FUTURE PERSPECTIVE OF AUTISM SPECTRUM DISORDER AND MOLECULAR PATHOGENESIS

A THESIS SUBMITTED TO THE GRADUATE SCHOOL OF HEALTH SCIENCES OF NEAR EAST UNIVERSITY

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In Partial Fulfillment of the Requirements for the Degree of Master of Science in Medical Biology and Genetics

NICOSIA, 2017

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The Director of Graduate School of Health Sciences,

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DEDICATION

To all those that believe and motivate me

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LIST OF ABBREVIATIONS

ASD:	Autism spectrum disorders.
ID:	Intellectual disability.
SRS:	Social responsiveness scale.
AQ:	Autism quotient.
PDD-NOS:	Pervasive developmental disorder not otherwise specific.
DSM:	Diagnostic and statistical manual for mental disorder.
AD:	Autistic disorder.
HFA:	High functioning autism.
LFA:	Low functioning autism.
IEM:	Inborn errors of metabolism.
FXS:	Fragile X syndrome.
UTR:	Un-translated region.
MECP:	Methyl CpG binding protein.
RS:	Rett syndrome.
DD:	Developmental delay.
ADOS:	Autism diagnostic observation schedule.
ADI-R:	Autism diagnostic interview review.
CARS:	Childhood autism rating scale.
MIA:	Maternal immune activation.
PSD:	Phenan- McDermid syndrome.
CNV:	Copy number variation.
CNS:	Central nervous system.
NRXN:	Neurexin.
NLGN:	Neuroligin.
OXTr:	Oxytocin receptor.
CNTN:	Contactin.
CAMs:	Cell adhesion molecules.
CNTNAP:	Contactin associated protein.
DAT:	Dopamine transporter.
APBA:	Amyloid precursion protein binding protein.

BDNF: Brain derived neurotrophic factor.

CADPS: Calcium dependent secretion activator.

IL1RAPL1: Interleukin 1 receptor accessory protein like 1.

XLID: X chromosome linked intellectual disability.

OPHN: Oligophrenin.

TM4SF2: Transmembrane 4 superfamily.

TSC: Tuberous sclerosis.

NF: Neurofibromin.

FMR: Fragile mental retardation.

FMRP: Fragile mental retardation protein.

ABSTRACT

Autism spectrum disorder (ASD) belongs to the group of neuropsychiatric developmental disorder which is usually characterised due to a complication in social disability, communication and repetitive impairment. Autism spectrum disorder is mostly common in male than female in a ratio of 4: 1. This neurodevelopmental disorder is also associated with medical symptoms and comorbidities such as epilepsy, sleeping disorder, oral health issues and gastrointestinal disorder. This thesis gives a proper definition regarding the history of Autism spectrum disorder (ASD) likewise providing information in regards to the various divisions of Autism spectrum disorder (ASD) which includes Autistic syndrome (AS), Asperger disorder (AD) and pervasive developmental disorder (PDD). This research work also provides detailing Genetic factors associated with ASD which is subdivided into two factors namely; Chromosomal abnormalities or gene alteration and genetic copy number variant which have a strong association and capacity to develop an autistic disorder.

Other techniques such as Fluorescence In Situ hybridization (FISH), Whole exome sequencing (WES), the selective candidate gene analysis and chromosomal microarray are widely explained in this research work to investigate specific sub-microscopic deletions and likewise the confirmation of a clinical diagnosis. This also shows some genetic disorder observed to be heavily linked with Austim spectrum disorder (ASD) such as Fragile X syndrome (FXS), Rett syndrome (RS), Tuberous sclerosis (TS) and 22q11 deletion syndrome. Amidst these genetic syndromes, some candidate genes have been observed to be related to Autism spectrum disorder (Table 3.1) due to their various networking functions in the brains which causes symptoms of ASD when altered.The presumed environmental effect of Autism spectrum disorder is caused due to autoimmunity, maternal infection during pregnancy, gastrointestinal dysfunction and altered micro-biome and immune dysfunction were likewise briefed. Huge research have been carriedout to contribute to the improvement of ASD through development of various diagnostic tools and criteria to improve evaluation of the behavioural features of ASD individuals which involves the following such as Autism diagnostic observation scheme (ADOS), Autism diagnostic interview revised (ADI-R), Childhood autism rating scale (CARS), Intelligence quotient (IQ), Magnetic resonance imaging (MRI) and diffusion tensor imaging (DTI).

ÖZET

Otizim spectrum bozukluğu (OSB) bir grup nörepskiyatrik gelişim bozukluğuna bağlı, sosyal yetersizlik, iletişimde zorluk ve tekrarlı impermanlarla ortaya çıkan bir hastalıktır. OSB erkeklerde kadınlara oranla 4 kat daha fazla görülür. Bu nörogelişimsel bozukluk, epilepsy, uyku bozukluğu, oral sağlık sorunları ve gastrointestinal bozukluklar gibi diğer bazı eşzamanlı hastalıklarla da ilişkilidir. Bu tez OSB tarihi ile otistik sendromu, asperger sendromu ve yaygın gelişimsel bozukluğun da içinde bulunduğu bölümleri hakkında detaylı bilgi vermektedir. Bu araştırmada ayrıca, OSB ile ilişkili genetic faktörler, kromozomal anomaliler yada gen değişiklikleri ve genetic kopya sayısı varyasyonları, detaylı şekilde ele alınmıştır.

Diğer yandan, Floresan İn Situ Hibridizasyon (FISH), tüm ekzon dizileme, microarray ve seçici aday en incelemesini detaylandırıp, bu tekniklerin spesifik delesyon analizinde ve klinik tanı koymadaki önemleri belirtilmiştir. Böylece, OSB ile ilişkili Frajil X sendromu, Rett sendromu, Tuberküloz ve 22q11 delesyonu diğer bozukluklar saptanmıştır. Bu genetic sendromlar yanı sıra, bazı aday genler (Tablo 3.1) beyin fonksiyonlarındaki rolleri ve mutasyona uğradıklarında OSB ile uyumlu semptomlar görülmesi nedeyile saptanmıştır. Genetic faktörler haricinde, otoimmünite, hamilelikte maternal enfeksiyon, gastrointestinal fonksiyon bozukluğu, değişmiş mikrobiom ve immune fonksiyon bozuklukları da çevresel faktörler olarak ele alınmıştır. Aralarında Autism diagnostic observation scheme (ADOS), Autism diagnostic interview revised (ADI-R), Childhood autism rating scale (CARS), Intelligence quotient (IQ), Magnetic resonance imaging (MRI) ve diffusion tensor imaging (DTI) gibi tanı yöntemleri ile OSB'li kişileri değerlendirmede kullanılar kriterler, çok uzun süreli araştırmalar sonucu ortaya çıkmıştır.

INTRODUCTION

Autism spectrum disorders (ASD) is among groups of neuropsychiatric disorders which are normally classified as a result of complications in communication behaviour and social skill likewise the presence of stereotyped and repetitive behaviours (Kanner 1943 and Asperger 1944). Autism is seen has the most common form of developmental disorder recognised in the United States, which is termed to affect an approximate of 1 in 68 children [Center for Disease Control (CDC) 2014, (APA), 2013]. Persons associated with ASD are also faced with other psychiatric and medical conditions which may include intellectual disability (ID), epileptic problems, difficulties in motor control , attention-deficit hyperactivity disorder (ADHD), sleep disorders,anxiety, depression or gastrointestinal problems [Gilberg., 2010]. The 'Early Symptomatic Syndromes Eliciting Neurodevelopmental Clinical Examinations' (ESSENCE) was formed by Christopher Gillberg for the sole aim of putting into consideration the syndromic overlap and clinical heterogeneity(Gilberg., 2010).

Males are 4–8 times of more affected with ASD than affected females, but the sex ratio is said to balance among patients suffering with intellectual disability (ID) and dysmorphic characteristics. Autism can as well be studied as a category of affected person'svs unaffected person or as a quantitative trait with the use of auto or hetero-questionnaires analysis such as the social responsiveness scale (SRS) or the autism quotient (AQ) respectively. With the use of this method and approach, autistic trait seems to be normally and adequately distributed in clinical cases likewise in a large population. The major causes of autism remain largely unknown and undetected, although twin research has repeatedly shown a relatively increasing genetic improvement and knowledge to ASD. Molecular genetic research have been able to identify more than 100 autism spectrum disorder risk genes which are seen to be highly responsible for carrying rare deleterious mutations in approximately 10–25% of the persons suffering with ASD [Huguet *et al*., 2013]. The genetic landscape of autism spectrum disorder is hereby caved by a complex reciprocation between a common as well as a rare variants and its most likely to hugely vary from one person to another (Bourgeon. 2015). Furthermore, the susceptibility genes are seen to relate judiciously in a fewer number of biological pathways which may include protein translation, chromatin remodelling, synaptic functions and actin dynamics (Bougeron. 2015).

Autism was first and originally mentioned and explained by Leo Kanner in 1943 which is considered as one among the most severe neurodevelopmental disorders [meaning it is usually found in the brain and shows its severity in early childhood] that has significant impact on social well-being, communication, cognitive and possesses a huge genetic factor [Rutter, Kim-Cohen, & Maughan, 2006].

Autism is examined has a developmental disorder and at such, symptoms and behavioural changes takes place over the course of development. With every autism spectrum individual showing a slightly different presentation of the disorder, the symptoms can be majorly grouped into 3 core domains which may include;

-Social interaction

-Speech/ communication

-Compulsive / repetitive behaviours

ASD are diagnosed primarily based on behavioural observation of individuals that shows symptom of the abnormalities. The diagnostic principle for ASD has been periodically revised due to the effective advancement in research. The Diagnostic and statistical manual of mental disorders fourth edition (DSM-IV) termed ASD to be a combination of triad symptoms involving impairment in social , communication interaction as well as repetitive behavioural pattern involved in the three specific subgroups of Autism spectrum disorder (ASD) involves Autistic disorder, Asperger disorder and pervasive developmental disorder not otherwise specified (PDD-NOS). The most recent version of DSM is the DSM-5 which was introduced in 2013(APA 1994), these three subgroups were merged together to form a single umbrella term called the “Autism spectrum disorder”. Also, the three symptoms of these disabilities from DSM-IV version were also conjoined into two categories, where social interaction and communication are joined together as a single or one diagnostic category.

In further explanation to these major symptoms, an approximate of 31% ASD affected persons showcase severe intellectual disability and 20-30% hasseizures (Canitano 2007). Gastrointestinal disorder (White *et al*.., 2007), sleeping disorder, anxiety disorder and abnormal responses to sensory stimuli are seen as common comorbidities associated with ASD.

Male are more likely affected than the female sex with the approximate ASD diagnostic ratio of male to females as 4:1. Most ASD cases are said to be idiopathic signifying absence of known causes such as brain injury and genetic disease. Apparently,genetic influence is strongly considered based on high risk of developing ASD if affected person possess a positive family history of the disorder. A lot of research are presently been conducted to examine the brain functionality of individual with Autism spectrum disorder.

Usually, family of children suffering from Autism spectrum disorder (ASD) normally report the symptom onset at about 12 and 18 month old of the child. In the year 2007, the centre for Disease control and prevention [CDC, 2007] reveals that1 in every 150 children to shows a prevalence rate of this disorder in the United States which explains a huge and remarkable increase from the previous prevalence rate of over a decade ago as 1 in 2,500.(E.g.Lotter 1966). The prevalence rate of this Autism spectrum disorder (ASD) later rose to about 1 in every 110 persons with this disorder [Autism and developmental disabilities monitoring network 2009] and the most increased rate of 1 in 90 parents reporting incidence of their children being diagnosis with the autism spectrum disorder (Kogan *et al*., 2009).

1.1 AIM AND OBJECTIVES

- To investigate the historical perspective and comorbidities of Autism spectrum disorder (ASD).
- To review the causes and molecular pathogenesis of Autism spectrum disorder.
- To review present research on present diagnostic techniques in observing Autism spectrum disorder.
- To detect the genes and environmental involvement to Autism spectrum disorder.
- To evaluate and if possible suggest future direction to improving knowledge and cure of Autism spectrum disorders

1.2 A BRIEF HISTORY OF AUTISM DISORDER

Dr. Eugen Bleuler was the first person to use the word*autism* was firstly used in the early 20th century to describe a person suffering from schizophrenia that was disarticulated from reality (Bleuler 1916). A Decades after, a child psychiatrist working at Johns Hopkins University called Dr. Leo Kanner used a particular term so as to describe and explain a childhood disability which he called “early infantile autism” showcasing difficulties in social and language impairment as well as the presence of a repetitive behaviours (Kanner 1943).

Dr. Kanner illustrated that the group of children he was dealing with also had a disconnect from reality as described by Dr. Bleuler, but did not also have schizophrenia. During the same time period in Europe, Dr. Hans Asperger who is an Austrian paediatrician practicing in Vienna, came up with a theory after observing a few group of boys in 1944 who posseses similar impairment in regards tosocial interaction, but they are not as severely affected as those described by Dr. Kanner (Asperger 1944). After the research, he called them "little professors" due to their tendency to discuss subjects in great detail. Dr. Asperger's work was revisited in the 1980s by a psychiatrist in the United Kingdom called Dr. Lorna Wing and after the research, he encourage autism experts researchers to see autism as a spectrum (ASD) of challenges other than one homogenous disorder. This thought gave a greater chance that encouraged the addition of Asperger's Disorder (AD) among other autism-related diagnoses such as PDD-NOS, which is to be considered as a separate diagnoses from autism in 1994 (APA 1994).

Knowledge and awareness of autism began to increase in the 1980's were many has greatly and partly contributed to this awareness through the movie '' Rain man'' (1988) which won numerous academy awards as well as best picture and best actor award for Dustin Hoffman who gave an accurate portrayal of an adult with Autism disorder. The media has greatly imparted by drawing a huge attention to people about Autism since the early 1990's. Many television reports, newspaper, articles, magazines and documentary have improved the dissemination of information about autism in recent years.

Autism spectrum disorder attributed a group of diagnosis that are considered clinically different from one another but are sometimes grouped together for intellectual purposes due to their overlapping characteristics.

Autism spectrum disorder is mainly diagnosed based on observation and parental interview regarding their children behavioural characteristics in the social character, communication interactions and repetitive behaviour domains. Specific symptom within each domain that is essential for a diagnosis on the Autism spectrum are majorly described in the diagnostic and statistical manual for mental disorder, fourth edition (DSM-IV; APA 1994). Meanwhile, autism was not mentioned as a diagnosis in the first edition of the DSM (DSM-1; APA 1952). Autism was continually recognised as a part of childhood schizophrenia until in 1980 when it was recognised has its own disorder called '' infantile autism'' in the publication of the DSM-111 (APA 1980). Subsequent editions of the DSM have further described autism

diagnosis and there are current different diagnostic criteria for Autistic disorders, Asperger disorder (AD) and PDD-NOS. Number of appropriately designed tools exist to help make a diagnosis of ASD and some of this tool for evaluation are; the Autism diagnostic observation schedules (ADOS) (Lord *et al.*, 1999) and the Autism diagnostic interview revised [ADI-R)(Lord, Rutter and Le contour 1994].

The disorders are listed and explained has below;

- PDD
- Autism
- PDD-NOS
- Asperger

1.3 CLINICAL CHARACTERISTICS

The main symptom of ASD is examined to be difficulty with reciprocal social interaction, such as limited potential to develop appropriate peer relationships and reduce shared enjoyment with others. Communication skills are also impacted in ASD and Individuals often try to maintain reciprocal conversations as well as constantly demonstrating unusual speech patterns. The last area of impairment is often the most seemingly striking and involves a series of behaviour, interests, and activities that are unusually repetitive or restricted in quality. Symptoms in this domain include hand flapping and insistence on maintaining a routine. Each of the domains will be thoroughly discussed in detail below. Meanwhile the major areas of challenges and difficulties are alike among individuals with autism spectrum disorder ASD, An observable reality among children and individuals with ASD is the significant difference in terms of symptom presentation. It became so rigid that researchers came up with the conclusion that individuals as not affected by autism disorder, but rather “autisms” this is because every child differs in his/her presenting abilities, strengths and weaknesses. For example, one child may be specifically good at maintaining consistent eye contact during social interactions, but struggle to maintain age appropriate friendships while another may use appropriate gestures, but have challenges with direct eye gaze. Taking into consideration of each child’s individual symptom profile is important in developing an adequate and appropriate case approach as well as focusing on suitable treatment targets.

1.3.1 Qualitative Impairment Social Interaction

The central features of autism within the social domain include disabilities in social mutuality (the give and take of social interaction); the incorporation of verbal with nonverbal aspects of

social discourse; selective friendship development likewise exchange of interests,excitement and enjoyment with others children (Filipek *et al*, 2000, Volkmar *et al*., 1999, Volkmar & Klin, 1999]. During the first months of life, disabilities are found in social reciprocity and social communication. Infants and toddlers with autism do not find it difficult to make steady and meaningful eye contact and they pay less attend to the voices and faces of others than their typically developing peers. Active and responsive smiling may be absent and social imitative games and may be largely one sided. In addition, toddlers with autism engage less often in social referencing and joint attention, rarely sharing observations, excitement, and achievements with others in a mutualized fashion (through the integration of speech, vocalizations, reciprocal eye contact, pointing, facial expressions, and gestures). Although they may point out something of interest, they typically do not use this as a springboard for a give-and-take interaction with others. They may not make reciprocal eye contact as they point and vocalize; monitor the expression of others to gauge interest, enthusiasm, and approval; or demonstrate curiosity about the interests, preferences, opinions, and experiences of others.

When in the centre of stimulating social activity, they may prefer to explore their inanimate environment or engage in a perseverative interest or behaviour [Bernabei *et al*., 1998] carried out a videotape study that uses an observational Checklist that targets social interaction, communication, and both functional and symbolic play. The researcher reported that 1.8 to 4.6 year old infants and toddlers who were later diagnosed with autism/PDD hardly made communicative gestures, played imaginatively, or get involved in conventional social games (Bernabei et al., 1998), they tend to remain on the boundary of social activity, respond solely to adults, or include other children in one-sided physical or highly scripted play (in which they direct the action). They may express a precocious aptitude for early academic tasks and an enthusiastic interest in exploring the details of the inanimate world around them, yet fail to understand or obtain pleasure from imaginative and interactive play. When they become older, they obviously prefer spending their time collecting authentic knowledge on narrow, esoteric topics rather than playing creatively with other children, participating in social events, or joining clubs and athletic teams. Their interests are often surrounded around taxonomy, classification, and categorization. Although they may enjoy participating in chess tournaments and Magic Card swaps, they ideally do not enjoy "hanging out" with peers, discussing about favourite teams, music, and clothing, or attending sports events or concerts.

Adolescents and young adults with autism may fail to showcase basic social etiquette, understand social intent; appreciate subtle emotional states within themselves and others; or presume the thoughts, feelings, and behaviour of others even in relatively straightforward

social situations. They may ask uncommon, overly personal, or rhetorical questions in order to obtain accurate information related to an enigmatic interest; seek repeated reassurance over a minor issue, or awkwardly attempt to demonstrate friendliness. Highly literal and tactile problem solving can lead to socially inappropriate comments and behaviour, with little appreciation of the need to accept justificatory circumstances, exceptions to the rule, or the unique needs and preferences of others.

1.3.2 Qualitative Impairment in Communication

Communication is also often influenced in persons with ASD and at such, retard language development; challenges in starting and maintaining conversations, presence of repetitive speech, and lack of counterfeit play skills are common. Many children with ASD are retarded in the development of their first words or phrases. Most typically developing and growing children have single words by 12 months of age and phrase speech which maybe two to three words in length within 24 months. Most children with ASD develop a single word at 24 months or later and phrase speech at 36 months or later. While some other children with autism spectrum disorder may not be able to develop concrete spoken language throughout their lifetime, never the less, there have been high increases in recent years pertaining to the percentage of children with ASD who remain non-verbal. For those children who develop fluent speech, an often observed and noted challenge is the inability to commence and maintain an appropriate age conversations.

In general, individuals tend to be better able to stick strictly to conversations about their own topics of interest rather than someone else's choice of topics of discussion. Some persons with ASD may provide an inappropriate acknowledgement rather than trying to ask questions about the other person or make a comment on an on-going topic. In most cases, comments thatare not relevant to the conversation at hand and inflexibility in the conversation may also be noticed from a person with ASD. All of these factors make the back-and-forth nature of conversation difficult to maintain.

Most unusual aspects of spoken language are also common in ASD. A Large number of persons with autism spectrum disorder (ASD) userepetitive language in their day to day lifeduring interactions with other individuals. For instance, they may repeat a phrases used by an adult either immediately it has been said (called echolalia) or sometime after they have been said (called delayed echolalia). Another usual type of repetitive speech involves repeating lines heard on movies, commercials, and television shows.

Usually, these phrases can be used at random times and they can also be used in a communicative manner. An instance would be when a parent asks a child a question (e.g., "Would you like something to eat?") and the child answers the question with a line that is verbatim from a movie, such as "Pokémon's energy is running low and needs replenishment." Meanwhile many typically developing children engage in this behaviour occasionally, individuals with ASD may usually or regularly use this "stereotyped" speech as part of their everyday spoken language.

Families of higher functioning children with ASD report that their speech can sound like they are giving a lecture when the children are relaying information about a topic of interest. Imaginative play skills are also often inculcated in ASD. Pretend playing skills is considered to be under the "communication" domain because playing skills are often associated to language development. Typically developing children starts to develop pretend play skills before 24 months of age. Early imaginative play skills involve pretending to talk on a telephone or play with dolls. Usually children with ASD prefer to use toys for their function rather than engage in interactive pretend play with the toys. For instance, they may press the buttons on a toy phone or move a doll's arms rather than pretend to talk on the phone or feed the doll with a toy spoon.

1.3.3 Repetitive or Restricted Interest and Behaviour

Repetitive pattern of behaviour is very common among several individual with ASD. Meanwhile, these features may be the most noticeable to the general public. They do not represent the major symptoms of ASD and are not sufficiently enough to diagnose an individual with ASD. Many of these behaviours are also present in person with developmental delays without ASD, such as intellectual disabilities (previously known as *mental retardation)*. As children with ASD grow older, nature of their repetitive /restricted behaviour usually changes. "Lower order" repetitive behaviours such as hand flapping and lining up toys are mostly common in younger and more cognitively delayed children with ASD while "higher order" repetitive behaviours often comprise intense interests and compulsive behaviours and are more pronounced in older and less cognitively disable children with ASD.

Usually, restricted and repetitive behaviours can impede with an individual's functioning, there are also some important advantage to these behavioural patterns. For instance, large individuals with ASD are bent on following guided rules and routine. This can be a positive

trait in that they often do not break the rules intentionally and generally attempt to obey or comply with a rule that has been taught.

Persons with ASD usually have high interests in stipulated and specific topics. Therefore, they often become experts in such a given area and have an in-depth knowledge of a particular or specified topic. This can be beneficial in building future careers and hobbies and may also be way to encourage learning of other related topics.

1.4 EARLY SCREENING AND DIAGNOSIS

ASD is characterised with neurobiological foundation and genetic factors play an important as well as significant role in its development. Diagnosis of ASD is strictly dependent on direct observation and parental interview about the child behaviour in terms of social, communication and repetitive behavioural features without the use of brain scans or blood tests .Particular symptoms within each domain that are needed for a diagnosis on the autism spectrum are explained in the Diagnostic and Statistical Manual for Mental Disorders, Fourth Edition (DSM-IV*;* APA 1994). Autism continued to be recognised a part of childhood schizophrenia until 1980s when it was recognized as its own disorder called "infantile autism" in the publication of the DSM-III (APA 1980). Other editions of the DSMhave further alienated autism diagnoses and recently, there are different diagnostic criteria for Autistic Disorder,Asperger's Disorder and PDD-NOS. A number of well- established tools are available to help in the diagnosis of ASD, such as the Autism Diagnostic Observation Schedule (ADOS)(Lord *et al*. 1999) and the Autism Diagnostic Interview-Revised (ADI)(Lord, Rutter, and Le Couteur 1994].

1.4.1 Pervasive developmental disorders not otherwise specific (PDD-NOS)

Autism spectrum disorder (ASD) falls under the group of pervasive developmental disorder in the diagnostic and statistical manual for mental disorder fourth edition (DSM-IV) domain and PDD is characterised due to a severe disabilities in many aspects of a person life, which involves severity in social interactions, communication skills and categorized behaviour. Some other diagnosis also exists without this category of clinical disorder that is not on the autism spectrum. Clinically, working with person with Autism spectrum is conscious to differentiate this disorder from an ASD. Clinician is a general term that is used to describe people such as psychologist, speech therapist and treatmentproviders, who works directly with people and clients. The term is majorly used in clinics and hospitals to differentiate those professional who works directly with patients and clients from administrative staff.

PDD-NOS is sometimes described as “a typical autism” due to lack of full criteria for autism od Asperger disorder but shows a similar autism like features.

Furthermore, children with PDD-NOS have improved language ability as well as cognitive skills comparing to autism children. In this regard, PDD-NOS are seen as a diagnosis that is mild and also have improved prognosis compared to autistic individual.

1.4.2 Autistic Disorder (AD)

Another name for Autistic Disorder is “autism,” or “strict autism,” or likewise called “early infantile autism.” A large individuals diagnosed with ASD have an autism percentage of (~60%) compared to Asperger’s Disorder with a percentage of (~24%) or PDD-NOS (~16%; Goin-Kochel, Mackintosh, and Myers 2006). Males are mostly diagnosed with autism than females having a sex ratio of approximately 4:1. There have been some reports that show that females have more severe symptom of Autism and less cognitive abilities as compared to males.

Autism disorder can be diagnosed at exactly about two years of age or more of the child during this period, it is generally more stable. When comparing the diagnosis of autism with the PDD category, persons with autism face a huge challenge in the three general domains which are; social interactions, communication disability, and restricted interests and behaviours. Criteria in the *DSM-IV*have shown that autism diagnosis is important for person who shows at least two particular symptoms in the social domain, one in the communication domain, and one in the repetitive behaviours domain. Furthermore, in respect to these criteria, six symptoms in totality across all three domains are needed to receive a diagnosis of autism. A part of these difficulties must have had an onset at three years of age or even earlier.

Epidemiological research have suggested that an average of 60–70% of children battling with autism are tends to have a re-occurrence in intellectual disability (Fombonne 2003) and it is also observed that one-third to one-half of person diagnosed with Autistic Disorder possesses severely disability in language or may remain non-verbal throughout their adulthood (Howlin *et al*., 2004). Intellectual disability is a diagnostic term which is mainly used to explain individuals with a below average intellectual abilities which are also followed and accompanied by some impairments in daily life behaviours such as self-care, independent living skills and interpersonal skills. Difficulties begin at the early stage of the individual life and it is majorly measured by cognitive abilities of the person.

Intellectual disability varies in stages and diagnoses for measuring intellectual disability has greatly and immensely reduced thereby increasing the rate of autism (Croen *et al*., 2002). In

the past several children diagnosed with autism are faced with co-occuring intellectual disability and likewise a vast percentage of this children experience a non-vocal life style throughout their lifetime (Rutter, Greenfield, and Lockyer 1967). Meanwhile several clinicians have categories autism individual into different classes based on their intellectual ability so as to enable them to adapt to their day to day known as adaptive functioning. Individuals who show little impairment and cognitive ability in adaptative function are termed High functioning autism(HFA)while low functioning autism are those that exhibit forms of intellectual disability and difficulties in their adaptive function.

Persons experiencing autism characteristic are known to have some features which are high temper, anger, sleeping difficulty, gastrointestinal abnormalities and psychological problems.

1.4.3 Asperger Disorder

Asperger's Syndrome or Asperger Syndrome is another name known for persons suffering from Asperger disorder and it is also known to have challenges in social and repetitive interests.

Basically, the DSM-IVrequires a minimum of two symptoms in the social domain and a minimum of one symptom at the behavioural domain respectively. Problems associated with Asperger individual are the inability to maintain age appropriate. Persons suffering from Asperger disorder are different from individual with Autism disorder in a number of ways.

One distinctive difference between Asperger disorder individual is that they do not possess language and communication delay impairment while Autistic disorder individual are faced with great impairment disorder and it's seen as a major challenging factor among Autism persons.

Furthermore, several children are faced with cognitive disability among autistic individual but may be average or above average among Asperger disorder individual when measured by the cognitive testing.If a person is not characterized by a delays in language development and shows normal cognitive ability, and at same time meet the full criteria for Autism, in this casesuch a person is termed has receiving the diagnosis of autism rather than Asperger disorder (AD).

An additional observable difference in regards to males and females sex ratio of Asperger's Disorder which is approximately 6:1, allowing it to be higher than the 4:1 sex ratio which is observed in autism. Asperger's Disorder is relatively said to be relatively diagnosed later in life than that of autism. Another observable feature observed between Asperger and autism is that, the average age stipulated for diagnosis testing for Asperger disorder is 7.2 years of age

while autism disorder individual is 3.1 years of age (Mandell, Novak, and Zubritsky 2005). This is probably seen has a result of lack of language delay among Asperger disorder individuals compared to autism individuals. Several parent with Asperger children describe their kids has strong aggressive individuals with a high strength and command of words.This characteristic among Asperger children are not earlier noticed except when children enter a schooling environment where direct comparisons are carried out with a typical child with great social behaviour.

ASD AND MEDICAL COMORBIDITIES

Several children and adult who are diagnosed of Autism spectrum disorder (ASD) have comorbid health problems and recent research have shown that persons with this disorder are said to be accompanied with several health challenges. Persons suffering with autism spectrum disorders (ASD) are accompanied with high risk for medical challenges or comorbidities which possibly or probably remain undetected and at such resulting into negative impact in their development process. These comorbidities are characterised by oral health challenges, sleep disability, gastrointestinal disorder and epilepsy disorder. A huge campaign in enabling awareness as well as treatment for this health challenges and comorbidities is of high importance and likewise very essential to improving the quality of life of persons affected by this disorder.

Many medical conditions show itself at an early time, at such preceding ASD diagnosis which may likely advance into adulthood thereby calling for intensive care and appropriate attention. Irrespective of the higher rate of medical care used compared to the general pediatric population (Gurney *et al*.., 2006, Liptak*et al*.,2006, Croen *et al*.., 2006), patients and families witness difficulties having access to specialised health care (Kraus *et al*., 2003, Thomas*et al* 2007). The unmet need tends to be very high in non-metropolitan area among minority families and among patient with limited or a lower family income individual.

Furthermore, children with autism spectrum disorder (ASD) needs extensive health care, free access to medical home provides an approach based on partnership between the patient, family, primary and specialty care providers and community support. For children who needs special health care, a designated medication is provided across the system of care to ensure comprehensive health care services for those individuals. The empirical benefits of coordinated care using the home care model are stilol being accessed and the support from this medical home are seen as an important source of support and care delivery to the families.

An improvement in the awareness of medical comorbidities helps to improve practitioners and family with extensive knowledge of common health problems faced by individuals with ASD. Limited empirical data for treatment of these common comorbidities brings awareness to professionals involved in the health care of individuals with ASD to readily make availability of the treatment to alleviate medical problems which can often exacerbate ASD symptoms.

Below entails the different types of medical comorbidities in Autism;

2.1 GASTROINTESTINAL DISORDERS (GI)

Gastrointestinal disorder varies in occurrence withchildren affected with ASD which range from 9 to 70% (Gurney *et al*, 2006, Buie *et al*., 2010, Valicenti *et al*, 2006). Gastrointestinal disorder is divided into the following types which involve chronic abdominal pain, severe constipation and chronic diarrhea and gastro-esophageal. Either of the different gastrointestinal conditions occurs at an increasing rate compared to the general pediatric population or compared to other neurodevelopmental conditions is unknown; moreover, gastrointestinal problems continue to require medical treatment till adulthood (Kohane *et al*., 2012).

Due to lack of reasonable amount of data regarding adequate practice and evaluation, autism expert have develop a guideline which is heavily recommended following children without (Buie *et al*., 2010) .Chronic abdominal pain is a common problem faced by ASD patient which is presumed to last for about 1-2 month among persons with autism spectrum disorder but due to delay in communication ability among ASD children, it may remain undetected at an early stage. Some negative feature observed among ASD affected person must be put in serious consideration in order to improve early diagnosis among patient suffering from ASD and this symptoms includes loss of weight, adequate growth pattern deficiency, Gastro-intestinal blood loss , emaciation, severe diarrhea, fever, persistent right upper- or lower-quadrant pain, inflammatory bowel disease family history or abnormal (AAP.., 2005). Some important test maybe recommended for ASD presumed patient such as abdominal radiographs, ultrasonography and collection of stool samples. Among this diagnostic test is invasive testing such as endoscopy which is carried out to to check for the level of severity of the symptom. As a result of lack of findings regarding the level of severity and disease level, family need assurance to enable the well-being of their children were most patient are advice to strictly avoid the intake of lactose for a period of 2 weeks in the sole aim of providing relief to the patient. Chronic retained stool symptom can also be drastically reduced by the trial of a stool softer for a period of 4 weeks. Constipation is another chronic symptom of ASD which is cause as a result of delayance or difficulty in defecation for a period of 2 weeks of even more among persons with ASD. Children affected with autism spectrum disorder (ASD) are observed to have the habit of holding on to faeces for a long duration of time which may eventually lead to constipation. Evaluation of children with constipation disability must be properly scrutinized in terms of history and physical examination which may provide guidelines and proves for further diagnosis. Physical examination is very difficult and challenging for some children with ASD most especially the examination of the

rectal to check for stool retention and if bleeding occurs.Treatment for constipation is addressed based on behavioural management and pharmacotherapy and some of the medications commonly used are lubricants such as mineral oil and laxatives as well as lactulose or sorbitol's.

Chronic diarrhea is also one of the most common gastrointestinal disorder which found among ASD patient and it is defined as the persistence loose of stool for a period of 2 weeks or more without an increase in the stool frequency which would help stop or hold the persistence. Acute diarrhea is usually caused due to infection while chronic diarrhea is caused as a result of inflammatory bowel disease and problem with absorption. Stool samples should also be collected for proper examination to investigate the possibility of chronic infection, bleeding, or mal-absorption.

2.2 ORAL HEALTH ISSUE DUE IN AUTISM SPECTRUM DISORDER

Children facing problem of ASD are usually faced with a high risk of oral health problem which involves caries and individual inflicted injuries.

The risk of having caries is at increase rate when individuals or person is highly dependent on others for dental hygiene (Kopycka *et al*., 2008). The delay in terms of communication among ASD children has a negative effect on children in terms of oral care (Weil *et al*., 2012). Oral hyper-sensitivity and hostility during tooth brushing can also leads to caries which can promote the formation of a bacterial plaque among ASD infected persons.

Additionally, the wearing of structural wear on tooth and the oral mucosa can cause children to afflict themselves with injuries but when mouth guide are wore by ASD affected individuals, it prevent self -infliction injuries to this persons with ASD as advised by the dentist.

Constant advice and consultation with a specialised dentist in regards to taking adequate and proper care of children embattling with special health difficulty may help in easing the child health condition.

2.3 SLEEPING DISORDER

Sleeping disorder is one of the major problems faced with individual suffering from ASD and this problem is linked to problem in going to bed, difficulty in finding sleep, reduction in sleep duration and interruption of sleep. The developmental process of persons with sleeping disorder lifespan has not been adequately studied but it is presumed that sleeping disorder appears in the childhood and persists till adulthood among ASD affected persons (Kohane *et al*., 2012). One of the major problems of disruption in sleep can increase the daytime activity,

behaviour regulation and immerse family stress. The Autism Treatment Network in the United State of American recommends that sleeping disorder should be part of the routine carried out for children with ASD during health screening [Malow et al., 2012]. The Children Sleep Habits Questionnaire (Glodlin *et al*., 2008) is a questionnaire that is frequently used basically in autism research for preschool age children and this research questionnaire carries a strong psychometric properties. Apart from this questionnaire, the Modified Simonds and Parraga Sleep Questionnaire (MSPSQ)(Johnson et al., 2012) is also used to compare a child sleeping habit which provide an extensive descriptions of sleep disorder. Adolescent sleep Wake Scale (LeBourgeois *et al*., 2005) is another sleep assessment option which is solely made available for older children.Lastly, the Family inventory of Sleep Habits (Malow *et al*.,2009) questions centre on various environmental factors which may be important for identifying sleep intervention targets (Morgenthaler *et al*., 2006).

In addition, once sleeping disorder has been noticed, the next line of action to be put into serious consideration is the treatment of behavioural action with the behavioural modification strategies.In regards to medical therapies for treating sleeping disorders in ASD, evidence has been very limited in which data are mostly focused on Melatonin (Garstang., 2006). Melatonin supplementation is simply explained has a form of complementary alternative medicine (CAM) that is administered to reduce the sleep latency time. This CAM supplement helps greatly or immensely to reduce family stress and also to improve sleep in children. Other commonly used sleep agents like mirtazapine and trazodone are accompanied with a severe side effect such as lowers seizure threshold and they also lack controlled studies.

2.4 EPILEPSY

Tuberous sclerosis (Mumis et al., 2011) or Dravet syndrome (Li et al., 2011) are genetically identical defined forms of Epilepsy syndrome which is found to be common among children with ASD. One of the reasons for the strong link between epilepsy and ASD is possibly as a result the severity and complexity of the brain lesions and epilepsy phenotypically age of onset.

The prevalence rate of epilepsy during childhood is relatively presumed and estimated to be 2–3%, while it is estimated to be 5-38% in children with ASD (Tuchman.., 1991, Spence.., 2009). With the high frequency rate between autism and Epilepsy, these has rules out any form of coincidence but rather has suggested that they both share common pathogenic mechanism and characteristic.

The treatment for epilepsy in children with autism spectrum disorder (ASD) is the same as the treatment with seizure treatment in children with epilepsy. Drugs such as Anti-epileptic drugs should be selected based on the type of seizure so as to control the minimum side effect seizure and its form of dosage.Effective blood testing should be carried out so as to monitor the treatment procedures and also the behavioural side effect of persons with ASD (Spence. 2009).

GENETICS CAUSES OF AUTISM

Autism is seen is a typical instance of a neurodevelopmental disorder that has a strong genetic evidence. A Distinctive difference should not be made between autistic disorder and autism spectrum disorder (ASD) due to their close relationship and similarity (Benvenuto *et al*., 2009).

Due to several survey that has been carried out between 1980s and 1990s, it has proven that monozygotic twin have a high percentage of genetic effect at about 69% - 95% than seen in dizygotic twin which have a percentage of 0% - 24%. The hereditary components are estimated at 90%. The sex ratio of male-female ratio is estimated between 3-1 (Brkanac *et al*., 2008,). There are several techniques that are put into consideration to help in improving the genetic efficiency and authentication of autistic disorder and this includes, molecular research and linkage studies.

The Linkage studies has greatly impacted in helping to search for those part of a chromosome that is same between affected family and showing a significant difference among non-affected members of a family.

Genetic studies have immensely contributed in providing genetic difference between healthy individuals of one and unhealthy individuals on another hand (Vorstman *et al*., 2006a).

If classic microscopic cytogenetic process is adequately used, it provides information on the structural chromosomal aberrations in 3%-7% of patients affected with autism and developmental disability. The fluorescence in situ- hybridization (FISH) technique is majorly used to investigate specific sub-microscopic deletions and it is likewise primarily used for the confirmation of a clinical diagnosis in 22q11 deletion syndrome. The major disadvantage of this technique is that it’s consume a lot of time and only one or few chromosomal regions can be examine per experiment.

The most common techniques which are extensively used to detect the susceptibility of ASD genes are the chromosomal microarray, whole- exome sequencing (WES), and selective candidate gene analysis (Klauck, 2006; Persico &Napolioni, 2013). The whole exome sequencing technique has successful to detect rare genetic defects in various heterogeneous situations as well as in Autism spectrum disorder (Rabbani *et al*., 2014). Whole exome sequencing is used to confirm the presence of a de novo which was extensively expressed in ASD persons in a recent research that was carried out on 928 individuals(Sanders *et al*., 2012). It was also observed in a recent research which involves the use of whole exome

sequence to detect ASD linked genes in fragile X protein (Iossifov *et al.,* 2012). Furthermore another technique used is the chromosomal microarray analysis which is used in the detection of chromosomal abnormalities found in patients who are diagnosed with ASD (Zilina *et al*., 2014).

Among these several abnormalities, it was noticed thatGABA receptor subunit genes in the likes of GABRA5, GABRB3 and GABRG3 are closely related with the pathophysiology of autism spectrum disorder (ASD) (Kim *et al*, 2006; Klauck, 2006, Vorstman *et al*, 2006). Severe implications may be caused as a result of the malfunction of any of the genes causing an inhibition of excitatory neural pathways that can cause an abnormality leading to brain development (Klauck., 2006). In conclusively, a selective candidate gene analysis is an important tool for screening and it is responsible for the identification of genes involve in autism spectrum disorder (ASD) (Holt *et al*, 2010, Klauck, 2006).

3.1 GENETIC SYNDROME AND AUTISM

Below will be discussed the most know genetic disorders linked with Autism;

3.1.1 Fragile X Syndrome

The fragile X syndrome (FXS) which is one of the most known genetic disorder, has been hugely linked to be the common cause of inherited mental retardation (Crowford. 2001)). The first persons that provided these detailing and knowledgeable information are Brown and colleagues (1982, Fragile XSyndrome was first described by Lubs (1969), who used the classical microscopic cytogenetic techniques in the detection of a fragile site at the end of the long arm of the X chromosome.

Clinically, patience with fragile X syndrome are face with problems of mental retardation, macro-orchidism, large ears as well as long faces. But in most cases, mental retardation is said to ranges from moderate to severe state with a frequent occurrence of autistic-like behaviours. Apparently, Persons associated with FXS at about 30% are categorized and grouped as being within the autistic spectrum (Roger.., 2001). Many report has postulated a linkage between FXS and autism which has of now, there have been no concrete evidence to confirm a link of fragile X syndrome to autism (Muhle. 2004).

FXS is majorly caused as a result of the expansion of the CGG repeat which is found to be located at the 50- un-translated regions (50-UTR) of the first exon of the fragile X mental retardation 1 gene (FMR1) at the chromosomal locus Xq27.3 (Fu *et al*., 1991). The number of CGG repeats is highly polymorphic but in normal individuals, it ranges from 6 to 50 triplets. When the expansion of the CGG repeat is more than 200 in the fragile X syndrome, it

is termed full mutation. Full mutation is primarily caused due to hyper-methylation of the CpG islands found within the FMRI promoter regions along with the gene silencing during transcription. Pre- mutation with a CGG repeat of 55-200 can lead to a full mutation when it is maternally transmitted. Larger repeat has been known to carry huge risk of expansion comparing to smaller repeats (Nolin *et al*.., 2003). Repeat ranging from 40-54 repeat are called intermediate allele and it has been observed to be slightly unstable upon transmission (Nolin *et al*., 2003; Sullivan *et al*., 2002). The formation of a full mutation is said to have been caused has a result of the expansion of intermediate allele over a span of two generation (Terraciano *et al*., 2004). The intermediate alleles is been recognised has the 'gray zone' alleles (Nolin *et al*., 1996) and the larger the size the greater its instability increases.

It has been understood for several years now that the severity in term of intellectual disability and the extent of related behaviour problems observed in individual is dependant of the number of repeats. The phenotypic psychopathological features of fragile X syndrome involves; developmental delay, obsessive-compulsive characteristics,multiform anxiety symptoms, hyperactivity / impulsivity, epileptic phenomena, aggression are frequently predominant, as social anxiety and withdrawal behaviour, stereotypies like flapping or biting of the hands, perseverations, extreme sensitivity to environmental stimuli, and in general, decreased social reciprocity with an avoidance of eye contact (Hagerman, 2005).

In conclusion, the target molecules of FMRP includes shank3, GluN2A, mTOR, TSC2, NF1, neuroligin2 and neurexin1 (Darnell *et al*.,2011) which are associated with autism spectrum disorder (ASD) pathogenesis.

3.1.2 Rett Syndrome

Rett syndrome (RS) was firstly discovered and described by Andreas Rett in 1966. Rett syndrome is said to have been caused as a result of a mutation in the Methy-CpG Binding protein 2 (MECP2) genes in chromosome Xq28. and it can only be inherited as an X-linked. Rett syndrome occurs solely in girls where the level of occurrence is estimated to be between 1/10,000 - 1/20,000. This syndrome is always very fatal in boys due to the presence of an extra X chromosome or mosaicism of the MECP2 mutation which can only be found in rare male cases.

RS individuals are characterised by normal developmental features during the first 6 to 18 month of their life after which they begin to experience developmental stagnation, loss of acquired skills and development of this persons comes to a standstill. During early childhood, an affected female lose the functional aim of their hands and begin to show sign of repetitive

wringing and clapping motions. These kids are characterized by slow response compared to a typically developing child and they are also known to exhibit a small head size called microcephaly. Aside from the signs mentioned above, other viable symptoms includes breathe abnormality, sleeping disturbance and seizures. The most severe symptoms of Rett syndrome persons are respiratory dysfunction, forelimb and hind limb clasping, stereotypy, hyperactivity, cognitive impairment, anxiety and affected (Shahbazian *et al* 2002; Moretti et al, 2005).

This stagnation of this developmental process result to a phenotypic retardation as well as an unequal growth in the circumference of the head, decrease in eye contact as well as motor degradation. During the first year of life with this Rett syndrome, autistic behaviour are said to be predominant with impairment in social behaviour, reduced communication skill and stereotypies behavioural (Ben Zeev, 2007; Gonzales & LaSalle, 2010). Patient facing Rett syndromes are faced with epileptic condition and at the age of 10 children are faced with a huge and severe intellectual disability. In approximately 80% - 90% of patients affected by RS, the mutation that is mostly likely to occur is a de novo mutation which is present in the MECP2 gene. This MECP2 gene is expressed most particularly in neurons and to a lesser degree in glial cells and it is likely to enable neuronal maturation in the postnatal period. MECP2 gene is also functional in the expression of the gene that is responsible for coding the brain derived neuro-trophic factor (BDNF) that is solely aimed in neuronal maturation and plasticity.

Rett Syndrome (RS) is a better example of an autism related disorder with a given and proven genetic Pathophysiological feature. Research in Rett syndrome (RS)could have well trigger an excellent knowledge of the involvement of central nervous system dysfunctions in autism.

3.1.3 Tuberous Sclerosis

Tuberous sclerosis complex (TSC) of the Bourneville-Pringle wasfirstly illustrated in the year 1880 by Bourneville and it is an autosomal dominant inheritance with a multi-organ disorder.

It is recognised as the syndromic forms of ASD that frequently takes place during transcription factors. This may be basically because of a defect in the transcription factor that has a result have significant influences on many genes and their downstream molecules causing diverse neuronal functions.

Tuberous sclerosis is seen as a genetic disorder which is explained by the growth of numerous non-cancerous (Benign) tumours in several part of the body. This tumour can be seen on the skin, kidney, brain and some other organs which may leads to a significant health

challenge. TSC can likewise lead to developmental problems with signs and symptoms varying from one person to another. The prevalence rate of this disorder is 1 in 6,000 - 10,000 births. TSC occurs due to mutations in two genes which are TSC 1 and TSC 2 gene. Tuberous sclerosis1(TSC1) gene is located on long arm of chromosome 9 (9q34.3) which is responsible for coding for hamartin while the Tuberous sclerosis 2 (TSC2) gene is located on short arm of chromosome 16 (16p13.3) and it is strictly responsible for coding for tuberin. A change in one of these two genes can be demonstrated when an approximately 85% of patients are clinically confirmed after diagnosis of Tuberous sclerosis (TSC). Normally, a *de novo* mutation is found to be present in this type of genetic disorder, meanwhile 30% the patient ratio is estimated to have one or more affected family members. A resultant mutation in both genes can leads to abnormality in cell growth and division in multiple organ systems. In the brain, this is showcased by the formation of cortical and subcortical hamartomas including tubers.

Furthermore, several organs can likewise be affected at such causing formation of cystic kidneys, angio-fibromas that affect the face and rhabdomyomas. Other structural abnormalities linked with the central nervous system are; cognitive dysfunctions,epilepsy and symptoms of autism (Datta *et al*, 2008).

In 1932 beforeKanner's publication, Critshley and Earl explained the autistic characteristics which is linked with TSC has a disorder that cause a decrease in social contact, stereotypies, disturbed speech as well as withdrawal behaviour. Researches carried out during the last few decades have explained that autism takes place in about 25-60 % of TSC patients. A major characteristic of patients affected with TSC is the increasing level of social cognitive function and a less stereotype. However, the male to female ratio of autism to TSC is the equality (Wiznitzer, 2004).

The neurobiological substrate for autism in tuberous sclerosis (TSC) is still very undetected and unclear. Both the hamartin and tuberin protein modulate important in playing a vital role in neuronal migration, differentiation and development (Asato *etal*., 2004). Over expression of the TSC1/TSC2 complex causes a suppression which leads to the formation of axons while under expression on the other hand is associated with the formation of tubers (Choi *et al*., 2008). The functional combination of TSC1/TSC2 also explains that a mutation in any of the two genes can cause similar phenotypic characteristic (Orlova & Crino, 2010).

3.1.4 22q11 Deletion Syndrome

The 22q11 deletion syndrome (22q11DS) was firstly described by a scientist called Shprintzen in 1978 and he describe it as a velocardio-facial syndrome which is caused as a result of the intestinal deletion of the long arm of chromosome 22 (22q11.2).

This velocardio-facial syndrome is linked with other abnormalities such as congenital heart defect, cleft palates, facial dysmorphisms and hypo-parathyroidism. The sex ratio of this syndrome is approximately equal. The T-box 1 (TBX1) gene is one of the important gene of 22q11DS which is responsible for the coding of other genes. The protein that is responsible for the encoding of this gene is responsible for the development and growth of the heart, face, limb and some part of the brain.

TABLE 3.1: SHOWING CANDIDATE GENES PROPOSED TO BE RESPONSIBLE FOR ASD

CHROMOSOMES	CANDIDATE GENES	FUNCTION/ NETWORK
2q12.3-q14.2	DPP10	Neuro-transmission
2q16.3	NRXN1	Synapse formation
3p26-p25	CNTN4	Synapse formation
3p24-26	OXTr	Neuro-transmission
7q31.1	ST7	Tumor suppression
4p14-q21.1	GABRG/GABRA	GABA neuro-transmission
8p23	DLGAP2	NMDA neuro-transmission
7q35-q36	CNTNAP2	Synapse formation
15q13	APBA2	Neuro-transmission
15q11-q14	GABRA/GABRB/GABRG	GABA neuro-transmission
22q11	PRODH	Neuro-modulation
16p11.2	DOC2A	Neuro-transmission
7q31	WNT2	Signal transduction
22q13	SHANK3	Synapse formation
7q31	CADPS2	Synapse formation
7q22	RELN	Neuronal migration
X227.3	FMR1	Neuronal development
Xp22.1-p21.3	IL1RAPL1	Interleukin receptor
Xq12	OPHN1	GTpase activation
Xq11.4	TM4F2	Cell proliferation

Xp22.3	NLGN4	Synapse formation
Xp11.4	TSPAN7	Neuronal growth and development

These table above shows the wide range of genes and their functions which are relatively involved in Autism.

SHANK gene

SHANK family genes is solely responsible for encoding of scaffolding proteins which is enriched in the postsynaptic density (PSD) of excitation synapses which carry out its function in the maturation, formation and maintenance of synapses. A postsynaptic membrane is a membrane that is responsible for multi-synaptic protein complexes (Sheng and Kim, 2000). The shank family is postulated to have three main members which includes; SHANK1, SHANK2, SHANK3, likewise known as ProSAP3, ProSAP1 and ProSAP2 respectively. The idea behind the involvement of shank in the etiology of ASD was from phenan-McDermid syndrome (PMS) or 22q13 deletion syndrome which is a neurodevelopmental disorder caused by a micro-deletion on chromosome 22 which causes a serious defect to the affected individual (Boeckers *et al*., 2002; Wilson *et al.*,2003; Phelan and McDermid, 2012).

The association between SHANK gene and autism spectrum disorder (ASD) becomes very pronounced due to the identification of numerous mutation at the SHANK3 locus found in autistic person (Durand *et al*.,2007) causing a severe impairment in the autism spectrum disorder pathogenesis involving a severe language expression, speech delay, Hypotonia, global developmental delay. It was also observed between an European and Canadian population that mutation in the SHANK2 and SHANK1 leads to de novo copy number variation (CNV) deletion and missense mutation causing intellectual disability (Berkel *et al*., 2010, Leblond *et al*., 2012, Sato *et al*., 2012).

NRXN1 gene (Neurexin)

Neurexins gene is a cell adhesion molecules and as well as also a receptors in vertebrate nervous system. Neurexin 1 observed as a cell surface receptor helps in joining neuroligins to enable it form a calcium dependent neurexin / neuroligin complex during synapses in the central nervous system (CNS).

NRXN1 is a gene that is very essential in neuro-transmission and it also help or function effectively in synaptic contact formation in SFARIGENE. Neurexin1 (NRXN1) is listed as a

strong candidate gene as a result of its heterozygous deletion nature and also because of its strong ability to detect point mutation in a small number of patient suffering from autism spectrum disorder (ASD)..

Neurexin proteins are also known as cell surface receptors that functions collectively with neuroligin (NLGN). Ca2+ dependent neurexin / neuroligin complex is present at synapses in the central nervous system (CNS) and it is majorly required for theefficient neuro-transmission as well as synaptic contact formation.

NRXN1 gene is known for the expression of NRXN1 (OMIM: 600565) protein which is located on the long arm of chromosome 2p16.3. These NRXN1 gene posseses 22 exons which codes 1477 amino acid which possesses 7505 base pairs (base pair).

Deletion either small or large in the NRXN1 gene plays a significant role in ASD etiology. In additional, when there is a missense and nonsense mutation in the Neurexin1 (NRXN1) gene, it plays a significant role in the pathogenesis of this disorder (Feng *et al* 2006, Kim *et al* 2008).

Mutation in the NRXN1 gene leads to the formation of 513L and L7481 mutation which are known to be inherited from the paternal.

OXTr gene

OXTr is the gene responsible for encoding the receptor for oxytocin. Further research carried out on a group of unrelated autistic individuals did not show OXTr deletion but it shows hyper-methylation of the promoter gene with a reduced expression of mRNA. Oxytocin is a candidate gene that is majorly responsible for modelling human behavioural characteristics and also epigenetic mechanism. Hyper-methylation of specific CpG islands is likely to cause a reduction in OXTr gene expression.

Duplication of OXTr gene is heavily linked with pervasive developmental disorder patient symptoms of obesity and behavioural issues (Bittel *et al*, 2006), as a result suggesting that an increase in OXTr expression is liable to causea fall in the behavioural phenotype of ASD individuals.

CNTN4 gene

The CNTN4 gene carryout an important function in the formation, maintenance as well as neuronal network plasticity. A disruption of this gene causes a serious problem in term of developmental delay and mental retardation.

Contactin 4 (CNTN4) is formed as a result of the mutation of Alu Y mediated unequal recombination event. Alu elements which is inserted into about one million of a human genome is said to account for 10% of the total genome of a DNA molecules(Codish *et al* 1998).

Contactin 4 is an axon associated cell adhesion molecule (AxCAMs) which is highly expressed in the human brain most especially the cerebellum, amygdala, thalamus and cerebral cortex which are directly interrupted by copy number variation (Fernandez *et al*., 2014

AxCAMs are important in playing vital roles in axonal elongation along specific pathways, fasciculation of specific axonal populations and the functioning, maintenance and plasticity of some synaptic connections.

The expression of CNTN4 gene in human tissue indicates that this protein may have significant role in both early growth of developing axons and adult nervous system maintenance (Hansford, 2003). It is also postulated that a loss of a single functional copy of CNTN4 leads to the developmental delay characterized by a 3p deletion syndrome.

The syndrome is characterized by the following phenotype;long philtrum, mental retardation, microcephally, hypertonia, digital anomalies and dysmorphic facial features including a triangular shape face, hypertelorism,ptosis, broad nasal root, down turned mouth, micrognathia and dysplastic ears (Fernandez *et al* 2004, Schwyzer *et al* 1987, Narahara *et al* 1990).

It is also explained that individuals with inappropriate CNV does not interrupt CNTN4 genes that causes classical 3p deletions syndrome phenotype and CNV polymorphic and not Pathologic. There has been few report of CNV affecting CNTN4 in normal individual. If mutation of CNTN4 is incomplete penetrant, disruption of the gene may not result in ASD in all detected cases.

Notably, mutation of contactin associated protein like 2 (CNTNAP2) have also been linked to ASD carrying the syndromic symptoms such as language and mental retardation. Aside disruption of CNTN4 being a factor that may likely the development of ASD, Imprinting, environmental interaction or other factors may determine how mutation in CNTN4 causes ASD.

GABRA

GABRA is a dopaminergic gene that encodes dopamine transporter (DAT), severe dopamine receptors, as well as enzymes of dopamine synthesis (DOPA decarboxylase, DDC) and catabolism.

Early biochemical studies have found lower plasma activity of dopamine-beta-hydroxylase which is an enzyme that convert dopamine to norepine leading to the reduction in the level of dopamine in isolated platelets (Launely *et al*, 1987) and urine (Martinean *et al*., 1992) in autistic patients.

ASD patients are known to show altered expression if GABRA1, GABRA2, GABRA3 in parental cortex, cerebellum and frontal cortex which are the three brain area commonly involved in ASD (Fatemi et al, 2009).

GABRA genes encodes multiple subunits (Alpha, beta and gamma) of gamma-aminobuxyric acid (GABA) a receptor channel, responsible for inhibitory action of GABA neurotransmission in the brain, GABA-ergic mechanisms are strongly implicated in ASD pathogenesis in both human and animal experimental models (Menold*et al*., 2001).

APBA2

Amyloid precursor protein binding protein A2 (APBA2) is a gene that is located at the long arm of chromosome 15q13.1 duplication and it is also responsible for encoding of a neuronal adaptor protein which is very important for synaptic transmission responsible for direct interaction with neurexin1 (NRXN1) gene at the pre-synaptic membrane.

APBA2 gene is among the four genes detected in duplication of 15q13.3 chromosomes from autism pro-band as well as in affected siblings. APBA2 are responsible for encoding neuronal adaptor protein which are also recognised as Mint2 or X11L present in mouse (Christian et al 2008).

The APBA2 protein importance is in synaptic transmission and direct interaction with the neurexins gene (Biederer and Sudhof 2000). APBA2 is an important candidate gene of autism that is responsible for the development of normal social interaction.

Deletion and duplication of this gene have leads to series of neuro-developmental phenotypes in human and at such, striking social interaction phenotype in a mice model.

WNT2 gene

The WNT2 is a gene that is located at the long arm of autism susceptibility chromosome locus 7q31 (Vincent *et al*, 2000; Warburton *et al.,* 2000) and single nucleotide polymorphism and several WNT2 locus variant are linked with autism (Wassink *et al*, 2001; Marvi *et al*, 2010).

WNT2 is an important growth factor which has been heavily associated with autism spectrum disorder (ASD) acting through the canonical Wnt pathways. WNT2 is majorly responsible for triggering a signal transduction cascade which is mediated by a Dishevelled (Logan and Nusse, 2004).

Meanwhile, the Wnt signalling pathway is associated with ASD and is regulated by chromodomain-helicase-DNA-binding protein 8 (CHD8).

BDNF gene

Brain derived neurotrophic factor (BDNF) gene belong to the member of neurotrophic family responsible for growth and also help in supporting axodendritic growth,neurogenesis, neuronal/ synaptic differentiation and brain dysfunction. It is also associated with ASD (Huang, Reichardt, 2001, Martinowich *et al*, 2007). Calcium dependent secretion activator 2 ($CADPS_2$) is a calcium binding protein in the pre-synaptic nerve terminal that is responsible for the interactions and regulation of the exocytosis of BDNF which contains dense core vesicle (Cisternas *et al*; 2003).

The location of $CADPS_2$ is at the autism susceptibility locus on chromosome 7q31 and its abnormal spice in autism patient's exhibit social interaction deficits (Sadakata *et al*; 2007)

RELN gene

Reelin is also found to be heavily linked to autism and it is seen as a large secreted extracellular matrix glycoprotein that functions as a serine protease for the extracellular matrix which is responsible for neuronal migration, cortical patterning and brain development (Forster *et al*; 2006). The location of the RELN gene is on the chromosome 7q22 locus in an autism susceptibility individual and it is also found in triplet GGC repeats in 5' untranslated region (5UTR). The RELN gene has been linked in a Cauasian population with autism (Persico *et al*, 2001; Skaar *et al*, 2005). Reelin has also been implicated in pathogenesis of various neuropsychiatric disorder including schizophrenia, bipolar disorder, Lissencephaly and epilepsy (Fatemi, 2001).

NLGN4 gene

NRXN1 and NLGN4 gene are important synaptic cell adhesion molecules which are enriched at pre and post synaptic membrane (Craig &Keng 2007; Sudhof 2008). Neurexins and neuroligins interact with each other leading to the regulation of different aspect of both excitatory and inhibitory synaptic development and function which has result, affecting the excitatory and inhibitory level of balance in post synaptic neuron.Mutation in genes that encodes neurexin (such as missense mutation and CNV deletion) and neuroligins(a frame shift insertion mutation for NLGN4) are heavily linked with ASD, intellectual disability and schizophrenia (Jamain *et al*., 2003; Lanmonnier *et al.,* 2004, Autism Genome project *et al*., 2007; Kim *et al*., 2008; Walsh *et al.,* 2008).

CNTNAP2

CNTNAP2 is a neuronal trans-membrane protein which belongs to the member of the neurexin family which is located at Juxtaparamodes of myelinated axons. CNTNAP2 are responsible for the regulation of neuron-glia interactions and potassium channel clustering in myelinated axons (Poliak *et al*; 1999). Several single nucleotide polymorphism such as rs2710102, rs7794745, rs17236239 and non-synonymous variants such as 1867T present in CNTNAP2 locus are found to be heavily linked with ASD with the following impairment in language disability and cortical dysplasia focal epilepsy syndrome in humans (Alarcon *et al*.,2008; Arking *et al*.,2008; Bakkaloglu *et al*.,2008; Vernes *et al*., 008).

3.2. X- LINKED GENES

IL1RAPL1

Interleukin 1 receptor accessory protein like 1 (IL1RAPL1) is a gene that is linked with X-chromosome associated with intellectual disability (XLID) and synaptic regulations that is linked with ASD. IL1RAPL1 is responsible for encoding a synaptic trans-membrane protein (Carrie *et al,* 1999]. Recent research have carried out a systematic sequencing screening of X-chromosomes on individual affected by ASD which may leads to the identification of a *de novo* frameshift mutation in IL1RAPL1 (Piton *et al*, 2008). This important gene performs an effective role in the stabilization and formation of excitatory synapses through scaffolding protein PSD-95 recruitment to excitatory postsynaptic sites (Pavlosky *et al* 2010). Furthermore, IL1RAPL1 induces the pre synaptic differentiation through its trans-synaptic interaction with protein tyrosine phosphate leading to the recruiting of $RhoGAP_2$ to the excitatory synapses and induces dendritic spine formation (Valnegri *et al* 2011b).

IL1RAPL1 is responsible for regulating the development of inhibitory circuits in the cerebellum, all ASD related brain regions and disrupts the excitatory and inhibitory balance (Gambino *et al*., 2009).

Oligophrenin1 (OPHN1)

OPHN1 is an X linked intellectual disability gene which is responsible for encoding a GTPase activation protein that prohibits Cdc42, Rac and RhoA small GTPase. Due to report regarding association between truncating mutation of OPHN1 with XLID, further research have assisted in linking non-synonymous rare missense variant in OPHN1 with autism spectrum disorder(ASD) and likewise schizophrenia. OPHN1 is responsible for regulating dendritic spine morphogenesis through the RhoA signalling pathway (Govek*et al*.,2004) as well as activity dependent synaptic stabilization of AMPA receptors (Nadif *et al*, 2009).

TM4SF$_2$ or Tetraspanin7 (TSPAN7)

Tetraspanin7 is an X-linked gene which is responsible for encoding a membrane protein belonging to trans-membrane 4 superfamily [TM4SF]. It is also responsible in playing an important role in cell proliferation, activation, growth, adhesion and migration (Maecher *et al* 1997).

TM4SF protein forms a complex with integrin which regulates cell motility and migration by modulating the actin cytoskeleton [Berditchevski and Odintsova 1999]. A balance translocation and mutation (a nonsense mutation and P172H missense mutation) of TM4SF2 was first discovered in individuals with XLID [Zemni *et al*, 2000]. In subsequently research, P172H missense mutation is found in XLID individuals. A micro-duplication in the TM4SF2 locus has been revealed but was also present in unaffected control persons as a result, suggesting it maybe a neutral polymorphism [Cai *et al.*, 2008].

3.3 SYNAPTIC SIGNALING

Tuberous sclerosis complex (TSC1/TSC2),Neurofibromin1 (NF1), phosphatase and tensin homolog (PTEN) are genes that are linked with neurological diseases with common autistic symptoms including neurofibromatosis, Tuberous sclerosis and Cowdea/ Lhermitte-Duclos syndrome These are tumour suppressor gene that share common function; they are responsible for negative regulation of the mammalian target of rapamycin (mTOR) signalling pathway mutation mice. TSC1 loss in the cerebellar purkinje cell display an autistic like

behaviours (Tsai *et al* 2012) and TSC 2 heterozygote mice exhibits abnormal social communication (Young *et al*., 2010).

NF1 mutant mice show aberrant social transmission of food preference and deficits in hippocampus-dependent learning (Costa *et al*., 2001, 2002). PTEN deficient mice show alteration in social interaction and macrocephaly with hyper-activation of mTOR pathway (Kwon *et al*., 2006).

NmDAR and MGLuR signalling pathways

ASD- related signalling molecules such as TSC2, Shank3, mTOR, NF1, GluN2A, neuroligin2, neurexin1 are known to be linked with NmDAR and mGLUR signalling pathway.

NMDAR and MGLUR carry out a very important function in the regulation of synaptic function and plasticity at excitatory synapses. NF1 is known to interact with NmDAR complex and also regulates GLuN2A phosphorylation (Husi *et al*., 2000).

A defect in NmDAR function and associated signalling are also observed in non-syndromic ASD model with shank mutation. Shank proteins are physically linked to both NmDAR and mGLuR causing the regulation of the signalling pathways downstream of mGLuR or NmDAR activation as well as interactions between the two receptors.

3.4 ENVIRONMENTAL INVOLVEMENT IN PATHOGENESIS OF AUTISM

Despite the reason that ASD has been heavily linked to heredity, other epidemiological and epigenetic factors have come to bring about an importance instance of consideration aside genetics. Epidemiological studies has identify numerous non genetic influence and condition that have serve as an eye opener and also providing more rooms and opportunity for extensive research in other to investigate the mechanism (Grabruchker., 2012).

Treatment of maternal with pharmaceutical drugs and medication such as valproic acid, thalidomide and antidepressant most especially during the first trimester period of pregnancy is seen has a means associated with an increase the chances of giving birth to a child with Autism spectrum disorder (ASD) as a result of the advance effect of this hazardous drugs to the pregnant mother (Croen., 2011, Christensen et al., 2011). Frequent exposure to several toxicant such as pesticide, polybrominated diphenyl ethers (PBDEs), polychlorinated biohenyl (PCBs) can lead to a detrimental effect on the development process of especially persons with genetic susceptibility (Newschaffer, 2007). Additionally, many neurotoxic

compounds are likewise suspected to interfere with neuro-transmitter systems which also implicated in ASD (Quaak, 2013). Maternal residential tendency in increasing the risk of ASD during pregnancy is relatively high due to agricultural applications of pesticide, but this scenario may be as a result of abnormally high exposure levels .These chemicals are toxic that it has further potential to cause immune-toxicity, which may lead to alteration in cytokine production frequently observed in individuals with ASD (Goines., 2015).

Advanced maternal along with paternal age is also seen as an establishing risk factors for autism which was recently reaffirmed by an international study which also found that autism spectrum disorder (ASD) risk is relatively high in advance age parent (Sandi., 2015) . Advanced parental age is thought to contribute to methylation defect in gametes, which can cause an increased in the oxidative stress leading to DNA damage and fragmentation (Menezo, 2015). Maternal nutritional and metabolic risk factors include diabetes, obesity and folic acid deficiency, meanwhile zinc deficiency has been observed in autistic children and it may contribute to pathophysiology (Grabrucker, 2012; Lyall, 2013; Ornoy, 2015). Air pollution has also align to cause an increasingly high risk of autism spectrum disorder (ASD) which may leads to a prenatal exposure to heavy metals such as Ozone,chlorinated solvents, small particulate matter, diesel and residential proximity to freeways (Lyall 2013; Ornoy., 2015). Exposure of humans to numerous external environmental effects may adversely cause a detrimental effect on fetal development.

3.5 IMMUNE CONSIDERATION

A largest majority of independent studies have brought about a role ofimmune system in ASD during the prenatal and postnatal periods and as a result of this, several research projects continue to concentrate more on investigate this topic. It will be critical to observe if these immune abnormalities are causes or consequences that lead to alterations in neurodevelopment, or if it’s just a mere epiphenomenon in ASD.

3.5.1 Maternal Infection during Pregnancy

Many epidemiological researches have proven to provide important information about the association that exist between maternal infection and symptoms or signs of a fever during pregnancy to be heavily associated to ASD (Patterson. 2011). More insight was given in regards to maternal infection during pregnancy by a Danish study when a pregnant woman was admitted in the hospital to be treated for a viral infection during the first trimester period for birth in the year 1995-2005 or for a bacterial infection during the second trimester period of pregnancy in year 1980-2015 and it was been observed that the child was infected with

ASD (Atladottir., 2010). Meanwhile, the study did not give full detailing information regarding the relationship between the infection and the total length during the pregnancy which is seen has a risk factor.

The study give for information regarding mother who are diagnosed after admission with any kind of infection most especially bacteria to have a higher chances or increase rate of giving birth to a child with Autism.

Cytokine carry out important function most especially during infection by causing a fever and also the activation of the immune system which causes diffusible factor and at such, increasing the tendency of being transfer from mother to fetus (Zaretsky, 2004). Cytokines is seen to have been strongly associated in neuro-developmental processes in a way that any disruption of the tightly controlled systems could possibly result in pathophysiological disruption in the development of the brain (Garay, 2010; Devreman, 2009).

3.5.2 Autoimmunity

Epidemiological studies of the incidence of familial autoimmunity also give more insight onthe association between activation of the maternal immune system and autism spectrum disorder (ASD) individuals. A systematic review and meta- analysis carried out on nine case control study and one cohort study has revealed that maternal immune disease formed during the stage of pregnancy have a significant and increasing risk of giving rise to a child with Autism and it also postulated that abnormal maternal immune activation can cause a detrimental effect on the fetus development (Chen, 2016). Review have also found a positivelink or association between the maternal thyroid disease and ASD patients as well as maternal diabetes, rheumatoid arthritis, celiac disease and systemic lupus erythematosus has a change in autoimmunity which may cause a detrimental effect on the child causing ASD (Vinet, 2015, Xu, 2014, Gesundheit *et al*, 2013; Atladoittir *et al*, 2009; Croen, 2015).

Autoimmune conditions can mainly be transferred to children from their mother through the maternal antibodies transfer just as in the case with neonatal lupus erythematosus (NLE), While the immunoglobulin G (IgG) antibodies are transfer from the placenta through the neonatal Fc receptor (FcRn) to give the fetus passive immunity and this takes place during the second trimester period of pregnancy. Observably, mothers having these antibodies are said to showcase an increasing rate of autoimmune diseases (Brimberg, 2013). Additionally, a combination of maternal antibodies specific to ASD that recognizes targets in the developing brain were linked with elevated stereotypical behaviours in ASD (Braunschweig *et al*, 2013).

3.5.3 Gastrointestinal Dysfunction and Altered Micro-biome

Many patient suffering from autism are reported to showcase a high complication in gastrointestinal comorbidity distress which is highly important considering the fact that the proper immune functioning and microbial symbiotic functioning are very important in gastrointestinal tract health.

Gastrointestinal symptoms which are observed in autism spectrum disorder individuals include abdominal pain, chronic diarrhea and constipation. This factor is very important for raising issues in term of nutritional intake and quality of diet. Patient with ASD are likewise observed to posseses an increase in the intestinal permeability which causes a huge effect on the mucosal immune system leading to a change in the gut flora.

In the microbial population, ASD can also be detected depending on the level of bacterial in the feces and urine sample of persons suffering from Autism spectrum disorder (Rosenfeld, 2015).

Observably, an increase in the level severity of autism symptoms has been found to be associated with a high risk of having gastrointestinal problems.

DIAGNOSIS OF ASD

4.1 DIAGNOSTIC TOOLS AND CRITERIA

The most commonly rating scales for the continuous and proper evaluations of the behavioural characteristic of children affected with ASD are; Autism diagnostic observation schedules (ADOS) and autism diagnostic interview-reviewed (ADI-R) (Akshoomoff *et al*., 2006; Gotham et al., 2008; Hu & Steinberg, 2009). The autism diagnostic observation schedules (ADOS) is a standardized diagnostic observational tool that is responsible for the evaluation of social and communication disorder linked with autism spectrum disorder behavioural features (Akshoomoff *et al*., 2006;Lord et al., 2000). The ADOS in also commonly used in combination to ADI-R due to the semi- structured interview which is carried out among parent with children suffering from ASD with the primary aim of detecting abnormalities linked with language ability, social skills and cognitive functions (Akshoomoff *et al*., 2006; Hu & Steinberg,2009).

Right from the time autism diagnostic observation schedule (ADOS) and autism diagnostic interview reviewed was implemented, it has been widely used simply because of it ability to different between a person suffering from autism and another individual with a different neurodevelopmental disorder (Reaven *et al*., 2008).

The childhood autism rating scale (CARS) is another common tool used by physicians to screen for ASD. The CARS is a behavioural rating scale that is majorly used to differentiate or distinguish among ASD individuals and other neurodevelopmental disable individuals such as differentiating between PDD-NOS and intellectual disability (Chlebowski *et al*., 2010; Geier *et al*, 2013). This rating scale is very important in explaining the severity of the children due to close behavioural observation of these children (Geier *et al*., 2013).

Furthermore, there have been revisions of the specific criteria for a diagnosis of ASD for the main aim of making it more reliable and precise. These changes have been put into consideration due to the increasing number of evidence showing typical differentiation between;AS, PDD–NOS and other forms of ASD (Huerta *et al*., 2013; Lord *et al*., 2012)

4.2 DIAGNOSTIC MARKERS OF ASD

There has been an increase in the number of investigators regarding the study of ASD in other to make new discovery and development of new ASD marker in other to improve the efficiency of autism spectrum disorder diagnosis. A marker is simply defined as a variable concern with the study of a particular disease of interest found among individuals and it can

likewise be observed from a particular patient through the use of sensitivity as well as a reliability quantitative approach (Ruggeri *et al*., 2014; Gabriele *et al*., 2014).

Several molecules which could be used in diagnosis of autism spectrum disorder (ASD are identified and this includes neuro-transmitters such as GABA, glutamate and serotonin.

Another important and efficient bio-marker which is also used for detecting ASD is the hormonal and immunological bio-marker (Ruggeri *et al*, 2014). Evidence has suggested that autism spectrum disorder (ASD) is been grouped by the alteration in the hormonal level including oxytocin and dopamine which are efficient and significant neuro-modulators in the brain (Ruggeri *et al*., 2014). PET scan is been used which show which evidently showed that the level of dopaminergic system in the brain of children suffering from ASD is altered. The dopaminergic system of the brain is also important or responsible for controlling motivation and (Nakamura *et al*., 2010). Furthermore, the hormonal plasma level of oxytocin which is responsible for the regulation of repetitive behavioural characteristics is altered among individuals with neuro-developmental and behavioural problems associated with autism spectrum disorder individuals (ASD) (Alabdali *et al*., 2014; Hammock *et al*., 2012).

Other typical characteristic of persons with autism spectrum disorder(ASD) are facial abnormalities, such as asymmetry in the face, and a prominent forehead. The development of the face and the brain are tightly interconnected and as a result leading to abnormalities in facial morphology indicating a dysfunction in brain growth (Ercan *et al*., 2008; Hammond *et al*., 2008). A typical facial asymmetry, especially in the right supraorbital and anterior periorbital regions can help clinicians discriminate and differentiate between the faces of ASD and healthy children (Hammond *et al*., 2008). The present development of morphometric analysis techniques such as 3D dense surface models (DSMs) of face shape has increasingly enable scientists to be able to accurately recognize the facial phenotype of individuals with ASD (Claes *et al*., 2011; Hammond,2007).

Other factors that can as well help out in the diagnoses of autism spectrum disorder(ASD) involves a reduced eye contact, which can be measured and observed through the use of an eye tracking technology (Boraston and Blakemore, 2007; Mercadante *et al*., 2006) while a reduction in communication and cognitive skill can be effectively evaluated by the intelligence quotient (IQ) as well as the presence or absence of language. These neuro-psychological and behavioural markers have proven beyond doubt to be very efficient in clinical practice in respect to the diagnosis of ASD.

4.3 FUTURE DIRECTION

4.3.1 Gene-Environmental Interaction

Epigenetics, especially interaction between gene and environment (G x E) is a subject matter that have become of high interest and examination in the research and study of Autism Spectrum Disorder (ASD) (Kim, 2015). Through the use of this model, a pathway that is genetically susceptible is liable to get another injury by an environmental insult which might be at a specific window where there is developmental vulnerability that could lead to causal changes of pathophysiology. Factors that are heritable or genetic can also be responsible for exposing an individual to some certain hazards in the environment. This helps to describe the difference in both phenotypes with the large array of genes and factors in the environment effects in individuals with Autism spectrum disorder (ASD). According to a proposal that says if these two factors are combined together in dysregulating same signalling pathways which underlie a critical process such as neuronal connectivity during periods that are critical, a strong probability in forming necessary combination of conditions to increase neurodevelopment is observed (Stamou, 2013). Furthermore, in a recent study which used a very large and well characterized Autism Spectrum Disorder cohort report that individuals having Autism Spectrum Disorder-associated copy number variants that their mothers witnesses fever or infections as at the time of pregnancy showcase a high intense of behavioural phenotypes as compared to individuals who have predisposition genetically of exposure on its own (Mazina et al., 2015).

4.3.2 Therapeutics

The vast and uncertain etiology of clinical heterogeneity of Autism Spectrum Disorder have quite made it hard in providing a generally accepted effective treatment which medication mostly are prescribed to mainly treat comorbid symptoms of ASD. Early intense behavioural intervention is the present best therapy to improve core deficits in Autism Spectrum Disorder and that is the reason it is fundamental and critically important to keep a set of biomarkers that will help in diagnosis of the disorder at an early age before abnormal behaviours is visible (Lai, 2013).

As a result of the constant gastrointestinal comorbidities and the modified brain-gut micro-biome that is observed in Autism Spectrum Disorder, there have been therapies that include diet intervention and probiotics which has been proposed that it helps at least a subset of patients. A gluten-free in particular and or casein-free food may be approved for administration in tackling intestinal inflammation with leaky gut. Some studies have also

suggest that behavioural improvements are followed with the intake of the diet but scientific evidence in respect to the effectiveness and efficiency of the alternative treatment remains very limited (Lange, 2015; Dougle et al., 2015).

The immune system describes another welcoming potential therapeutic target that function in easing the fever effects or swellings at the stage of pregnancy likewise immune alterations is addressed in ASD patients. At this juncture, drugs that immune-modulate and are also anti-inflammatory like corticosteroids has showcase sign of great behaviour adjustments that was in an open-label trial of patients affected Autism spectrum disorder (ASD). It is suggested that these treatment types should be targeted towards a particular subtypes with validation in wide and mostly intense trials. Pharmacogenomics is another type of emerging personalized treatment that is aimed at the use of a person's genetic information that will provide intervention pharmacologically which will increase benefit of therapy and at the same time reducing any side effects (Bower, 2015).

4.3.3 Animal Model

The use of animal models is on the increase to find out the complex mechanism and process that is behind Autism Spectrum Disorder behaviours with phenotypes. The animal models are readily available for drug testing or therapeutic testing of any kind (Servadio, 2015). In both non-human primate alongside rodent models, maternal immune activation (MIA) showcase that even if there is no infection, there is still behavioural changes in the offspring as a result of maternal immune system activation using bacterial and viral mimics more so the cytokine IL-6 (Bauman, 2014; Boksa, 2010 and Smith, 2007). Some measures which involves factors in fetal immunity, gene expression patterns and changes in morphology of the fetal brain has been observed to depend majorly on mouse strain, maternal immune activation (MIA)-inducing agent alongside exposure timing which highly suggests a gene-environment (GxE) role of interactions and relationship (Schwartzer, 2013; and Garbett, 2012). Auto-antibody maternal effect exposure has been observed and studied in animal models in a process that there is a human IgG transfer from maternal parents of Autism Spectrum Disorder children into females that are pregnant which eventually will lead to increased anxiety, motor development and slow sensory and alteration in the ability to socialize in mice and modified brain and social development in primates such as monkeys comparing it with the IgG from mothers that were used as control (Brauschwerg et al., 2013). A more recent research is the injecting of a purified IgG in the cerebral ventricular of a mouse embryo which indicate that there is a result of binding to radial glial cells in the developing brain of embryos by the

autism-specific maternal autoantibodies, at such giving or suggesting a possible mechanism for these antibodies (Martinez et al., 2016).

POTENTIAL TREATMENTS FOR AUTISM SPECTRUM DISORDER

The United State food and drug administration have approved only two medicines for the treatment of autism spectrum disorder (ASD) which includes; Risperidone and Aripiprazole which all act as a dopamine and 5-HT receptor antagonists. These drugs are majorly useful for correction of movement and stereotypy behaviours but not for social and communication defects.

5.1 CONCLUSION

The knowledge and understanding of Autism spectrum disorder has significantly improve beyond all odds over the last few years as a result of the increasing and overwhelming research that is been put into consideration in this field of great interest. Notwithstanding, further research work is highly welcome in this field not just to understand its origin and its comorbidities but it has likewise improve on knowing the difference among affected person with this disorders. Autism spectrum disorder (ASD) is termed a neuro-developmental disorder that calls for a risk for failure of persons to adapt at the social skills, psychological and educational level of endeavours.

Autism spectrum disorder (ASD) is seen has a complex neurodevelopmental disorders that is seen to have been caused by both environmental factors and genetic which may be liable to cause changes in the functionality and connectivity of the brain.

Affected person with autism spectrum disorder(ASD) lack the sole ability to understand feelings, intentions, thought and emotional states but has a result in the advancement in sequencing technology, it has greatly contributed in the identification of several target genes found in ASD patients. Research has exposed phenotypic abnormalities seen in the chromosomes of persons suffering from ASD and at such, supporting the idea that genetics plays an important and vital role in Autism spectrum disorder predisposition.

The selected candidate gene analysis is a screening tool which is viably important in the identification of genes responsible in Autism spectrum disorder individual. The selected candidate genes are mostly involved in the screening for genes that are clinically important for human behaviour as well as belonging to a neuro-developmental pathway in the brain.

Extensive research has shown that a mutation in serotonergic gene and neuroligins are likely to cause implications and difficulties in depression and synaptogenesis respectively thereby increasing the risk to ASD.

Epigenetic also plays a significant role as a result of changes due to gene- environmental interactions leading to the alteration in the anatomical potential of the brain and connectivity that are solely responsible for consistent abnormal cognitive and social deficiency noted in patient with ASD.

5.2 RECOMMENDATION

With huge, effective and continuous study, it would immensely increase the ability to develop new diagnostic tools for early detection and evaluation of children with high risk factor of autism spectrum disorder (ASD). Huge advancement in research will facilitate an effective and personalize treatment at an early age of the child and likewise contribute as well as help in terms of brain development and changing the course of early behavioural features of the individual with ASD.

It is also recommended that parent should show active and early response to sign and symptoms of Autism spectrum disorders (ASD) observed in their young child for early diagnosis and prompt urgency to the disorder to avoid future complications.

Candidate genes and environmental epidemiological factors observed to have a strong effect on Autism spectrum disorder individuals should be put into serious considerations by research to bring a pathway in the detection of the major cause of Autism spectrum disorder as well as a lasting remedy to this disorder that has caused discomfort and instability to both parents and ASD affected persons.

It is advised that future research should be more focused on identifying new therapeutic targets likewise the development of effective strategies for ASD treatment through extensive study of animal models that have same abnormalities as noticed in ASD individuals.

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