DE GRUYTER OPEN

Folia Medica 2015; 57(1): 19-28

doi: 10.1515/folmed-2015-0015

REVIEW

AUTISM SPECTRUM DISORDER - A COMPLEX GENETIC DISORDER

Hristo Y. Ivanov[1], Vili K. Stoyanova[1,2], Nikolay T. Popov[3], Tihomir I. Vachev[1,4]
[1]Department of Pediatrics and Medical Genetics, Medical Faculty, Medical University, Plovdiv, [2]Department of Medical Genetics, St. George University Hospital, Plovdiv, [3]Psychiatric Ward for Men, State Psychiatric Hospital, Pazardzhik, [4]Department of Plant Physiology and Molecular Biology, Paisii Hilendarski University, Plovdiv, Bulgaria

РАССТРОЙСТВО АУТИСТИЧЕСКОГО СПЕКТРА КАК КОМПЛЕКСНОЕ ГЕНЕТИЧЕСКОЕ ЗАБОЛЕВАНИЕ

Христо Й. Иванов[1], Вили К. Стоянова[1,2], Николай Т. Попов[3], Тихомир И. Вачев[1,4]
[1]Кафедра педиатрии и медицинской генетики, Факультет медицины, Медицинский университет, Пловдив, [2]Отделение медицинской генетики, Университетская больница "Св. Георги", Пловдив, [3]Мужское психиатрическое отделение для активного лечения, Государственная психиатрическая больница, Пазарджик, [4]Кафедра физиологии растений и молекулярной биологии, ПУ „Паисий Хилендарски", Пловдив, Болгария

ABSTRACT

Autism spectrum disorder is an entity that reflects a scientific consensus that several previously separated disorders are actually a single spectrum disorder with different levels of symptom severity in two core domains - deficits in social communication and interaction, and restricted repetitive behaviors. Autism spectrum disorder is diagnosed in all racial, ethnic and socioeconomic groups and because of its increased prevalence, reported worldwide through the last years, made it one of the most discussed child psychiatric disorders. In term of aetiology as several other complex diseases, Autism spectrum disorder is considered to have a strong genetic component.

Key words: *autism spectrum disorder, whole exome sequencing, genome wide expression*

Folia Medica 2015; 57(1): 19-28

РЕЗЮМЕ

Расстройство аутистического спектра представляет собой объединяющее понятие, которое отражает научный консенсус по вопросу о том, что несколько, считающихся до этого отдельными, заболеваний являются континуумом одного расстройства с варьирующей степенью тяжести симптомов в двух основных областях – дефицита в социальном взаимодействии и коммуникации и стереотипного поведения, интересов и деятельностей. Расстройство аутистического спектра диагностицируется во всех рассовых, этнических и социально-экономических группах и по причине повышения частоты проявления становится одним из наиболее часто обсуждаемых психиатрических расстройств в детском возрасте. Считается, что этиологические причины расстройства аутистического спектра, как и ряд других комплексных заболеваний имеют генетическую основу.

Ключевые слова: *расстройство аутистического спектра, экзомное секвенирование, экспрессия генов*

Folia Medica 2015; 57(1&2): 19-28

INTRODUCTION

The term and the diagnosis of 'infantile autism' were introduced into the world of science by an American psychiatrist, Leo Kanner.[1] In an article of 1943, Kanner described a condition in 11 children that he called 'early infantile autism'.

A year later the word 'autism' in its modern sense (e.g. developmental disorder) was also used by the Austrian physician Hans Asperger. Working at the Vienna University Hospital, he observed children with normal (and sometimes higher) intelligence, who had difficulties communicating,

Article's history: Received: 1 Dec 2014; Received in a revised form: 29 Jan 2015; Accepted: 1 March 2015
*Correspondence and reprint request to: *H. Ivanov, Department of Pediatrics and Medical Genetics, Faculty of Medicine, Medical University, Plovdiv; Mobile: +359 884 827 070;* E-mail: *doctorhristoivanov@yahoo.com*
15 A, Vassil Aprilov Blvd, 4002 Plovdiv, Bulgaria

failed to demonstrate empathy, did not understand body language and had poor language in terms of sounds or verbal statements. He gave a description of a syndrome which was later named after him, the syndrome of Asperger.

Over the past 65 years etiological paradigms within psychiatry have changed significantly in parallel with the development of the concept that cognitive and behavioral disorders have organic 'brain-based' etiology. Initially, autism was thought to be largely a condition resulting from the way children were brought up by their parents. The first suggestion that rare genetic and biomedical conditions can lead to autism and that it is not associated with 'bad parenting', was voiced by phenylketonuria, but then these syndromic cases of autism were considered exceptions.[2,3] In the 60s and 70s of the last century autism was considered a form of psychosis or childhood schizophrenia. The separation of autism from childhood-onset psychoses, particularly schizophrenia, was an important advance in the study of childhood psychopathology. Clinically, this has been a basic and self evident distinction as age of onset, differential diagnosis, and treatment of the two conditions differed.[4] In 1980s autism was classified as a developmental disorder and the thesis of its biological nature was accepted. The term 'pervasive developmental disorders' (PDD) was used and introduced into the Diagnostic and Statistic Manual of Mental Disorders - III (DSM - III) and later included in the International Classification of Diseases - 10 (ICD). In the next version of DSM, fourth edition (DSM-IV)[5], PDD was conceptualized as a manifestation of triad symptoms: impairment in social interaction, qualitative impairment of communication, restricted and repetitive behavior pattern, and included five specific subgroups: Autistic Disorder; Pervasive Developmental Disorder, Not Otherwise Specified; Asperger's Disorder; Rett's Disorder; and Childhood Disintegrative Disorder. In the newest version of DSM-V released in 2013[6], these subgroups were merged into a single umbrella term as 'autism spectrum disorders' (ASD), and the triad of impairments has been folded into two, with social communication and social interaction combined as a single diagnostic domain.

PREVALENCE

ASD is diagnosed in all racial, ethnic and socioeconomic groups, five times more common in boys than in girls.[7]

Prior to 1990, most studies estimated general population prevalence for autism of four to five per 10,000 (1/2000-1/2500).[8] Increased prevalence of ASD have been reported worldwide over the last few years. According to the Center for Disease Control (CDC) and Autism and Developmental Disabilities Monitoring (ADDM) Network in the USA, 1 in 88 children are identified with the ASD[7] although this increase might merely reflect improved diagnostic means.

Due to the high social significance of autism and the arising frequency, clinical practice has long sought to shade a light on etiology and pathogenesis of this disorder which still remain elusive. Until the etiology of ASD is known, it will be difficult to identify effective prevention and treatment. Current belief holds that ASD derive generically from a complex interaction between genetic, epigenetic and environmental factors[9] (Fig. 1).

Further, it is suggested that some of the associated sequence variations detected in ASD are common in the general population. However, it is not clear whether the involvement of single genes in combination with nongenetic factors or multiple genes through locus heterogeneity or multiple genes through allelic heterogeneity is needed to result in the ASD phenotype. It is also suggested that multiple genes in combination with nongenetic factors may be in operation and that is needed to result in the ASD phenotype.[10] An important fact to note is that environmental factors are probably connected somehow to genetic makeup and epigenetic mechanisms and the non-coding RNAs can be the link between, even though there is no clear evidence for that. All those questions still remain unclear.[11]

THE ENVIRONMENTAL MODIFIERS

Environmental exposures and stressors act through their impact on the organism. Effects may impact brain development, development of other organs and systems, and ongoing physiological processes.[12] Such environmental modifiers include advanced parental age, oxidative stress, neuroinflammation and mitochondrial dysfunction as well as environmental pollutants (e.g., air pollution, organophosphates and heavy metals) and biochemical disturbances.[10] Other modulating factors include an interaction between immune dysfunction and genetic predisposition, as evident from the reports of cytokine-mediated influences on neuronal development and links between genes that encode for immune-related proteins and ASD.[13]

As there is controversy, a recent study showed

Folia Medica 2015; 57(1): 19-28

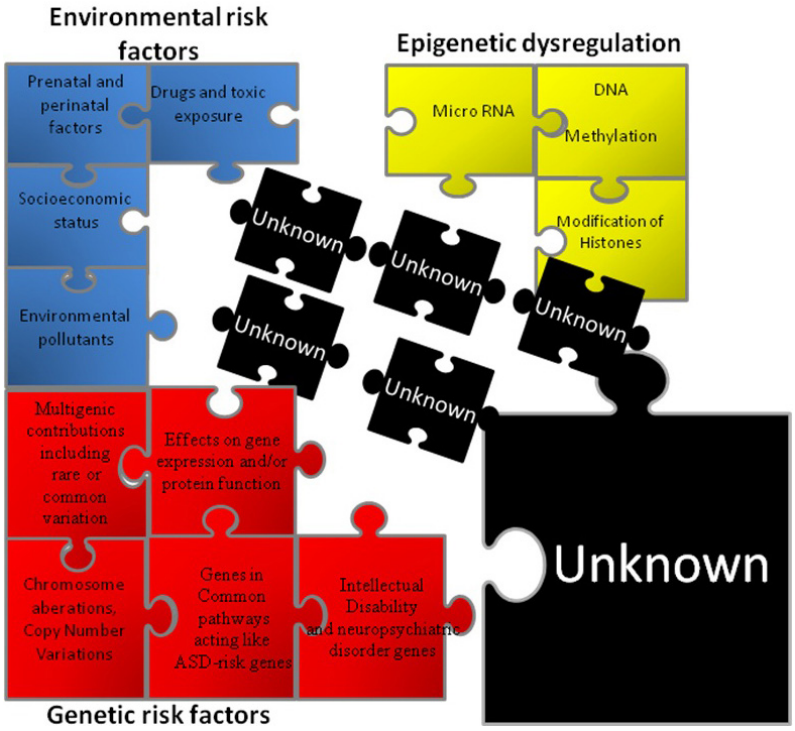

Figure 1. The puzzling etiology of ASD.

that part of the individual risk of ASD and autistic disorder increased with increasing genetic relatedness. Heritability of ASD and autistic disorder was estimated in this study to be approximately 50%.[14]

Environmental factors do contribute to the etiology of ASD but they are beyond the scope of the present review, and we shall concentrate on the genetic basis of ASD.

ASD has a strong genetic basis. In twin studies, the concordance rate of broad ASD phenotype in monozygotic twins is 70–90%, but 0–30% in dizygotic twins.[15,16] A very recent prospective study reported that if there was an older sibling with ASD, the likelihood of subsequent offspring having autism was 18.7% overall and 32.2% if the child had more than one older sibling with autism.[17] Despite being highly heritable, ASD is genetically complex and the underlying genetic architecture is still not well understood.

THE TERMS 'SYNDROMIC' AND 'NONSYNDROMIC'AUTISM

An important distinction in the genetics of autism is that between 'syndromic' and 'non-syndromic' (or idiopathic) autism (Fig. 2). 'Non-syndromic autism' is a term used to describe cases where autism is the primary diagnosis and is caused by unknown genetic or environmental cause, oligogenic, polygenic, and multifactorial mechanisms.[18] Moreover, many genes involved in nonsyndromic intellectual disabilities (ID) and in epilepsy have also been implicated in the etiology of nonsyndromic ASD. These genes probably belong to a continuum of neurodevelopment disorders that manifest in different manners depending on associated genetic and environmental factors.[19]

The term syndromic or secondary autism is used to refer to a condition caused by a well-known genetic variant, such as tuberous sclerosis, Rett syndrome, fragile X syndrome or other medical genetic conditions. It is typically associated with malformations and/or dysmorphic features and unlike 'idiopathic' ASD, it shows a different male: female sex ratio.[18]

However, none of these etiologies is specific to autism because each of them encompasses a variable proportion of individuals with and without autism.[20]

CAUSES OF 'SYNDROMIC' AUTISM

Currently a genetic cause can be identified in 20% to 25% of children with autism. The known genetic causes (Table 1) include cytogenetically visible chromosomal abnormalities (~5%), copy number variants (CNVs) (i.e. submicroscopic deletions and duplications) (10-20%) and single gene disorders in which neurologic findings are associated with ASD (~5%).[21]

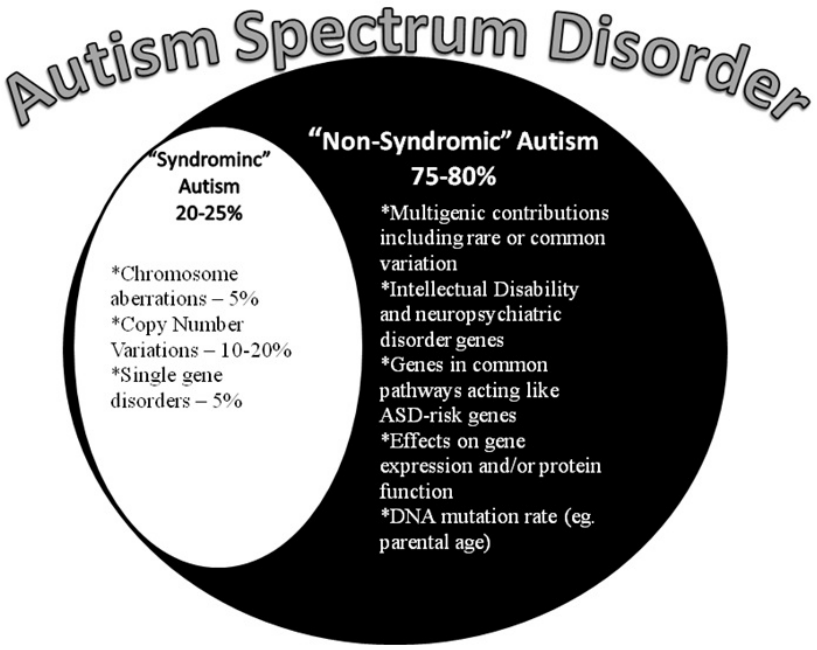

Figure 2. Causes of 'syndormic' and 'non-syndromic' autism.

CHROMOSOME ABERRATION AS A CAUSE

Cytogenetic changes are consistently reported as one of the most common identifiable causes of autism. Cytogenetic abnormalities visible with karyotype analysis are found in approximately 5% of children with ASD and another 3-5% can be identified by fluorescence in situ hybridization (FISH) techniques.[21] The most commonly reported (recurrent) cytogenetic abnormalities found in persons with autism are the 15q11–q13 duplication (of the maternal allele) of the Prader-Willi/Angelman syndrome region (1-3%)[24], deletions involving 7q, 22q13, 2q37, 18q, Xp and sex chromosome aneuploidies (47, XYY and 45, X / 46, XY mosaicism)[25].

COPY NUMBER VARIATIONS (CNV) AS A CAUSE

Array comparative genomic hybridization (aCGH) is steadily replacing high-resolution chromosome analysis and FISH in the evaluation of children with autism. Array CGH is designed to test for known deletion/duplication syndromes on the entire genome plus assessment of subtelomeric regions.[21] Rare de novo and some inherited CNVs can contribute to the genetic vulnerability to ASD in as many as 10% of examined cases. CNVs can involve a single gene and act much as a sequence-level mutation or they can encompass several genes as part of a genomic disorder.[24] Some CNVs associated with autism include 1q21 deletion, 2p15-2p16.1 deletion, 15q13 deletion/duplication, 16p11.2 deletion/duplication.[23]

Weiss et al.[26] reported 16p11.2 deletions or duplications in approximately 1% of individuals with autism and 1.5% of children with developmental or language delays. The 16p11.2 deletion often occurs de novo, but may be transmitted from parent to child in an autosomal dominant manner. Of note, however, is that the same 16p11.2 CNVs can be observed in a variety of other disorders including schizophrenia, bipolar disorder, seizures, ADHD, and dyslexia, as well as apparently unaffected family members; thus, interpretation of the significance of this CNV can be difficult.[21]

Overall, CNVs are linked to a broad variety of clinical features, including major or minor malformations, facial dysmorphisms, severe neurological symptoms, full-blown autism, milder autism-spectrum traits, or even behavioral disorders outside of the autism spectrum. Thus, the variable penetrance and great phenotypic heterogeneity characterizing CNV expressivity make it often difficult to determine whether in a given patient a CNV is the sole cause of autism, confers vulnerability to the disease, or represents a chance finding. The majority of CNVs are inherited from either one of the parents, who may display some autistic traits, but clearly without satisfying the criteria for autistic disorder. Notably, many CNVs found in ASD patients have been found also in patients with other psychopathologies, especially intellectual disability and schizophrenia.[23]

Folia Medica 2015; 57(1): 19-28

Table 1. Some genetic syndromes associated with autism[22,23]

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

SINGLE GENE DISORDERS AS A CAUSE

Well-known genetic disorders can encompass autistic features in their clinical presentation, such as fragile X syndrome, tuberous sclerosis, neurofibromatosis, untreated phenylketonuria, and Rett syndrome.[23] The most prevalent single gene disorders as a cause are fragile X syndrome (around 3-5%) and tuberous sclerosis (TSC1/TSC2 - around 1%).[27]

Whereas 1% to 3% of children ascertained on the basis of an autism diagnosis have fragile X syndrome, at least half of the children with fragile X syndrome have some autistic behavior.[21]

The situation is similar with tuberous sclerosis (TSC) - 25-50% of intellectually disabled individuals with TSC fulfill autism diagnostic criteria but only 1.1-1.3% of individuals initially diagnosed with ASD have TSC.[21]

Notably, the clinical manifestations of 'syndromic' autism can be highly heterogeneous, even in the presence of the same well characterized mutation or genomic rearrangement, likely due to differences in genetic background and epigenetic influences.[23]

THE SEARCH FOR CAUSE OF 'NON-SYNDROMIC' AUTISM

Many genes and environmental factors are likely to contribute to the etiology of autism, making it difficult to isolate disorder genes. The search methods of the disorder susceptibility genes include linkage studies, genome-wide association studies, whole-exome sequencing, expressions analysis and epigenetic studies.

LINKAGE STUDIES

Substantial effort in autism genetics over the last 10 years has been focused on genetic linkage analysis using an affected sibling-pair design in multiplex families. Linkage can be defined as the tendency for alleles close together on the same chromosome to be transmitted together, as an intact unit, through meiosis. Linkage studies are either performed as full genome screens with a dense set of genetic markers covering all chromosomes, or locally (fine-mapping) at a certain chromosomal area of interest.[27]

To date, only loci on 17q11–17q21 and 7q22-7q32 have been replicated at levels that could be considered genome-wide significant. Both loci contain some of the most intensively studied candidate genes associated with ASD - CNTNAP2, RELN and MET.[28]

The linkage data indicate that many loci may underlie risk of autism, which is consistent with the well-accepted hypothesis that many genes may be associated with autism. To address the limitation of reduced linkage signals due to marked genetic heterogeneity, some studies have made an effort to increase sample homogeneity by focusing on selected characteristics (or endophenotypes) of autism, such as language measures (e.g., age at first word), developmental milestones (e.g., bladder and bowel control), developmental regression, and restricted repetitive and stereotyped patterns of behavior, interests, and activities (e.g., obsessive-compulsive behavior). Such endophenotypes, which can also be found in nonautistic family members and in the general population, facilitate the mapping of quantitative trait loci (QTL) that individually contribute to the overall autism phenotype.[29] Alarcon et al. used a measure of language delay to identify a QTL on 7q34–7q36.[30] Schellenberg and colleagues identified a locus at 9q34 using the same age-at-first word measure.[31]

QTL studies suggest that 'subtyping' autism patients will help narrow the search for specific autism susceptibility genes. The fact that linkage studies have not yet identified 'the ASD gene' is not a failure of these methods but rather an accurate reflection of the complexity of this disorders and the need for larger homogenized sample sizes.[28]

GENOME-WIDE ASSOCIATION STUDIES (GWAS)

GWAS studies identify single-nucleotide polymorphisms (SNPs) and other common genetic variants in DNA that may be associated with a disease or trait by investigating the entire genome using an unbiased hypothesis-free search. The first GWAS was carried out by Weiss et al.[32] and they have found no statistically significant SNP association in over 1000 families. The authors then carry out a genotyping of the most significant SNPs in additional autistic families and a point mutation in 5p15 have shown statistical significance. This SNP fell between genes encoding SEMA5A (a member of the semaphorin family of proteins involved in axon guidance) and TAS2R1 (a bitter-taste receptor). Independent evidence was presented of reduced expression of SEMA5A in lymphoblastoid cell lines, whole-blood and brain samples of autistic individuals compared to control.[32]

Wang et al.[33] carried out another GWAS. They used two populations: one largely overlapping with the sample used by Weiss et al.[32] and the other including over 1200 cases and nearly 6500 controls. One region of genome-wide significance was identified at 5p14. The association signal came

Folia Medica 2015; 57(1): 19-28

from a region between CDH10 and CDH9, two genes encoding neuronal cell-adhesion molecules.[33]

In 2010 the Autism Genome Project (AGP) Consortium genotyped 1 million SNPs and analyzed 1558 defined ASD families[34], identifying genome-wide association with rs4141463, located in the MACROD2 gene, as crossing a preset significance threshold of $P < 5\times10(-8)$.

The different results gathered from the independent GWAS, despite the great wealth of data gained from each single study, have been interpreted as stemming from the large phenotypic and genetic heterogeneity of ASD. Thus, current efforts are aimed at finding autism subtype-related genetic variants, also by searching for QTL. Nevertheless, GWA studies may help identify targets for more in-depth studies of etiological mechanisms.

WHOLE-EXOME SEQUENCING (WAS)

Since 2005, next-generation sequencing (NGS) technologies have been improving as rapid, high-throughput and cost-effective approaches to fulfill medical sciences and research demands.[35] Whole-exome sequencing (WES) has recently been introduced to identify rare or novel genetic defects from genetic disorders. Particularly, ASD is a model disease to apply WES because multiple loci are involved in its development with relatively weak genotype-phenotype correlation.[23] The data gathered from WES[35-38] demonstrates the importance of de novo mutations in the etiology of ASD. There may be several hundred genes in which high risk-conferring de novo mutations can occur and the vast majority of them may increase the risk but does not "cause" the disease, further supporting an oligogenic/polygenic model. Many of the disrupted genes were found to impact important gene networks (synaptic plasticity, -catenin/chromatin remodeling), and several de novo mutations were found in genes previously implicated in other neurodevelopmental disorders and in intellectual disability (e.g., SCN1A, SCN2A, GRIN2B).[35-38]

Other use of WAS is to find candidate recessive mutations in autistic probands with known shared parental ancestry.[23] Chahrour et al.[39] used homozygosity analysis to identify probands from non-consanguineous families that showed evidence of distant shared ancestry, suggesting potentially recessive mutations. They identified four novel candidate genes, namely UBE3B, CLTCL1, NCKAP5L and ZNF18, which encode proteins involved in proteolysis, GTPase-mediated signaling, and cytoskeletal organization.[39] Puffenberger et al.[40] found a homozygous missense mutation in HERC2 (c.1781C>T, p.Pro594Leu) associated with global developmental delay and ASD. Novarino et al.[41] screening families with autism, epilepsy, and intellectual disability identified homozygous inactivating mutations in the BCKDK gene (Branched Chain Ketoacid Dehydrogenase Kinase).

The data acquired by WES shows that lots of genes have been identified and associated with ASD but no common etiology can be proposed. All aspects such as oligogenic, de novo mutations, recessive inherited mutations, polygenic, multifactorial, pleiotropic effects, combination of locus heterogeneity should be considered when thinking of ASD etiology.

GENOME WIDE EXPRESSION STUDIES

The advent of microarray technologies allows the design and implementation of genome-wide expression profiling which helps to unravel the molecular basis of phenotypic variation in many disease states. Global expression profiling provides the opportunity to evaluate possible transcriptomic deviations in ASD at both genes and gene-network levels. Currently, several published studies describe genome-wide expression patterns in biomaterials derived from ASD patients and controls.[42] So far, only a handful of studies have investigated the ASD transcriptome in post-mortem brain samples[43,44] and other studies have used RNAs from lymphoblastoid cell lines or blood[45-50] demonstrating their usefulness for this purpose. Lymphoblasts provide access to larger sample sizes and unlimited source of RNA. Moreover, for technical reasons lymphoblasts can be better controlled for various experimental confounds that may influence the fidelity of measuring gene expression data.[51]

Most of the studies found different expression in genes that are involved in the neurodevelopment in ASD samples compared to controls. NAGLU, FLAP/ALOX5AP, CHL1, and ROBO1 that are involved in neuronal differentiation, brain development and axon guidance were found downregulated by Hu et al.[47] FGF12, MYT1L, and GAS7 that are associated with neuronal differentiation and outgrowth were found downregulated by Garbett et al.[43] Semaphorin 4C (SEMA4C) and glutamate dehydrogenase (GLUD1) that are involved in neural development and in glutamatergic neurotransmission were discovered upregulated by Gregg et al.[46]

Several of these studies[43,46,47,49,52] also found different expression in the genes involved in the immune system: pro-inflammatory cytokines, NK

and CD8+ cell-related genes including receptor genes and genes involved in the cytotoxicity pathways - ASS, DAPK1, FLT1, IL6ST[47], IL2RB, TH1TH2, FAS[43], SPON2, IL2RB, PRF1, EAT2/SH2D1B, GZMB, CX3CR1 PAM[46], suggesting neural inflammation model for ASD.

The data acquired so far suggest that genes involved in the nervous system development and the immune system may play a role in the pathogenesis of ASD. Analysis of peripheral tissues such as blood and lymphoblast cell lines are important as a complement to SNP genotyping and CNV analyses in order to assess the functional significance of genetic variants, and identify ASD biomarkers.

EPIGENETIC STUDIES

Epigenetic mechanisms, such as DNA methylation, modifications of histone proteins and miRNA, regulate chromatin structure and/or gene expression without changing DNA sequence. Epigenetic abnormalities are associated with several neurodevelopmental diseases including ASD.[19] Regulation of neuronal structure and function through epigenetic mechanisms is believed to be critical in the development of the nervous system.[53]

Regulation of gene expression through DNA methylation has been found in several genes associated with ASD. Hyper-methylation of specific CpG sites in the promoter regions of BCL-2 and RORA that leads to downregulation of these genes was identified in autistic children compared to healthy developing twins.[54] SHANK3 is another gene that is associated with ASD and has an epigenetic regulation.[55]

Alternation in the modifications of the histone proteins is another epigenetic mechanism that could be related to ASD. SMCX gene, which encodes histone 3 lysine 4 (H3K4) me3 - specific demethylase, regulates through demethylation of the histones other genes, i.e., SCN2A, CACNA1H, BDNF, SLC18A1, associated with ASD and cognitive dysfunction.[56]

Another aspect of epigenetic control of gene expression is through miRNA. There are only a handful of studies that have analyzed the expression profile of miRNA in ASD samples compared to healthy controls.[57-60] They all have found differently expressed miRNA and their predicted target genes in the ASD samples suggesting that miRNA may play a role in the etiology of this disorder.

The role of epigenetics in ASD has only emerged in the last few years, and represents a growing area of research.

CONCLUSION

The latest advances in the discovery of the genetic factors involved in the etiology of ASD have increased significantly over the past years. A genetic factor in the etiology of this disorder is certain, as certain as its complexity is. Understanding the genetic factors involved is crucial to establishing future intervention strategies. It is expected that high-throughput molecular screenings, such as high resolution array-CGH, whole-exome and whole-genome sequencing, as well as transcriptomic and epigenetic analysis, will further increase our understanding of the genetics of ASD. The interpretation of this newly acquired genetic data will require multidisciplinary approach, but still clinical geneticists have an important role in the diagnosis and research of autism.

REFERENCES

1. Kanner L. Autistic disturbances of affective contact. Nervous Child 1943;2:217-50.
2. Friedman E. The autistic syndrome and phenylketonuria. Schizophrenia 1969;1:249-61.
3. Cantwell DP, Baker L, Rutter M. Families of autistic and dysphasic children. I. Family life and interaction patterns. Arch Gen Psychiatry 1979;36:682-7.
4. Rutter, M, Taylor E. Child and adolescent psychiatry. London: Blackwell; 2002.
5. American Psychiatric Association. The Diagnostic and Statistical Manual of Mental Disorders. Washington DC: American Psychiatric Publishing; 1994: 66-78.
6. American Psychiatric Association. The diagnostic and statistical manual of mental disorders: DSM 5, Washington DC: American Psychiatric Publishing; 2013:50-59.
7. Baio J. Prevalence of autism spectrum disorders - Autism and developmental disabilities monitoring network, 14 Sites, United States, 2008. Surveillance Summaries 2012 March 30;61(SS03):1-19.
8. Fombonne E. Is there an epidemic of autism? Pediatrics 2001;107:411-2.
9. Engel SM, Daniels JL. On the complex relationship between genes and environment in the etiology of autism. Epidemiology 2011;22(4):486-8.
10. Eapen V. Genetic Basis of Autism: Is There a Way Forward? Curr Opin Psychiatry 2011;24(3):226-36.
11. Chaste P, Leboyer M. Autism risk factors: genes, environment, and gene-environment interactions. Dialogues Clin Neurosci 2012;14(3):281-92.
12. Herbert MR. Contributions of the environment and environmentally vulnerable physiology to autism spectrum disorders. Curr Opin Neurol 2010; 23(2):103-10.

13. Ashwood P, Krakowiak P, Hertz-Picciotto I, et al. Elevated plasma cytokines in autism spectrum disorders provide evidence of immune dysfunction and are associated with impaired behavioral outcome. Brain Behav Immun 2011;25:40-5.
14. Sandin S, Lichtenstein P, Kuja-Halkola R, Larsson H, Hultman CM, Reichenberg A. The Familial Risk of Autism. JAMA 2014;311:1770-7.
15. Rosenberg RE, Law JK, Yenokyan J, et al. Characteristics and concordance of autism spectrum disorders among 277 twin pairs. Arch Pediatr Adolesc Med 2009;163:907-14.
16. Ronald A, Hoekstra R. Progress in understanding the causes of autism spectrum disorders and autistic traits: twin studies from 1977 to the present day. New York: Springer; 2014:33-65.
17. Ozonoff S, Young GS, Carter A, et al. Recurrence risk for autism spectrum disorders: a baby siblings research consortium study. Pediatrics 2011;128:e488-e495.
18. Lintas C, Persico AM. Autistic phenotypes and genetic testing: state-of-the-art for the clinical geneticist. J Med Genet 2009;46(1):1-8.
19. Fett-Conte AC, Bossolani-Martins AL, Pereira-Nascimento P. Genetic etiology of autism. In: Fitzgerald M, editor. Recent advances in autism spectrum disorders - Volume I. InTech. Available from: http://www.intechopen.com/books/recent-advances-in-autism-spectrum-disorders-volume-i/genetic-etiology-of-autism.
20. Benvenuto A, Moavero R, Alessandrelli R, et al. Syndromic autism: causes and pathogenetic pathways. World J Pediatr 2009;5(3):169-6.
21. Miles JH, McCathren RB, Stichter J, Shinawi M. Autism spectrum disorders. In: Pagon RA, Adam MP, Ardinger HH, et al, editors. SourceGeneReviews® [Internet]. Seattle (WA): University of Washington, Seattle; 1993-2015.
22. Cohen D, Pichard N, Tordjman S, et al. Specific genetic disorders and autism: Clinical contribution towards their identification. J Autism Dev Disord 2005;35(1):103-16.
23. Persico AM, Napolioni V. Autism genetics. Behavioural Brain Research 2013;251:95-112.
24. Devlin B, Scherer SW. Genetic architecture in autism spectrum disorder. Current Opinion in Genetics & Development 2012;22:229-37.
25. Schaefer BG, Mendelsohn NJ. Genetics evaluation for the etiologic diagnosis of autism spectrum disorders. Genet Med 2008:10(1):4-12.
26. Weiss LA, Shen Y, Korn JM, et al. Association between microdeletion and microduplication at 16p11.2 and autism. N Engl J Med 2008;358:667-75.
27. Freitag CM, Staal W, Klauck SM. Genetics of autistic disorders: review and clinical implications. Eur Child Adolesc Psychiatry 2010;19:169-78.
28. Abrahams BS, Geschwind DH. Advances in autism genetics: on the threshold of a new neurobiology. Nat Rev Genet 2008;9:341-55.
29. Kumar RA, Christian SL. Genetics of autism spectrum disorders. Current Neurology and Neuroscience Reports 2009;9:188-97.
30. Alarcon M, Cantor RM, Liu J, et al. Evidence for a language quantitative trait locus on chromosome 7q in multiplex autism families. Am J Hum Genet 2002;70:60-71.
31. Schellenberg, GD, et al. Evidence for multiple loci from a genome scan of autism kindreds. Mol Psychiatry 2006;11:1049-60.
32. Weiss LA, Arking DE, Daly MJ, et al. A genome-wide linkage and association scan reveals novel loci for autism. Nature 2009;461:802-8.
33. Wang K, Zhang H, Ma D, et al. Common genetic variants on 5p14.1 associate with autism spectrum disorders. Nature 2009;459:528-33.
34. Anney R, Klei L, Pinto D, et al. A genome-wide scan for common alleles affecting risk for autism. Hum Mol Genet 2010;19(20):4072-82.
35. Sanders SJ, Murtha MT, Gupta AR, et al. De novo mutations revealed by whole-exome sequencing are strongly associated with autism. Nature 2012;485:237-41.
36. Neale BM, Kou Y, Liu L, et al. Patterns and rates of exonic de novo mutations in autism spectrum disorders. Nature 2012;485:242-5.
37. O'Roak BJ, Vives L, Girirajan S, et al. Sporadic autism exomes reveal a highly interconnected protein network of de novo mutations. Nature 2012;485:246-50.
38. Iossifov I, Ronemus M, Levy D, et al. De novo gene disruptions in children on the autistic spectrum. Neuron 2012;74:285-99.
39. Chahrour MH, Yu TW, Lim ET, et al. Whole exome sequencing and homozygosity analysis implicate depolarization regulated neuronal genes in autism. PLoS Genetics 2012;8:e1002635.
40. Puffenberger EG, Jinks RN, Wang H, et al. A homozygous missense mutation in HERC2 associated with global developmental delay and autism spectrum disorder. Human Mutation 2012;33:1639-46.
41. Novarino G, El-Fishawy P, Kayserili H, et al. Mutations in BCKD-kinase lead to a potentially treatable form of autism with epilepsy. Science 2012;338:394-7.
42. Lintas C, Sacco R, Persico AM. Genome-wide expression studies in Autism spectrum disorder, Rett syndrome, and Down syndrome. Neurobiol Dis 2012;45(1):57-68.
43. Garbett K, Ebert PJ, Mitchell A, et al. Immune transcriptome alterations in the temporal cortex of sub-

jects with autism. Neurobiol Dis 2008;30(3);303-11.
44. Purcell AE, Jeon OH, Zimmerman AW, et al. Postmortem brain abnormalities of the glutamate neurotransmitter system in autism. Neurology 2001;57:1618-28.
45. Baron CA, Tepper CG, Liu SY, et al. Genomic and functional profiling of duplicated chromosome 15 cell lines reveal regulatory alterations in UBE3A-associated ubiquitin-proteasome pathway processes. Hum Mol Genet 2006;15(6):853-69.
46. Gregg JP, Lit L, Baron CA, et al. Gene expression changes in children with autism. Genomics 2008;91:22-9.
47. Hu VW, Frank BC, Heine S, et al. Gene expression profiling of lymphoblastoid cell lines from monozygotic twins discordant in severity of autism reveals differential regulation of neurologically relevant genes. BMC Genomics 2006;7:118.
48. Hu VW, Nguyen A, Kim KS, et al. Gene expression profiling of lymphoblasts from autistic and nonaffected sib pairs: altered pathways in neuronal development and steroid biosynthesis. PLoS One 2009a;4:e5775.
49. Hu VW, Sarachana T, Kim KS, et al. Gene expression profiling differentiates autism case-controls and phenotypic variants of autism spectrum disorders: evidence for circadian rhythm dysfunction in severe autism. Autism Res 2009; (2):78-97.
50. Nishimura Y, Martin CL, Vazquez-Lopez A, et al. Genome-wide expression profiling of lymphoblastoid cell lines distinguishes different forms of autism and reveals shared pathways. Hum Mol Genet 2007;16:1682-98.
51. Mohammad M, Ghahramani S, Pingzhao H, et al. Gene and miRNA expression profiles in autism spectrum disorders. Brain Research 2011;380:85-97.
52. Enstrom AM, Lit L, Onore CE, et al. Altered gene expression and function of peripheral blood natural killer cells in children with autism. Brain Behav Immun 2009;23:124-33.
53. Rangasamy S, D'Mello SR, Narayanan V. Epigenetics, Autism Spectrum and Neurodevelopmental Disorders. Neurotherapeutics 2013;10:742-56.
54. Nguyen A, Rauch TA, Pfeifer GP, et al. Global methylation profiling of lymphoblastoid cell lines reveals epigenetic contributions to autism spectrum disorders and a novel autism candidate gene, RORA, whose protein product is reduced in autistic brain. FASEB J 2010;24:3036-51.
55. Durand CM, Betancur C, Boeckers TM, et al. Mutations in the gene encoding the synaptic scaffolding protein SHANK3 are associated with autism spectrum disorders. Nat Genet 2007;39:25-7.
56. Siniscalco D, Cirillo A, Bradstreet JJ, et al. Epigenetic Findings in Autism: New Perspectives for Therapy. Int J Environ Res Public Health 2013;10:4261-73.
57. Talebizadeh, Z, Butler MG, Theodoro MF. Feasibility and relevance of examining lymphoblastoid cell lines to study role of microRNAs in autism. Autism Res 2008;1:240-50.
58. Sarachana T, Zhou R, Chen G, et al. Investigation of post-transcriptional gene regulatory networks associated with autism spectrum disorders by microRNA expression profiling of lymphoblastoid cell lines. Genome Med 2010;2:23.
59. Seno GMM, Hu P, Gwadry FG, et al. Gene and miRNA expression profiles in autism spectrum disorders. Brain Res 2011;1380:85-97.
60. Abu-Elneel K, Liu T, Gazzaniga FS, et al. Heterogeneous dysregulation of microRNAs across the autism spectrum. Neurogenetics 2008;9(3):153-61.