A Review on Cytogenetic Biomarkers in Autism Spectrum Disorder and Probable Protective Function of Vitamin E

By

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A thesis submitted to the Department of Pharmacy in partial fulfillment of the requirements for the degree of Bachelor of Pharmacy (Honors)

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Declaration

It is hereby declared that

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2. The thesis does not contain material previously published or written by a third party, except where this is appropriately cited through full and accurate referencing.
3. The thesis does not contain material which has been accepted, or submitted, for any other degree or diploma at a university or other institution.
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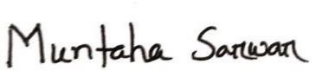

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Approval

The thesis/project titled “A Review on Cytogenetic Biomarkers in Autism Spectrum Disorder and Probable Protective Function of VitaminE” submitted by Muntaha Sarwar (16346003) of Summer, 2016 has been accepted as satisfactory in partial fulfillment of the requirement for the degree of Bachelor of Pharmacy (Hons.)on Summer 2020

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Ethics Statement

This study does not involve any kind of animal trial or human trial.

Abstract

Autism spectrum disorder (ASD) is a neurodevelopmental disorder with impaired sociability, disabled verbal and nonverbal interaction and repetitive or restrictive stereotypical behaviors. According to some assessments, worldwide, 1 in 160 children are affected by ASD. About 90% individuals have idiopathic autism and all responsible genes have not been found yet. Since treatment options for ASDs are very limited, clinical indicators like biomarkers offer greater significance on ASDs as a treatment choice. A variety of cytogenetic biomarkers have been addressed in this comprehensive review study in order to examine the hypothesis that ASD is more than 90% susceptible to genetics and many cytogenetic modifications contribute to the development of ASD. In addition to that, this study also addressed the antioxidant property of vitamin E to stabilize the genetic material. These biomarker findings may have important implications for patients and their families with respect to etiological diagnosis, genetic therapy and patient care.

Keywords: Autism; Chromosomal alteration; Gene; Loci; Cytoband;Vitamin E.

Dedication

Dedicated to my parents

Acknowledgement

I would like to express my gratitude and honor to those who have played a major role in my academic accomplishments. First and foremost, to God Almighty, who helped me to complete my academic journey.

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List of Acronyms

ASD	Autism Spectrum Disorder
VPA	Valproic Acid
ROS	Reactive Oxygen Species
SNV	Single Nucleotide Variation

Chapter 1

Introduction

Autism spectrum disorders (ASDs) is a complex condition that includes unwanted physical consequences spreading extensively throughout an area and involves several factors causing it. Symptoms of this condition can be identified by diverging from the normal type. Those direct to the specifications that includes inability in social interactions, communication as well as decreased and recurring sensory- motor behavioral patterns(Abrahams & Geschwind, 2008). Patients having ASDs commonly possess social anxiety disorder, intellectual disability, attention-deficit/hyperactivity disorder (van Steensel et al., 2013). Moreover, anomalism in immune system, in gastrointestinal function along with epilepsy, insomnia, mitochondrial dysfunction may occur (Masi et al., 2017).

Globally, the generality of autism is becoming larger on each successive year. According to some assessments, 1 in 160 children is afflicted by ASD around the world. It can be identified in less than 2-3 years after birth and remains throughout lifetime. In the first 5 years of life, the state is noticeable in the majority of cases. The degree of impairment in ASD can be intensely unpredictable broadening from mild to serious. The rate of autism is outlined extreme in males as compared in females with an general recorded proportion of around 4:1 (Fombonne, 1999; Lord et al., 1982; Volkmar et al., 1993). The condition can be lenient or it can be acute (Marrus & Constantino, 2016).

Treatment options are very limited in ASDs and to aid the welfare of most treatments there are not maximal indications (Lord et al., 2012). In order to obtain successful treatment for ASDs countless challenges exist (Constantino & Charman, 2016). However, objective indicators such as biomarkers place increased emphasis as a treatment option in ASDs. Hence, some biomarkers that can enhance the sign of treatment are Genomics, Neuroimaging, Patho-

physiological markers concerning mitochondrial operation, oxidative stress and function of immune system (Bent & Hendren, 2010).

Taking account into its increasing prevalence, the pathophysiology of ASD is not completely understood. The etiology of ASD is still not clear but some studies exhibits strong multiple interacting genome factors with complicated pattern of transmission as the reason of this disorder(Spence, 2004). Although environmental, dietary and gastrointestinal factors are also responsible as pathogenesis of autism in addition with genetic susceptibility as stated by some study reports(Frye et al., 2015).Environmental factors that can impact on genetic factors to induce autism includes pesticides, infection, toxins, parenteral exposure to thalidomide (Miller, et, al., 2004), valproic acid(G. Williams et al., 2001), ethanol(S. Bishop et al., 2007).

Over and above, neurotransmitters such as- glutamate (Glu), serotonin (5-HT), γ- aminobutyric acid (GABA), dopamine (DA), histamine (HA) can also develop ASD during early maturation of brain (Bacchelli et al., 2015).

Epilepsy is one of the most common symptoms in Autism Spectrum Disorder. To treat epileptic patients, valproic acid (VPA) is a frequently used medicament. Regardless it's efficiency as medicament it exhibits teratogenicity in humans and animals. Thrombocytopenia, hepatotoxicity, accumulation of platelets, pancreatitis are some of the adverse effects that may occur due to administration of valproic acid(Catalgol & Ozer, 2012).There are significant evidences offering valuable insights into the fact that, if VPA is exposed to rodent off-springs parentally, it causes deficiency in motor performance, in social behavior like developmental delays similar to those described in human autistic patients (Favre et al., 2013; Kataoka et al., 2013).

VPA exhibits teratogenicity in humans and animals and thrombocytopenia, hepatotoxicity, oxidative stress, accumulation of platelets, pancreatitis are some of the adverse effects that may

occur due to administration of valproic acid(Catalgol & Ozer, 2012). Toxicity may causes from the overproduced reactive oxygen species (ROS) along with reduced antioxidant potential. Genetic abnormalities may occur as a result of this toxicity and from the analysis of several researches it can be noted that oxidative stress in patients and in animal models can take place if valproic acid is used as medicament (Catalgol & Ozer, 2012).Embryofetopathy occurs if VPA is subjected during first trimesters of pregnancy that implies higher chances of autistic symptoms in children (Chandane & Shah, 2014). Furthermore, some correlative symptoms that occurs in VPA exposures are malformation of organ, delay in neurodevelopment, anomalies in craniofacial, reduced activity in social functioning (Chomiak et al., 2013).

The probable event of interaction between VPA and oxidative stress in animal models and in patients has signified by many case studies (Cengiz et al., 2000). To lessen the impairments of oxidants and withstand the possible damaging effect of free radicals, a number of natural substances have tested as anti-oxidants. Vitamin- E functions as anti-oxidant and it is one of the most fundamental fat-soluble nutrients in human body. According to many *in vivo* and *in vitro* trials, DNA damages can be reduced if vitamin E supplements are taken (Mozdarani & Salimi, 2006).

Vitamin E was first discovered by Herbert Evans and Katherine Bishop, researchers of University of California in green leafy vegetables (Catalgol & Ozer, 2012). Human body is incapable to produce this essential and fat soluble Vitamin. Therefore, Vitamin E rich foods along with supplements must be made available (Baran et al., 2006). Naturally derived elements named α, β, γ,δ derivatives of tocopherol and tocotrienol are known as Vitamin E. RRR-α-tocopherol remains in increased number in human body amongst them. Vitamin E potentially shows the ability to reduce oxidative stress, lipid peroxidation, reactive oxygen species toxicity in body system(Abdella et al., 2014).

To design medical therapeutics and diagnostics, biomarkers are essential (Cordero et al., 2010). Biomarkers of autism are indicated diversely in autistic patients. Because patients with autism can have different symptoms, biomarkers can be overlapped. Studies shows that patients with ASDs have anomalies in hormones, peptides, metabolites from neurologic, gastrointestinal (GI), immunologic and toxicologic systems(Ratajczak, 2011).

Cytogenetic alterations after administration of VPA in subjects are reported as ASD biomarkers in various studies. To determine cytogenetic changes, chromosomal aberrations are analyzed (Abdella et al., 2014). It is believed that many cytogenetic alterations contribute to develop autism. The susceptibility of ASD is estimated to be more than 90 percent genetic (Pickles et al., 1995). The cytogenetic anomalies in many ways result in functional genetic changes such as- dosage effects can take place in some genes as a result of modifications in the number of gene copies for instance in deletions or duplications; a gene perhaps directly disrupted by breakpoints associated with re-arrangement event; as a result of re-arrangement genes maybe isolated from gene-regulatory sequences; deletions may result in unmasking of a point mutation in a gene situated in the respective region of the nondeleted homologous chromosome (Vorstman et al., 2006). This review is designed to present an overview of potentially notable regions for positional candidate genes to researchers in the field of autism.

1.1 Aim

The aim of this study was to evaluate the change of biomarkers in the cytogenetic system due to possible protective effect of Vitamin E in autistic individuals.

1.2 Objectives

The objectives of this study were:

1.2.1 to find out the biomarkers of cytogenetic system.

1.2.2 to evaluate the changes in the cytogenetic system in autistic individual.

Chapter 2

Methodology

A literature review process can be composed by different type of manners such as structured literature review or systematic review. This study is a structured literature review and it presents an overview of previous studies as well as interprets literature that addresses a specific topic.

All information including data, statistics, figures, facts of this review paper was obtained by thorough literature review. Several credible sources were used to gather the information about cytogenetic alterations in ASD and protective effect of vitamin E including various peer-reviewed journals, online scholarly database, books etc. To collect all the journals and articles, an electronic search was conducted. Here, common medical literature database such as- NCBI resources, Google scholar, PubMed, Medline, Science Direct, ResearchGate and Elsevier were followed. To expand the research and find out additional information, references were cross-checked. The relevant articles were selected by screening the abstract of different articles that have information required for this study.

By this method, probable protective effect of vitamin E on ASD and available case reports of cytogenetic alterations occurred on ASD were reviewed and summarized with particular emphasis. Along with that, studies that consist of comparison between autistic and control group regarding chosen biomarkers and vitamin E on ASD were also evaluated.

Chapter 3

Discussion

3.1 Effect of vit-E on Chromosome anomaly in bone marrow cells of autistic mice

According to some studieschanges in cytogenetic system have found among individuals diagnosed with ASD. Abdella and colleagues performed such a study in which VPA induced autistic subjects showed diminished chromosomal alterations. Abnormalities like- removal of chromatid, centromeric mitigation, polyploidy, endomitosis were present in reduced extent on those subjects when treated with vitamin E at specified doses of 50,100 and 200 mg/kg clearly specifying cytotoxicity reduction (Abdella et al., 2014). Chromatid breaks, deletions, ring chromosomes, association of end-to-end, centromeric mitigation, centric fusion and much other impairment were found when vit-E was not given to the subjects.

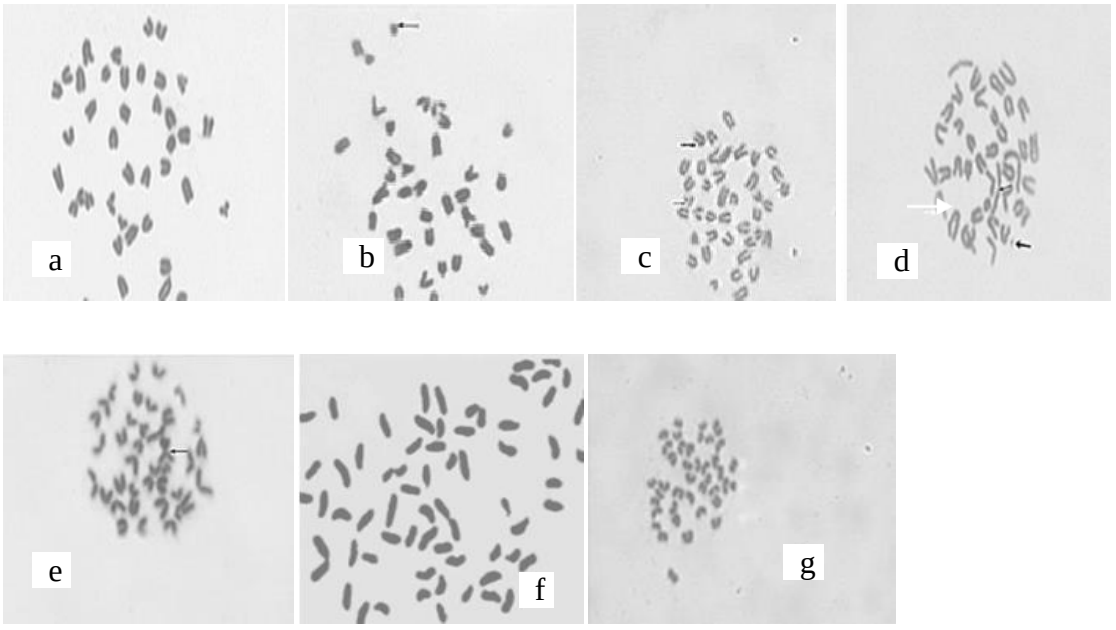

Figure 1: (a) Normal chromosome spread in bone marrow cell of the subject on metaphase spread. (*b) Ring chromosome (arrow) in bone marrow cell of the subject on metaphase spread indicating cytotoxicity. (c) Chromatid break (thin arrow) and chromatid gap (bold arrow) in*

bone marrow cell of the subject on metaphase spread indicating cytotoxicity.

(d) Deletion (white arrow), fragment and end-to-end association (black arrow) in bone marrow cell of the subject on metaphase spread indicating cytotoxicity.(e) Centric fusion in bone marrow cell of the subject on metaphase spread indicating cytotoxicity. (f) Centromeric mitigation in bone marrow cell of the subject indicating cytotoxicity. (g) Endomitosis in bone marrow cell of the subject indicating cytotoxicity (Abdella et al., 2014).

When antioxidant enzyme activities make alterations (Graf et al., 1998) or when VPA metabolites are created in the VPA treated subjects, chromosomal abnormalities and alteration in cells as well as in some tissues such as liver tissue are found.Defoort et al.(2006) have shown in their study that valproic acid can generate cytogenotoxicity. Sister chromatid interchanges in peripheral lymphocytes and this causes through homologous recombination repair pathway. That shows chromosomal vulnerability after subjection to a capable mutagenic agent. Although Marchion et al.(2005) showed in their study that VPA can perform as a histone deacetylase inhibitor and responsible for genomic vulnerability, activates acetylation of histone tails. DNA conformation alters as chromatin structure changes and causes DNA damage including break down of double strands (Coyle et al., 2005).

3.2 VPA induced autism

Schneider & Przewlocki (2005) first evaluated behavioral changes in rats receiving VPA injection in 12.5th day of gestation. They observed recurring and decreased sensory- motor behavioral patterns, diminished social behavioral pattern, and information processing deficit.

Moreover, detainment in motor development, ageing and growth, nest-seeking response, reduced body weight is also observed in their study (Schneider & Przewłocki, 2005).

Although the mechanism of action of Valproic acid is known partially, it is found in studies that

pregnant mice treated with Valproic acid disclose temporary hyperacetylation of H3 and H4 histones in the embryonic mouse brain and cause autism. Besides, Valproic acid involves with inhibition of an enzyme that causes GABA degradation, GABA transaminase results in increasing activity of the enzymes glutamic acid decarboxylase that is associated with GABA synthesis. Consequently, increased in GABA levels occur when the pregnant mice are treated with Valproic acid at the early stage of brain development and induced autism in offspring (Nicolini & Fahnestock, 2018).

Other mechanism of action of valproic acid includes activation of the β-catenin-Ras-ERK-p21 pathway(Jung et al., 2008), indirect inhibition of GSK-3β(Chen et al., 2000; Hall et al., 2002). By impediment of GSK3β, β- catenin activation is propagated that controls Ras and results in increased level of phpsphorylated ERK. In consequence, uninterrupted subsequent induction of p21 by ERK regulates stimulation of neural cell primogenitor separation and disrupts their propagation. Thus stimulation of neural cell primogenitor separation and impediments of its propagation is regulated through subsequent induction of p21 aided through ERK (Jung et al., 2008).

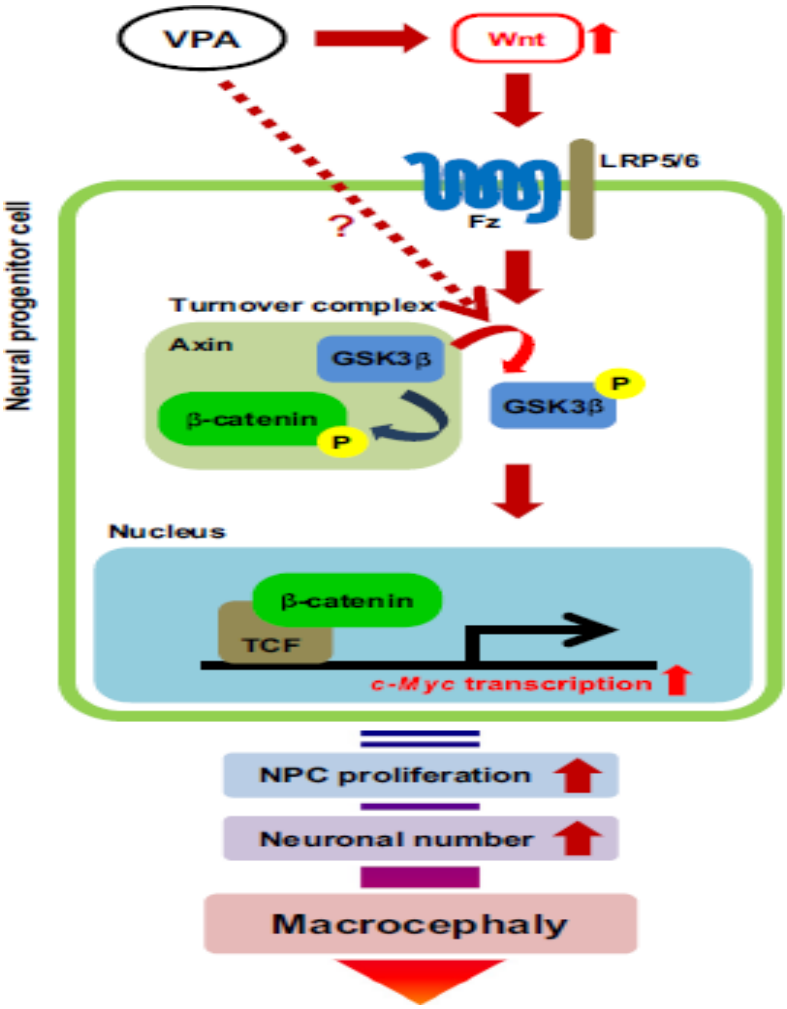

Figure 2: Schematic mechanism of VPA induced enhanced neurogenesis in the rat cortex. Prenatal exposure to VPA has increased Wnt in the embryonic brain, triggering Wnt/Fz (Frizzled) signaling to engage the membrane destruction complex, resulting in the phosphorylation of cytoplasmic tail of the lipoprotein receptor-related protein (LRP) (Go et al., 2012).

Disablement in olfactory system due to prenatal exposure of VPA was found in the several studies(D'Mello & Stoodley, 2015; McCracken, 2002; Tager-Flusberg, 1999).(Yeargin-Allsopp et al., 2003)had shown implementation of rodent pups olfaction in the developmental

process of social behavior. The three-chambered sociability test in both VPA exposed mice and rats have shown the social interaction deficiency (Kim et al., 2011; Moldrich et al., 2013; Roullet et al., 2010). It is a systematized test that has widespread use for assessing sociability in mice and rats(Crawley, 2004; Yang et al., 2011). There are interconnection between social phobia and hyper-active amygdala assisting the fact that asymmetrical recognition of fear and increased anxiety might aggravate aversion to the stimulant and gives rise to environmental fear that activates defective social interactions(Tillfors, 2004). These studies presents the significance of VPA subjected animal model in carrying on research to inspect autistic behaviors.

Rodier and colleagues (1996) proposed the VPA animal model where the rodents were exposed to VPA at single dose of 350 mg/kg for detecting the molecular pathway associated with autistic behavior and assessing possible therapeutics ofautism. Prenatally VPA subjected rodent model have been used widely from then onwards.

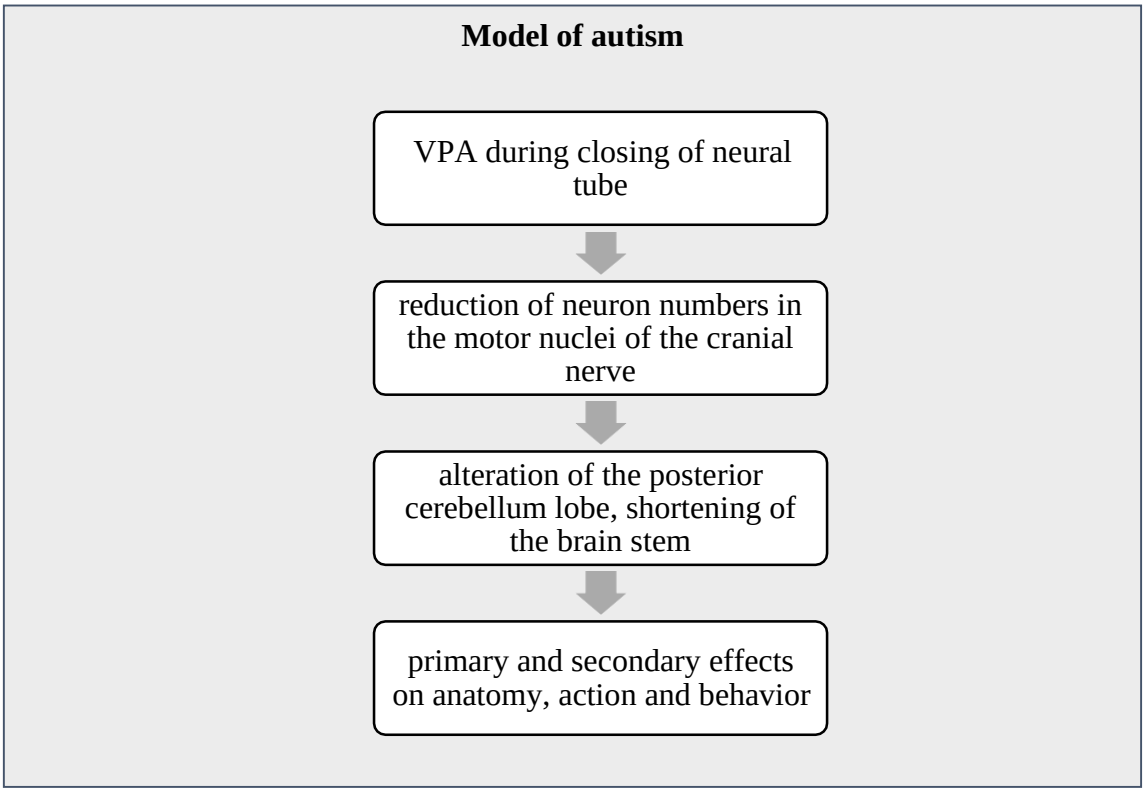

Figure 3: An animal model of autism on the basis of human evidence that at a very particular stage of CNS development, the condition can be triggered(Rodier, 1996).

3.3 Genetic alterations associated with ASD

It has been evaluated that more than 500 different genetic loci maybe associated with ASD (Stessman et al., 2014). According to State & Levitt, 2011, ASD may be developed in someone because of variations in many genes but none of them definitively accountable for it although someone with single gene disorder like fragile X can meet the criteria of ASD. With replication, variations that can be responsible for ASD have identified with genome-wide association studies.

In comparison to multiplex families (families with multiple affected offspring) the alteration of copy-number has revealed the over- expression of *de novo*, exceptional structural change in the simplex families (families with one affected offspring) genome. Moreover, in subsequent studies these results have been imitated, supporting the certainty where findings can be

established about genetic ties with common diseases and ASD (State & Levitt, 2011).

Iossifov and colleagues (2012) ran a study on a 343 families subset of Simons Simplex Collection where no considerable number of *de novo* missense mutation were noticed in affected compared to unaffected children. However, presence of gene-damaging mutations such as frame shifts, splice site and nonsense were noticed two-times as frequent (59 versus 28). They observed the parent of origin for *de novo* mutation is more frequently the father than the mother (50/17) for single nucleotide variation(SNV). Besides that mutation rate is also influenced by parent's age (Iossifov et al., 2012).

Table 1:Genetic biomarkers associated with ASD (State & Levitt, 2011).

Neurexin 1 (NRXN1) cancellation
7q11.23 duplicating actions
15q11-13 duplicating actions
16p11.2 duplication and deletion
SHANK 3
SHANK 2
SNC2A
CHD8
DYRKIA
POG2
GRIN2B
KATNAL2
CNTN4 cancellation
CNTNAP2
5p14.1
SEMA5A
TAS2R1
2q22.1
3p26.3
4q12
14q23
NLGN4

Cancellation at Neurexin 1 (NRXN1) locus, duplicating actions at 7q11.23 and at 15q11-13, duplication and deletion at 16p11.2 these are associated with ASD. Infrequent, functional

alteration in nucleotide sequence of genome encoding for NRXN1,SHANK3 and SHANK2 are linked with this genetic disorder (State & Levitt, 2011). Along with that four reports have verified genetic mutation responsible for ASD includes SNC2A, CHD8, DYRKIA, POG2, GRIN2B and KATNAL2 in whole exome sequencing method (Murdoch & State, 2013). Contacin 4 (CNTN4) aids in social and intellectual disability in a repetitive deletion syndrome and mutation in it is determined in idiopathic ASD. Alterations in CNTNAP2 are also found in ASD as stated in the following table (State & Levitt, 2011).

Wang and colleagues (2009) published a study in Nature on cytogenetic research. Their findings suggested that frequent genetic variants on 5p14.1 were responsible for ASD. Chromosome 5p14 contributes in cell adhesion and concludes that distinct gene in this class generates connectivity and structure of the brain that results in ASD (Wang et al., 2009).

Cadherin 9 (CDH9), cadherin 10 (CDH10), semaphorin 5A (SEMA5A), and taste receptor, type 2, member 1 (TAS2R1)- these four genes are associated in ASD in considerable amount of studies(Chango et al., 2000; Ma et al., 2009; Ronald et al., 2010; Weiss et al., 2009). As claimed by some GWAS they exist on chromosome 5p14, by which cell adhesion and growth of axon is controlled. These reports suggest that those genes and disruption in synaptic connection possibly a crucial factor in ASD when gene networks could not be established from a few number of genes (Lee et al., 2012).

A recent report assessed a higher concern in the number and size of deletions in individual with ASD in comparison to controls (Griswold et al., 2012). Overlapping in well-established autism related regions and candidate genes were identified in several copy number variations. Four large, novel deletions were found on 2q22.1, 3p26.3, 4q12, and14q23 which involve new regions and genes associated with ASD. NLGN4 related disordered findings has identified across cultures. No notable findings in connections with SNPs along NLGN4 gene was

observed in the Chinese ASD cases (Liu et al., 2013). However, nine nucleotide alterations in NLGN4X were observed to be connected with autism in Greek ASD cases (Volaki et al., 2013).

3.4 Some recently discovered cytogenetic biomarkers of ASD

Following table (Table 2) contains some recently discovered genetic biomarkers.

Table 2:Genetic biomarkers in individual with ASD/autistic characteristics

Gene	**Cytoband**	**Description**
SCN1A	2q24.3	Sodium channel, voltage-gated, alpha subunit 1
SATB2	2qq33.1	SATB homeobox 2
FOXP1	3p14.1	Forkhead box P1
NIPBL	5p13.2	Delangin (Nipped-B like protein)
ALDH7A1	5q23.2	Aldehyde dehydrogenase 7, member A1
AHI1	6q23.3	Abelson helper integration site 1
CHD7	8q12.2	Chromodomain helicase DNA binding protein 7
VPS13B	8q22.2	Vacuolar protein sorting 13 homolog B
TSC1	9q34.13	Tuberous sclerosis 1
EHMT1	9q34.3	Euchromatic histone lysine N-methyltransferase 1

Table 2 (continued)		
PTEN	10q23.31	Phosphatase &tensin homolog
DHCR7	11q13.4	7-Dehydrocholeterol reductase
CACNA1C	12p13.33	Calcium channel, voltage gated, subunit alpha1 C
PTPN11	12q24.13	Protein tyrosine phosphatase, non-receptor type 11
DMPK	19q13.32	Monotonic dystrophy protein kinase
NLGN4X	Xp22.31-p22.32	Neuroligin 4, X-linked
PTCHD1	Xp22.11	Patched domain containing 1
DMD	Xp21.1-21.2	Dystrophin
IQSEC2	Xp11.22	IQ motif & sec7, domain-2
FGD1	Xp11.22	Faciogenital dysplasia protein
MED12	Xq13.1	Mediator complex subunit 12 homolog
NLGN3	Xq13.1	Neuroligin-3
GRIA3	Xq25	Glutamate receptor, ionotropic, subunit 3, AMPA type
FMR1	Xq27.3	Fragile X mental retardation 1

Table 2 (continued)		
SLC6A8	Xq28	Solute carrier family 6, creatinine neurotransmitter transporter, member 8

Currently genetic testing of autism is offered by many companies on the basis of gene clusters with a strong clustering for ASD risk. There may be biomarkers in the coming years that can be used to detect high risk of ASD diagnosis, take for instance a mother with a high risk of immune dysfunction that probably contributed to ASD in second child after the first child has ASD (Goines & Van De Water, 2010) or a rise in the Akt-mTOR pathway seen in the fragile-X syndrome and other subtypes of ASD (Hoeffer et al., 2012).

Multiple genes have been reported to be deleted, mutated, disrupted or duplicated by translocation breakpoint in autistic individuals. For instance, alteration in SCN1A have been repored in individuals with ASD (Riva et al., 2009); disruption in SATB2 in autistic individual carrying a balanced translocation have been stated in several studies (Riva et al., 2009); impairements in FOXP1 (Hamdan et al., 2010); cognitive and growth retardation, malformation in upperlimbs occurs in 60% NIPBL muation, in 5% SMC1A mutation and SMC3 muation in one autistic patient (Oliver et al., 2008); at least 3 autistic patients have been found with alteration in ALDH7A1 mutation (Mills et al., 2010).

Patient with Joubart syndrome are frequently found to develop ASD (13%-36%), 10 genes have been involved in Joubart syndrome but only 4 have been reported to have mutated in subjects with ASD/autistic characteristics (Takahashi et al., 2005). CHD7 (chromodomain helicase DNA binding protein 7) variation causes heart anomaly, coloboma, retardation, choanal atresia, genital and ear anomalies therewith 68% (17/25) have ASD/autistic traits (Hartshorne et al., 2005). 49% (22/45) individuals who have variation in VPS13B meet autism

criteria (Howlin et al., 2005). Mutations of the genes TSC1 or TSC2 triggers tuberous sclerosis. The prevalence of tuberous sclerosis in epidemiologicak studies of patients with ASD is ~1%; the prevalence of ASD in subjects with tuberous sclerosis ranges from 16% to 60% (Mazaubrun, 2010).

Several studies have found that EHMT1 gene is responsible for central phenotype of the Kleefstra syndrome (9q subtelomeric deletion syndrome); 23% (5/22) of people with Kleefstra syndrome had ASD/autistic characteristics due to deletion or mutation (Anderlid et al., 2002; Dawson et al., 2002; Iwakoshi et al., 2004). The prevalence of PTEN mutation in children with ASD and macrocephaly is not known; 15 percent of children with PTEN mutations had ASD in one sample(4/26) (Butler et al., 2005; Buxbaum et al., 2007). Smith-Lemli-Opitz syndrome is an inborn metabolism deficiency that causes biosynthesis of the cholesterol (DHCR7 gene alteration). According to two studies the rate of ASD in this syndrome is really high, 53 percent (9/17) meet autism criteria and 71 percent (10/14) have ASD (Doco-fenzy et al., 2006; Tierney et al., 2006). Alteration in calcium channel,voltage-dependent,L-type,alpha 1c subunit cause timothy syndrome which is long QT syndrome with syndactyly. Five children identified with timothy syndrome, three had autism,one had severe language delay (Splawski et al., 2004).

Noonan syndrome (heart defects, short stature, craniofacial anomalies) was reported in individuals with PTPN11(Protein tyrosine phosphatase, non-receptor type11) alterations. 8 percent had ASD diagnosis in a sample of 65 children with Noonan (Ghaziuddin et al., 1994; Paul et al., 1983; Pierpont et al., 2009). In a study of 57 myotonic dystrophy-1 children and adolescents, 49 percent had autistic traits (Jr & Finir, 1994). Reports of both mutations and deletions is observed in ASD patients on NLGN4X (Doco-fenzy et al., 2006; Marshall et al., 2008) and PTCHD1 (Marshall et al., 2008; Noor et al., 2010). DMD is the largest known gene in human and alteration in it causes masular dystrophy and in one sample, 19 percent (16/85) met ASD tratits (Erturk et al., 2010); IQ motif and sec7 domain 2 is protein which is encoded

in humans by the gene IQSEC2. Alteration in this gene develops autistic traits (Shoubridge et al., 2010). Gene FGD1 is found on the X-chromosome's short arm which is very important for regular embryonic mammalian growth. Some mutation in it causes faciogenital dysplasia and four cases were identified with a clinical diagnosis of faciogenital dysplasia alongside autism characteristics (Taub & Stanton, 2008); aletration on MED12 gene results Lujan-Fryns syndrome. According to some studies, 62.5 percent (20/30) Lujan-Fryns syndrome cases had an autism condition (Schwartz et al., 2007). NLGN3 gene mutation were identified only in one family of two non-syndromic ASD members, one had Asperger syndrome where the oher had autistic traits in them (Jamain et al., 2003).

There are several reports about mutations along with partial duplication of GRIA3 gene in some autistic individuals(Doco-fenzy et al., 2006; Goldenberg, 2015; Jacquemont et al., 2006). Fragile X mental retardation protein or FMRP is encoded by FMR1 gene in human and fragile X condition is observed in ~2% ASD individuals. In some cases, ~60% of completely mutated males and ~20% of females had ASD; premutaion often correlated with a greater incidents of ASD: 10-15% for males and 5% for females (Clifford et al., 2007). The gene SLC6A8 at Xq28 encodes a trasporter of creatinine and mutaion in it results brain creatinine deficiency; autistic characteristics appear common in syndromes of creatinine deficiency (Cheillan et al., 2006; Li et al., 2002).

3.5 Some frequently occurred cytogenetic disorder and chromosomal anomalies in ASD.

Following table (Table 3) contains frequent cytogenomic disorders and chromosomal anomalies in ASD individuals-

Table 3: Frequent cytogenomic disorders and chromosomal anomalies in ASD individuals

Disorder	**Cytoband**
1p36 microdeletion disorder	1p36.32-p36.33
1q21.1 microduplication or microdeletion disorder	1q21.1
2p15-p16.1 microdeletion disorder	2p15-p16.1
2q37 monosomy	2q37.3
5q35 deletion, 5q35.2q35.3 duplication disorder	5q35.2-q35.3
7q11.23 deletion and duplication disorder	7q11.23
8p23.1 deletion or duplication disorder	8p23.1
10q22-q23 deletion disorder	10q22.3-q23.2
Distant 10q deletion	10q26.2-q26.3
11p15.5 duplication disorder	11p15.4-p15.5
11p13 deletion	11p13
11q deletion disorder	11q23.3
15p24 microdeletion disorder	15p24.1-p24.2
16p13.3 duplication disorder	16p13.3

Table 3 (continued)	
16p11.2-p12.2 microdeletion or microduplication disorder	16p11.2-p12.2
17p11.2 microdeletion and duplication disorder	17p11.2
17q21.31 microdeletion or microduplication disorder	17q21.31
Down syndrome	21
21q11 deletion and duplication disorder	21q11.21-q11.22
Turner syndrome	X
Klinefelter syndrome	X
XYY syndrome	Y
XXYY syndrome	X-Y
45,X/46,XY mosaicism	X

Some frequent chromosomal anomalies and genomic disorders have mentioned in individuals with autistic characteristics on multiple studies. Few cases linked to autistic features have recorded on 1p36 microdeletion that is a contiguous gene syndrome known as the most frequent syndrome of subtelomericmicrodeletion(Blennow et al., 1996; Bruno et al., 2008).

Neurodevelopmental disorders have been reported on microdeletion and/or microduplication on 1q21.1; 7 percent (3/42) with deletion and 30 percent (7/13) with duplication had autistic symptoms (Brunetti-Pierri et al., 2008; Mefford et al., 2008). On 6 recorded cases, 4 have autistic traits on recently represented 2p15-p16.1 microdeletion syndrome (Rajcan-Separovic et al., 2007). In ASD subjects multiple 2q37 deletions have been recorded; 24 percent (16/66) of patients with 2q37 reported autistic behavior, 63 percent (5/8) had autism in a smaller sample (S. R. Williams et al., 2010).

Sotos syndrome 5q35 deletion is caused by NSD1 mutations or deletions. Many reports of Sotos syndrome and ASD were documented, some clinically diagnosed before the genetic disorder was detected and others molecularly validated, although it was not known if they had deletions or NSD1 mutation (Deodato et al., 2006; Kielinen et al., 2004; Miles & Hillman, 2000).Williams syndrome (Williams-Beuren syndrome) is a contiguous condition of the genome that arises from a deletion of 7q11.23. Reciprocal duplications were identified in persons with extreme developmental delay and ASD; fifty percent (15/30) of Williams syndrome cases follow ASD parameters; forty percent (11/27) duplication of 7q11.23 have autism (Berg et al., 2007; Challman et al., 2003; Gallo, 2010). 7 deletions and 2 duplications case of 8p23.1 have been reported in some ASD related studies; 57% (4/7) of patients with 8p23 deletion had autism in one study (Fisch et al., 2010; Glancy et al., 2009). Frequent deletion of 10q22-q23 of different sizes was correlated with cognitive and behavioral disorders similar as ASD and hyperactivity (Alliman et al., 2010; Gallo, 2010). Deletion differ in size and the main region is not fixed; many literature have identified more than 100 cases of distal 10q deletion but only 4 cases with autistic features have recorded yet (Colleaux et al., 2001; Yatsenko et al., 2009).

Impaired expression of imprinted genes on chromosome 11p15.5 induces an overgrowth disease named Beckwith- Wiedemann syndrome (BWS) or a pre- and post-natal development retardation known as Silver-Russell syndrome (SRS). Maternal duplication on 11p15.5 of the

genes H19 and IGF2 cause SRS while paternal duplication causes BWS. Within ASD both conditions were identified; 7% (6 children) had an ASD diagnosis in a sample of 87 children with BWS (Kent et al., 2008).

WAGR syndrome (Wilms tumor, aniridia, genitourinary abnormalities and retardation of mental state) is a compact gene syndrome triggered by deletion of the region 11p13 and ASD is firmly related to it; in a study 52% (16 participants) of the 31 reported ASD where other 14 had some major autism traits (Xu et al., 2008). Jacobsen syndrome results from distal 11q gene condition, deletion range in size from 4-30 Mb. The breakpoint typically occurs inside or distal to 11q23.3 also generally stretch to the telomere. More than 200 cases were documented but very few cases were identified in accordance with ASD features. Nonetheless, 33 percent (3/9) of Jacobsen syndrome patients have autism in a new survey(Bernaciak et al., 2008; Fisch et al., 2010). 22% (4/18) of informed cases have autistic characteristic with 15q24 microdeletion syndrome (Marshall et al., 2008; McInnes et al., 2010).

16p13.3 deletions and duplications both have been found in ASD individuals (Hellings et al., 2002; Thienpont et al., 2010). 3 duplications of 16p11.2-p12.2 have been reported in ASD (Engelen et al., 2002).

16p11.2 microdeletion and microduplication both forms are related with partial penetration and expressiveness on autism (Bijlsma et al., 2009; Fernandez et al., 2010). 17p11.2 microdeletion(Smith-Magenis syndrome) and 17p11.2 microduplication (Potocki-Lupski syndrome) are triggered by alterations in copy numbers or by mutations in RAI1; both are often related to ASD. 90 percent (18/20 of people) with Smith-Magenis syndrome had ASD in one sample (Hicks & Ferguson, 2008; Laje et al., 2010; Nakamine et al., 2008).

In various ASD cases microduplication of 17q21.31 have been found (Grisart et al., 2009). In number of studies the percentage of patients of Down syndrome who meets autism requirements ranges between 5%-15% (Carter et al., 2007; Kent et al., 1999).

ASD requirements are fulfilled by 28 percent (84/299 of people) with 11q22 deletion syndrome. Newly identified duplication syndrome 22q11 has been documented in many ASD cases. Significant phenotypical heterogeneity have revealed in both deletions and duplications (Bucan et al., 2009; Peebles et al., 2007). As reported by some studies 5/150 people with Turner syndrome (3.3 percent) have autism (Creswell & Skuse, 1999). People with Klinefelter syndrome (XXY) have been consistentlt reported in ASD samples (D. V. M. Bishop et al., 2011). Epidemiological, clinical and academic studies of ASD have reported males with XYY syndrome (D. V. M. Bishop et al., 2011; Challman et al., 2003). ASD also happens in presence of XXYY syndrome (Tartaglia et al., 2008). In some studies mosaicism with ASD were reported in 45,X/46,XY (Fontenelle et al., 2004).

The above cytogenetic alterations have identified by literature review. We may presume other cases are likely to occur in other situations that either not have been published or got away my observation. As most ASD individuals are not regularly screened for the genetic disorders, the genes that are included in this study is just the tip of the iceberg on the basis of what we see documented in the literature. This should be also remembered that only a handful of cases have been documented in the literature as some of the alterations discussed above are very exceptional or uniquely identified. Evidence for the presence of a particular gene for ASD derives from the identification of other people with the same genetic disease and associated gene mutations. To classify most of these mutations sequencing would be appropriate but only very specific sequencing strategies have been implemented in ASD research to date. It can be assumed that in additional cases mutations in these genes will be found with whole-genome sequencing and more cytogenetic alterations will be discovered.

3.6 Cytogenetic biomarkers and gene editing techniques

Previously discussed cytogenetic alterations can be modified using gene editing techniques that enable genome modifications at single nucleotide level (Driehuis & Clevers, 2017). To date,

several innovative and important genome editing techniques have been developed as disease therapy, including RNA interface (RNAi), Zinc-Finger Nucleases (ZFNs), CRISPR/Cas9 etc. (Savić & Schwank, 2016) and among them CRISPR/Cas9 is better because this system is the most efficient (Wyman et al., 2013). SHANK3 gene is mainly considered as an important biomarker for ASD (Table 1). Zebrafish has been used to model ASD as it can exhibit a higher degree of efficacy during genetic experiment (C. X. Liu et al., 2018). In the experiment, CRISPR/Cas9 was used in loss of function mutation with a series of behavioral study, morphological estimation and molecular examination which efficiently charectarized the molecular and behavioral alterations of the mutant Zebrafish. This adaptable Zebrafish model will play an important role in the drug screening and neurodevelopment in the future ASD and SHANK3 functional studies (Hwang et al., 2013).

3.7 Protective effect of Vitamin E

To stable the genetic materials inside the cell the antioxidant properties of vitamin E is significant as autoxidation products of unsaturated fatty acids and lipids are greatly noxious mutagenic substances (Vaca et al., 1988).DNA damages can be decreased if vitamin E supplements is taken according to many *in vivo* and *in vitro* studies (Mozdarani & Salimi, 2006).

A current study done by Girgis(2011), verifies the reduced chromosomal damages and cytotoxic results due to administration of vitamin E in ASD. In the study it is also established that antioxidants like vit E protects against diazinon genotoxic effect. It disables or scavenges free radicals and provides protection as well as prevents DNA damage (Aly et al., 2009).

Vitamin E is categorized as naturally derived elements named α, β, γ,δ derivatives of tocopherol and tocotrienol. After absorption in gut, Vitamin E is carried to the liver by chylomicrons, a low density lipoprotein. Storage of Vitamin E in the biological system happens as a result of action of hepatic-α-tocopherol-transfer-protein when α-tocopherol integrates into plasma lipoproteins.

On the other hand, other forms of vitamin E are more prone to eliminate through bile or urine. Nonetheless, another form of Vitamin E, γ-tocopherol exhibits more efficiency in reactive nitrogen species detoxification. It shows greater antioxidant feature in food lipids than α-tocopherol.α-tocopherol rich diet sources are wheat germ or sunflower and γ-tocopherol rich diet sources are corn oil, sesame, soybean (Cordero et al., 2010).Vitamin E will show its activity properly when adequate amount will be absorbed from the gut, carried into the blood,
to tissues and will be stored in the cellular membrane (Burton et al., 1983).

In another article it was mentioned that α-tocopherol is favorably functional as a chain breaking anti-oxidant and has led to the biological properties of Vitamin-E molecule by both the phenolic head and phytyl tail (Burton et al., 1983). Tocotrienols have suggested possessing efficacious cholesterol lowering, neuroprotective and anticancer properties. It is reported that Vitamin-E decreases lipid peroxidation, oxidative stress and toxic effect of reactive oxygen species in biological system. Moreover, teratogenicity activated by VPA in animal models and in mitigating VPA induced hepatotoxicity has been protected by Vitamin-E, primarily by its antioxidant and anti-inflammatory properties. Neural tube defects caused by VPA were decreased by Vitamin-E supplementations. It also has demonstrated to have beneficial effects on immune system (Catalgol & Ozer, 2012).

As reported by Abdella and colleagues, most potential doses of vitamin E is 50 and 100 mg/kg that verified considerably limited array of chromosomal abnormalities and 200 mg/kg was not as effective dose as 50 and 100 mg/kg on the preceding criteria. It turned out 50 and 100 mg/kg has roughly the same impacts and efficacy compared to 200 mg/kg (Abdella et al., 2014).

Chapter 4

Conclusion

The purpose of this review was to represent the available cytogenetic biomarkers of ASD and to report the protective function of vitamin E against cytogenetic alterations in ASD individuals. These findings of biomarker, with regard to etiological diagnosis, genetic counseling and patient treatment will have significant implications for patient and their families. Further targets on the neurobiological research pathway for beginning pharmacotherapy can also be provided by this result of cytogenetic alterations. The evidence provided in this review clearly indicates that autism is the ultimate common pathway for many genetic brain disorders. These results also suggest that, on the basis of other genetic, environmental or stochastic influences, these cytogenetic biomarkers generate a spectrum of neurodevelopmental disorders. There is now strong evidence for importance of unusual and *de novo* variation in DNA sequence and structure, for the convergence of numerous separate mutations on functions from synaptic activity to chromatin alteration and for considerable phenotypic diversity involved in mutations having even significant biological consequences. Diagnosis of ASD is important to ensure that the autistic individual is receiving appropriate clinical treatment and educational placement that is not always accessible to people with genetic syndrome. Several SNPs are mentioned in this review and about their presence in ASD as pharmacogenetic biomarker. It can be said that soon scanning large population to find out cytogenetic mutations will be easier and classification of additional ASD genes will be less time consuming and less expensive with the new innovative methods. It is important to remember that in the presence of genetic disorder many practitioners are hesitant to provide an additional diagnosis of ASD.

Looking at this review, it can also be stated that ASD has a complex cytogenetic architecture and difficulties associated with categorical diagnosis. Fortunately, a fast increasing pool of well-established genes and loci and the recent development of systematic approaches to gene discovery provide the basis for real progress in the next step of ASD research to examine these considerations. In the review it is also referred that vitamin E has been observed to restore cytogenotoxicity. It can be concluded that vitamin E, as an antioxidant, has a protective effect against cytogenotoxicity and vitamin E supplementation maybe prenatally beneficial. However, further studies must be done on this as very limited data is available about protective function of vitamin E.

4.1 Limitation of the study

Although some limitations must be mentioned about how difficult it is to determine the extent of variations in terms of a biomarker that are indicative unless further studies are done. In the studies, the number of the subject significantly varies. Single markers are insufficient to express autism as it is a spectrum of disorders. It is therefore, conceivable that a combination of multiple biomarker arrays will differentiate between different autism spectrum disorders. This literature review shows that more research is required in the evaluation of different genes associated with ASD as the present data are insufficient. Certainly, considerable important issues remain to be explained, comprising the degree to which ASD represents developmental rather than ongoing functional deficits, the spatial and temporal features of human brain ASD pathology and the contribution of gene-environment, immune system, microbiomic and other cytogenetic and epigenetic considerations in the risk and outcome of diseases.

4.2 Future research plan

Diagnosis of ASD requires several biomarkers because biomarkers can be overlapped in ASD. Toxicologic, neurologic, metabolic, hepatic alterations and protective role of other anti-oxidants like vitamin E can be potential future research plan on autism spectrum disorder.

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