cerebrospinal fluid, serum carnitine deficiency and/or enhanced oxidative stress [113].

Other disorders such as Rett syndrome, Angelman syndrome, tuberous sclerosis, cerebellar ataxia and cerebellar palsy etc., also have overlapping features with ASD [119].

Apart from these three domains synaptic cleft- influence of genetic and environmental factors is also considered as risk factor for ASD.

Individuals with autism have differences in brain connectivity. Different areas of the brain coordinate with each other in a different way. Synapses are the physical connections through which brain cells communicate with each other and synapses decrease as children grow (normally). The number of synapses is usually high in teenagers but decreases in adults. However teenagers with autism possess more synapses than is typically. Mutation in the gene for tuberous sclerosis increases the risk for autism in people. Rapamycin decreases the number of synapses and improves social behaviours. It is called sirolimus and is used in patients with organ transplants to prevent rejection [120]. However, it has severe side effects including suppression of immune system, lung inflammation and risk for diabetes thus cannot be recommended to autistic children. A very similar drug, everolimus is being studied in patients with TS, a genetic disorder that causes tumours, cognitive impairment and increased risk for autism. Lack of pruning in autistic individuals is that, the brain cells of these individuals were filled with damaged parts and deficient in signs of a normal breakdown pathway called “autophagy”. In mouse, pruning defect related to a protein called mTOR. When mTOR is overactive, brain cells lose much of their self-pruning ability. Rapamycin is known to inhibit mTOR and showed effectiveness even in older mice that had fully developed autism-like behaviour [121]. More than 100 genes have reported in cases with autism. Mostly associated ones are RELN, SLC6A4, GABR, NLGN, OXTR, MET, SLC25A12, GRIK1 or GluR6, Glyoxalase 1, TPH1 and TPH2 [122]. (Table 1)

**CONCLUSION:-**
In present article, multifarious causes of ASD have been discussed with major emphasis on three major domains along with various biomarkers. The actual cause for ASD might be only genetic or only environmental or more than one reason may remain involved. However, there are other cases wherein these mutations or modifications may not have any implications on the individual. Owing to its complexity, the actual reason and the start point of these risk factors have not yet been discovered. Even for those that have been stated regarding the cause of autism is still not prevalent in all reported cases of ASD. This explains the necessity of further research to be carried out even in already stated causes of ASD and also on further new ways of diagnosis.

**Funding Details:-**
This work is not supported by any grant.

**Conflict of Interest:-**
Authors declare no conflict of interests among them.
| Gene                          | General role                                                                                     | Role in ASD                                                                                                                                                                                                 |
|-------------------------------|--------------------------------------------------------------------------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| **Reelin**<br>(RELN)          | Extracellular glycoprotein that help in migration of neural cells and in establishing neural connections. It plays an important role in positioning neuronal cells in the inferior olivery complex, cerebral cortex, cerebellum in the early embryonic stages. | Reduced levels of this protein and its m-RNA concentration were observed in the Autistic individuals. It is located at 7q22 (an autism critical region). A GGC repeat polymorphism immediately after 5’ ATG was observed in RELN gene. |
| **Human serotonin transporter**<br>(SLC6A4) | It localizes to chromosome 17q11.1-q12 and consists of 15 exons. | A HTTLPR polymorphism was found in promoter region of SLC6A4 gene. It is identified by elevated blood serotonin level due to no activity of serotonin inhibitors as a result of mutation. |
| **Gamma-aminobutyric acid receptor**<br>(GABR) | Chief inhibitory neurotransmitter in the brain, acts by binding to GABA receptor. | Located in 15q11-q13 which is complex region in the genome involved in gene expression, genome instability, imprinting and recombination. Duplication of this region was observed in many ASD individuals. |
| **Neuroligin**<br>(NLGN)      | Family of cell adhesion molecules that are essential for functional neuron synapses formation. | A de novo mutation in NLGN4 leads to premature STOP codon which causes premature termination of protein. A C TO T transition was also observed in NLGN3 which changed a highly conserved arginine residue into cysteine residue; this was inherited from the mother. |
| **Human oxytocin receptor**<br>(OXTR) | Synthesized in the hypothalamus; it acts as a neuromodulator in the CNS. It also plays a role in social and repetitive behaviors. | Mutation in this gene increases the risk of autism.                                                                                                                                                    |
| **MET**                       | Encodes a trans membrane receptor tyrosine kinase of the hepatocyte growth factor/scatter factor (HGF/SF). Primarily it is an oncogene, however plays an important role neurodevelopment. | Impaired MET leads to abnormal interneuron migration and signaling between them.                                                                                                                                 |
| **SLC25A12**                  | Located in the 2q31 region, encoding mitochondrial/glutamate carrier (AGC1) a key protein involved in ATP synthesis and mitochondrial function. | Several SNPs have been reported, yet the findings so far are not conclusive.                                                                                                                                 |
| **Glutamate receptor gene (GRIK1 or GluR6)** | Located at chromosome 6q21, as the glutamate is the principle excitatory neurotransmitter in brain | The actual relation unknown but it is known to be associated.                                                                                                                                              |
| **Glyoxalase 1**              | Cytosolic, ubiquitously expressed zinc metalloenzyme involved in scavenging toxic alpha-oxoaldehydes formed during cellular metabolic reactions. | GLO1, A419 polymorphisms can lead to autism.                                                                                                                                                             |
| **TPH1 and TPH2**             | It encodes rate-limiting enzymes that control serotonin biosynthesis. | Susceptible gene for ASD. TPH1 is expressed peripherally, however TPH2 is found in brain tissue.                                                                                                          |
References:
1. Muhle R, Trentacoste SV, RapinI. The genetics of autism. Pediatrics. 2004; 113:e472-486.
2. Goldani AA, Downs SR, Widjaja F, Lawton B, Hendren RL. Biomarkers in autism. Frontiers in psychiatry. 2014; 5:100.
3. Ste ssman HA, Bernier R, Eichler EE. A genotype-first approach to defining the subtypes of a complex disease. Cell. 2014; 156:872-877.
4. State MW, Levitt P. The conundrums of understanding genetic risks for autism spectrum disorders. Nature neuroscience. 2011; 14:1499-1506.
5. Belmonte MK, Bourgeron T. Fragile X syndrome and autism at the intersection of genetic and neural networks. Nature neuroscience. 2006; 9:1221-1225.
6. Murdoch JD, State MW. Recent developments in the genetics of autism spectrum disorders. Current opinion in genetics & development. 2013; 23:310-315.
7. Chango A, De Courcy GP, Boisson F, Guillard JC, Barbe F, Perrin MO, Christides JP, Rabbi K, Pfister M, Galan P, Herbergs S. 5, 10-methylfetaldehyde METHYL-REDUTASE common mutations, folate status and plasma homocysteine in healthy French adults of the Supplementation en Vitamines et Mineraux Antioxydants (SU. VI. MAX) cohort. British Journal of Nutrition. 2000; 84:891-896.
8. Lee TL, Raygada MJ, Rennert OM. Integrative gene network analysis provides novel regulatory relationships, genetic contributions and susceptible targets in autism spectrum disorders. Gene. 2012; 496:88-96.
9. Wong CC, Meaburn EL, Ronald A, Price TS, Jeffries AR, Schalkwyk LC, Plomin R, Mill J. Methylicon analysis of monozygotic twins discordant for autism spectrum disorder and related behavioural traits. Molecular psychiatry. 2014; 19:495-503.
10. Ma DK, Marchetto MC, Guo JU, Ming GL, Gage FH, Song H. Epigenetic choreographers of neurogenesis in the adult mammalian brain. Nature neuroscience. 2010; 13:1338-1344.
11. Gregory SG, Connelly JJ, Towers AJ, Johnson J, Biscocho D, Markunas CA, Lintas C, Abramson RK, Wright HH, Ellis P, Langford CF. Genomic and epigenetic evidence for oxtocin receptor deficiency in autism. BMC medicine. 2009; 4:62.
12. Nagarajan R, Hogart A, Gwye Y, Martin MR, LaSalle JM. Reduced MeCP2 expression is frequent in autism frontal cortex and correlates with aberrant MECP2 promoter methylation. Epigenetics. 2006; 1:172-182.
13. Shulha HP, Cheung I, Whittle C, Wang J, Virgil D, Lin CL, Guo Y, Lessard A, Akbarian S, Weng Z. Epigenetic signatures of autism: trimethylated H3K4 landscapes in prefrontal neurons. Archives of general psychiatry. 2012; 69:314-324.
14. Wong CC, Caspi A, Williams B, Craig IW, Houts R, Ambler A, Moffitt TE, Mill J. A longitudinal study of epigenetic variation in twins. Epigenetics. 2010; 5:516-526.
15. Lowe TL, Tanaka K, Seashore MR, Young JG, Cohen DJ. Detection of phenylketonuria in autistic and psychotic children. JAMA. 1980; 243:126-128.
16. Bottini N, De Luca D, Saccucci P, Fiumara A, Elia M, Porfiri MC, Lucarelli P, Curatolo P. Autism: evidence of association with adenosine deaminase genetic polymorphism. Neurogenetics. 2001; 3:111-113.
17. Zaffanello M, Zamboni G, Fontana E, Zoccante L, Tato L. A case of partial biotinidase deficiency associated with autism. Child Neuropsychology. 2003; 9:184-188.
18. Moretti P, Peters SU, Del Gaudio D, Sahoo T, Hyland K, Bottiglieri T, Hopkin RJ, Peach E, Min SH, Goldman D, Roa B. Brief report: autistic symptoms, developmental regression, mental retardation, epilepsy, and dyskinesias in CNS folate deficiency. Journal of autism and developmental disorders. 2008; 38:1170-1177.
19. Wang L, Angley MT, Gerber JP, Sorich MJ. A review of candidate urinary biomarkers for autism spectrum disorder. Biomarkers. 2011; 16:537-552.
20. Thomas RH, Foley KA, Mepham JR, Tichenoff LJ, Possmayer F, MacFabe DF. Altered brain phospholipid and acyl Carnitine profiles in proprionic acid infused rodents: further development of a potential model of autism spectrum disorders. Journal of neurochemistry. 2010; 113:515-529.
24. Gorrindo P, Lane CJ, Lee EB, McLaughlin B, Levitt P. Enrichment of Elevated Plasma F 2t-Isoprostane Levels in Individuals with Autism Who Are Stratified by Presence of Gastrointestinal Dysfunction. PloS one. 2013; 8:e68444.
25. Rossignol DA, Frye RE. Mitochondrial dysfunction in autism spectrum disorders: a systematic review and meta-analysis. Molecular psychiatry. 2012; 17:290-314.
26. Haas RH, Parikh S, Falk MJ, Saneto RP, Wolf NI, Darin N, Cohen BH. Mitochondrial disease: a practical approach for primary care physicians. Pediatrics. 2007; 120:1326-1333.
27. Poling JS, Frye RE, Shoffner J, Zimmerman AW. Developmental regression and mitochondrial dysfunction in a child with autism. Journal of child neurology. 2006; 21:170-172.
28. Deth R, Muratore C, Benzecry C, Power-Charnitsky VA, Waly M. How environmental and genetic factors combine to cause autism: A redox/methylation hypothesis. Neurotoxicology. 2008; 29:190-201.
29. James SJ, Cutler P, Melnyk S, Jernigan L, Gaylor DW, Neubrander JA. Metabolic biomarkers of increased oxidative stress and impaired methylation capacity in children with autism. The American journal of clinical nutrition. 2004; 80:1611-1617.
30. Ashwood P, Krakowiak P, Hertz-Picciotto I, Hansen R, Pessah I, Van de Water J. Elevated plasma cytokines in autism spectrum disorders provide evidence of immune dysfunction and are associated with impaired behavioral outcome. Brain, behavior, and immunity. 2011; 25:40-45.
31. Onore C, Careaga M, Ashwood P. The role of immune dysfunction in the pathophysiology of autism. Brain, behavior, and immunity. 2012; 26:383-392.
32. AshwoodP, Wills S, Van de Water J. The immune response in autism: a new frontier for autism research. Journal of leukocyte biology. 2006; 80:1-5.
33. Al-Ayadhi LY, Mostafa GA. A lack of association between elevated serum levels of S100B protein and autoimmunity in autistic children. Journal of neuroinflammation. 2012; 9:54.
34. Singh VK. Phenotypic expression of autoimmune autistic disorder (AAD): a major subset of autism. Annals of Clinical Psychiatry. 2009; 21:148-161.
35. Connolly AM, Chez MG, Pestronk A, Arnold ST, Mehta S, Deuel RK. Serum autoantibodies to brain in Landau-Kleffner variant, autism, and other neurologic disorders. The Journal of pediatrics. 1999; 134:607-613.
36. Ashwood P, Van de Water J. Is autism an autoimmune disease?. Autoimmunity Reviews. 2004; 3:557-562.
37. Cabanlit M, Wills S, Goines P, Ashwood P, Van De Water J. Brain-Specific Autoantibodies in the Plasma of Subjects with Autistic Spectrum Disorder. Annals of the New York Academy of Sciences. 2007; 1107:92-103.
38. Mostafa GA, AL-ayadhi LY. Increased serum levels of anti-ganglioside M1 auto-antibodies in autistic children: relation to the disease severity. Journal of neuroinflammation. 2011; 8:39.
39. Mostafa GA, Al-Ayadhi LY. The relationship between the increased frequency of serum antineuronal antibodies and the severity of autism in children. European journal of paediatric neurology. 2012; 16:464-468.
40. Mostafa GA, Kitchener N. Serum anti-nuclear antibodies as a marker of autoimmunity in Egyptian autistic children. Pediatric neurology. 2009; 40:107-112.
41. Singh VK, Warren R, Averrett R, Ghaziuddin M. Circulating autoantibodies to neuronal and glial filament proteins in autism. Pediatric neurology. 1997; 18:88-90.
42. Singh VK, Warren RP, Odell JD, Warren WL, Cole P. Antibodies to myelin basic protein in children with autistic behavior. Brain, behavior, and immunity. 1993; 7:97-103.
43. Mostafa GA, AL-ayadhi LY. A lack of association between hyperserotonemia and the increased frequency of serum anti-myelin basic protein auto-antibodies in autistic children. Journal of neuroinflammation. 2011; 8:71.
44. Mostafa GA, Awad El-Sayed Z, Mohamed Abd El-Aziz M, Farouk El-Sayed M. Serum anti-myelin—associated glycoprotein antibodies in Egyptian autistic children. Journal of child neurology. 2008; 23:1413-1418.
45. Connolly AM, Chez M, Streif EM, Keeling RM, Golumbek PT, Kwon JM, Riviello JJ, Robinson RG, Neuman RJ, Deuel RM. Brain-derived neurotrophic factor and autoantibodies to neural antigens in sera of children with autistic spectrum disorders, Landau-Kleffner syndrome, and epilepsy. Biological psychiatry. 2006; 59:354-363.
46. Taurines R, Segura M, Schecklmann M, Albantakis L, Grünblatt E, Walitza S, Ians T, Lyttwin B, Haberhausen M, Theisen FM, Martin B. Altered peripheral BDNF mRNA expression and BDNF protein concentrations in blood of children and adolescents with autism spectrum disorder. Journal of Neural Transmission. 2014; 121:1117-1128.
47. Kowal C, Athanassiou A, Chen H, Diamond B. Maternal antibodies and developing blood–brain barrier. Immunologic research. 2015; 63:18-25.
48. Samuelssohn AM, Jennische E, Hansson HA, Holmång A. Prenatal exposure to interleukin-6 results in inflammatory neurodegeneration in hippocampus with NMDA/GABA A dysregulation and impaired spatial
learning. American Journal of Physiology-Regulatory, Integrative and Comparative Physiology. 2006; 290:R1345-1356.
49. Tordjman S, Somogyi E, Coulon N, Kermarrec S, Cohen D, Bronsard G, Bonnot O, Weismann-Arcahe C, Botbol M, Lauth B, Ginchat V. Genex Environment interactions in autism spectrum disorders: role of epigenetic mechanisms. Frontiers in psychiatry. 2014; 5:53.
50. Oddi D, Crusio WE, D’Amato FR, Pietropaolo S. Monogenic mouse models of social dysfunction: implications for autism. Behavioural brain research. 2013; 251:75-84.
51. Oberman LM. mGluR antagonists and GABA agonists as novel pharmacological agents for the treatment of autism spectrum disorders. Expert opinion on investigational drugs. 2012; 21:1819-1825.
52. Verpelli C, Montani C, Vicidomini C, Heise C, Sala C. Mutations of the synapse genes and intellectual disability syndromes. European journal of pharmacology. 2013; 719:112-116.
53. Chia N, Wang L, Lu X, Senuf MC, Brenner CA, Ruden DM. Hypothesis: environmental regulation of 5-hydroxymethylcytosine by oxidative stress. Epigenetics. 2011; 6:853-856.
54. James SJ, Shplyeva S, Melnyk S, Pavliv O, Pogribny IP. Elevated 5-hydroxymethylcytosine in the Engrailed-2 (EN-2) promoter is associated with increased gene expression and decreased MeCP2 binding in autism cerebellum. Translational psychiatry. 2014; 4:e460.
55. Castro GD, Gómez MI, Castro J. 5-Methylcytosine attack by hydroxyl free radicals and during carbon tetrachloride promoted liver microsomal lipid peroxidation: structure of reaction products. Chemico-biological interactions. 1996; 99:289-299.
56. Brownstein MJ, Russell JT, Gainer H. Synthesis, transport, and release of posterior pituitary hormones. Science. 1980; 207:373-378.
57. Ludwig M, Leng G. Dendritic peptide release and peptide-dependent behaviours. Nature Reviews Neuroscience. 2006; 7;126-136.
58. Neumann ID. Brain oxytocin: a key regulator of emotional and social behaviours in both females and males. Journal of neuroendocrinology. 2008; 20:858-865.
59. Ross HE, Young LJ. Oxytocin and the neural mechanisms regulating social cognition and affiliative behavior. Frontiers in neuroendocrinology. 2009; 30:534-547.
60. Russell JA, Leng G, Douglas AJ. The magnocellular oxytocin system, the fount of maternity: adaptations in pregnancy. Frontiers in neuroendocrinology. 2003; 24:27-61.
61. Carter CS, Boone EM, Pournajafi-Nazarloo H, Bales KL. Consequences of early experiences and exposure to oxytocin and vasopressin are sexually dimorphic. Developmental neuroscience. 2009; 31:332-341.
62. Guastella AJ, Mitchell PB, Dadds MR. Oxytocin increases gaze to the eye region of human faces. Biological psychology. 2008; 63:3-5.
63. Ditzen B, Schaar M, Gabriel B, Bodenmann G, Ehlert U, Heinrichs M. Intranasal oxytocin increases positive communication and reduces cortisol levels during couple conflict. Biological psychiatry. 2009; 65:728-731.
64. Zak PJ, Stanton AA, Ahmadi S. Oxytocin increases generosity in humans. PloS one. 2007; 2:e1128.
65. Kosfeld M, Heinrichs M, Zak PJ, Fischbacher U, Fehr E. Oxytocin increases trust in humans. Nature. 2005; 435:673-676.
66. Rimmelse U, Hediger K, Heinrichs M, Klaver P. Oxytocin makes a face in memory familiar. Journal of Neuroscience. 2009; 29:38-42.
67. Higashida H, Yokoyama S, Kikuchi M, Munese T. CD38 and its role in oxytocin secretion and social behavior. Hormones and behavior. 2012; 61:351-358.
68. Denu JM. Vitamin B 3 and sirtuin function. Trends in biochemical sciences. 2004; 21:1057-1063.
69. vanWimersmaGreidanus TB, van Ree JM, de Wied D. Vasopressin and memory. Pharmacology & therapeutics. 1983; 20:437-458.
70. Hu VW. Is retinoic acid-related orphan receptor-alpha (RORA) a target for gene–environment interactions contributing to autism?. Neurotoxicology. 2012; 33:1434-1435.
71. Hu VW, Sarachana T, Kim KS, Nguyen A, Kulkarni S, Steinberg ME, Luu T, Lai Y, Lee NH. Gene expression profiling differentiates autism case–controls and phenotypic variants of autism spectrum disorders: Evidence for circadian rhythm dysfunction in severe autism. Autism research. 2009; 2:78-97.
72. Nguyen A, Rauch TA, Pfefier GP, Hu VW. Global methylation profiling of lymphoblastoid cell lines reveals epigenetic contributions to autism spectrum disorders and a novel autism candidate gene, RORA, whose protein product is reduced in autistic brain. The FASEB Journal. 2010; 24:3036-3051.
74. Sarachana T, Xu M, Wu RC, Hu VW. Sex hormones in autism: androgens and estrogens differentially and reciprocally regulate RORA, a novel candidate gene for autism. PloS one. 2011; 6:e17116.
75. Gold DA, Gent PM, Hamilton BA. RORα in genetic control of cerebellum development: 50 staggering years. Brain research. 2007; 1140:19-25.
76. Hadj-Sahraoui N, Frederic F, Zanjani H, Delhaye-Bouchaud N, Herrup K, Mariani J. Progressive atrophy of cerebellar Purkinje cell dendrites during aging of the heterozygous staggerer mouse (Rora+/sg). Developmental Brain Research. 2001; 126:201-209.
77. Boukhtouche F, Vodjdani G, Jarvis CI, Bakouche J, Staels B, Mallet J, Mariani J, Lemaigre-Dubreuil Y, Brugg B. Human retinoic acid receptor-related orphan receptor α1 overexpression protects neurones against oxidative stress-induced apoptosis. Journal of neurochemistry. 2006; 96:1778-1789.
78. Delerive P, Monté D, Dubois G, Trottein F, Fruchart-Najib J, Mariani J, Fruchart JC, Staels B. The orphan nuclear receptor RORα is a negative regulator of the inflammatory response. EMBO reports. 2001; 2:42-48.
79. Sato F, Kawamoto T, Fujimoto K, Noshiro M, Honda KK, Honma S, Honma KI, Kato Y. Functional analysis of the basic helix-loop-helix transcription factor DEC1 in circadian regulation. European Journal of Biochemistry. 2004; 271:4409-4419.
80. Gardener H, Spiegelman D, Buka SL. Prenatal risk factors for autism: comprehensive meta-analysis. The British journal of psychiatry. 2009; 195:7-14.
81. Goines PE, Croen LA, Braunischweig D, Yoshida CK, Grether J, Hansen R, Kharrazi M, Ashwood P, Van de Water J. Increased midgestational IFN-γ, IL-4 and IL-5 in women bearing a child with autism: a case-control study. Molecular autism. 2011; 2:13.
82. Cate PT, Ineke M. Prenatal maternal stress and child developmental outcome: implications for health care provision?. Developmental Medicine & Child Neurology. 2014; 56:204-205.
83. Gardener H, Spiegelman D, Buka SL. Perinatal and neonatal risk factors for autism: a comprehensive meta-analysis. Pediatrics. 2011; peds-2010.
84. Currenti SA. Understanding and determining the etiology of autism. Cellular and molecular neurobiology. 2010; 30:161-171.
85. Almeras L, Eyles D, Benech P, Laffite D, Villard C, Patatian A, Boucraut J, Mackay-Sim A, McGrath J, Féron F. Developmental vitamin D deficiency alters brain protein expression in the adult rat: implications for neuropsychiatric disorders. Proteomics. 2007; 7:769-780.
86. Moore ME, Piazza A, McCartney Y, Lynch MA. Evidence that vitamin D3 reverses age-related inflammatory changes in the rat hippocampus. Biochemical Society transactions. 2005; 573-577.
87. Burne TH, Féron F, Brown J, Eyles DW, McGrath JJ, Mackay-Sim A. Combined prenatal and chronic postnatal vitamin D deficiency in rats impairs prepulse inhibition of acoustic startle. Physiology &behavior. 2004; 81:651-655.
88. Garcia E, Wion-Barbot N, Montero-Menei CN, Berger F, Wion D. New clues about vitamin D functions in the nervous system. Trends in Endocrinology & Metabolism. 2002; 13:100-105.
89. Kern JK, Jones AM. Evidence of toxicity, oxidative stress, and neuronal insult in autism. Journal of Toxicology and Environmental Health, Part B. 2006; 9:485-499.
90. Dean L. Blood Groups and Red Cell Antigens [Internet]. Bethesda (MD): National Center for Biotechnology Information (US). Blood and the cells it contains. 2005.
91. Bennett DJ, Ogloff JR, Mullen PE, Thomas SD, Wallace C, Short T. Schizophrenia disorders, substance abuse and prior offending in a sequential series of 435 homicides. ActaPsychiatricaScandinavica. 2011; 124:226-233.
92. Fazel S, Gulati G, Linsell L, Geddes JR, Grann M. Schizophrenia and violence: systematic review and meta-analysis. PLoS Med. 2009; 6:e1000120.
93. Large M, Smith G, Nielsen O. The relationship between the rate of homicide by those with schizophrenia and the overall homicide rate: a systematic review and meta-analysis. Schizophrenia research. 2009; 112:123-129.
94. Nielsen O, Large M. Rates of homicide during the first episode of psychosis and after treatment: a systematic review and meta-analysis. Schizophrenia bulletin. 2010; 36:702-712.
95. Bo S, Abu-Akel A, Kongerslev M, Haahr UH, Simonsen E. Risk factors for violence among patients with schizophrenia. Clinical psychology review. 2011; 31:711-726.
96. Elbogen EB, Johnson SC. The intricate link between violence and mental disorder: results from the National Epidemiologic Survey on Alcohol and Related Conditions. Archives of general Psychiatry. 2009; 66:152-161.
97. Wallace C, Mullen P, Burgess P, Palmer S, Ruschena D, Browne C. Serious criminal offending and mental disorder. Case linkage study. The British Journal of Psychiatry. 1998; 172:477-484.
98. McGuire J, Langdon R, Brüne M. Moral cognition in schizophrenia. Cognitive neuropsychiatry. 2014; 19:495-508.
99. Hamlyn J, Duhig M, McGrath J, Scott J. Modifiable risk factors for schizophrenia and autism—shared risk factors impacting on brain development. Neurobiology of disease. 2013; 53:3-9.
100. Budimirovic DB, Kaufmann WE. What can we learn about autism from studying fragile X syndrome?. Developmental neuroscience. 2011; 33:379-394.
101. Muhle R, Trentacoste SV, RapinI. The genetics of autism. Pediatrics. 2004; 113:e472-486.
102. Schaefer GB, Mendelsohn NJ. Genetics evaluation for the etiologic diagnosis of autism spectrum disorders. Genetics in Medicine. 2008; 10:4-12.
103. Cohen D, Pichard N, Tordjman S, Baumann C, Burglen L, Excoffier L, Lazar G, Mazet P, Pinquier C, Verloes A, Héron D. Specific genetic disorders and autism: clinical contribution towards their identification. Journal of autism and developmental disorders. 2005; 35:103-116.
104. Budimirovic DB, Bukelis I, Cox C, Gray RM, Tierney E, Kaufmann WE. Autism spectrum disorder in Fragile X syndrome: differential contribution of adaptive socialization and social withdrawal. American journal of medical genetics Part A. 2006; 140:1814-1826.
105. Hatton DD, Sideris J, Skinner M, Mankowski J, Bailey DB, Roberts J, Mirrett P. Autistic behavior in children with fragile X syndrome: prevalence, stability, and the impact of FMRP. American journal of medical genetics Part A. 2006; 140:1804-1813.
106. Hernandez RN, Feinberg RL, Vaurio R, Passanante NM, Thompson RE, Kaufmann WE. Autism spectrum disorder in fragile X syndrome: a longitudinal evaluation. American Journal of Medical Genetics Part A. 2009; 149:1125-1137.
107. Sabaratnam M, Murthy NV, Wijeratne A, Buckingham A, Payne S. Autistic-like behaviour profile and psychiatric morbidity in Fragile X Syndrome. European child & adolescent psychiatry. 2003; 12:172-177.
108. Merenstein SA, Sobesky WE, Taylor AK, Riddle JE, Tran HX, Hagerman RJ. Molecular-clinical correlations in males with an expanded FMR1 mutation. American Journal of Medical Genetics Part A. 1996; 64:388-394.
109. Freund LS, Reiss AL, Abrams MT. Psychiatric disorders associated with fragile X in the young female. Pediatrics. 1993; 91:321-329.
110. Bailey DB, Mesibov GB, Hatton DD, Clark RD, Roberts JE, Mayhew L. Autistic behavior in young boys with fragile X syndrome. Journal of autism and developmental disorders. 1998; 28:499-508.
111. Cohen IL, Fisch GS, Sudhalter V, Wolf-Schein EG, Hanson D, Hagerman R, Jenkins EC, Brown WT. Social gaze, social avoidance, and repetitive behavior in fragile X males: a controlled study. American Journal on Mental Retardation. 1988 Mar.
112. Hagerman RJ, Berry-Kravis E, Kaufmann WE, Ono MY, Tartaglia N, Lachiewicz A, Kronk R, Delahunt C, Hessl D, Visootsak J, Picker J. Advances in the treatment of fragile X syndrome. Pediatrics. 2009; 123:378-390.
113. Streck EL, Gonçalves CL, Furlanetto CB, Scaini G, Dal-Pizzol F, Quevedo J. Mitochondria and the central nervous system: searching for a pathophysiological basis of psychiatric disorders. Revista Brasileira de Psiquiatria. 2014; 36:156-167.
114. Mattson MP, Gleichmann M, Cheng A. Mitochondria in neuroplasticity and neurological disorders. Neuron. 2008; 60:748-766.
115. Herrero-Mendez A, Almeida A, Fernández E, Maestre C, Moncada S, Bolaños JP. The bioenergetic and antioxidative status of neurons is controlled by continuous degradation of a key glycolytic enzyme by APC/C=Cdh1. Nature cell biology. 2009; 11:747-752.
116. Williams JM, Thompson VL, Mason-Parker SE, Abraham WC, Tate WP. Synaptic activity-dependent modulation of mitochondrial gene expression in the rat hippocampus. Molecular brain research. 1998; 60:50-56.
117. Li Z, Jo J, Jia JM, Lo SC, Whitcomb DJ, Jiao S, Cho K, Sheng M. Caspase-3 activation via mitochondria is required for long-term depression and AMPA receptor internalization. Cell. 2010; 141:859-871.
118. Sokol DK, Maloney B, Long JM, Ray B, Lahiri DK. Autism, Alzheimer disease, and fragile X APP, FMRP, and mGluR5 are molecular links. Neurology. 2011; 76:1344-1352.
119. Frye RE, Delatorre R, Taylor H, Slattery J, Melnyk S, Chowdhury N, James SJ. Redox metabolism abnormalities in autistic children associated with mitochondrial disease. Translational psychiatry. 2013; 3:e273.
120. Gurk-Turner C, Manitpisitkul W, Cooper M. A comprehensive review of everolimus clinical reports: a new mammalian target of rapamycin inhibitor. Transplantation. 2012; 94:659-668.
121. Beecham JE, Seneff S. The possible link between autism and glyphosate acting as glycine mimetic—A review of evidence from the literature with analysis. Journal of molecular and genetic medicine. 2015; 9:4.
122. Li X, Zou H, Brown WT. Genes associated with autism spectrum disorder. Brain research bulletin. 2012; 88:543-552.