AUTISM – GENETICS, ELECTROPHYSIOLOGY AND CLINICAL SYNDROMES

Nada Pop-Jordanova, Dijana Plasevska-Karanfilska

Macedonian Academy of Sciences and Arts, Skopje, R. Macedonia

Corresponding Author: Nada Pop-Jordanova, Macedonian Academy of Sciences and Arts, Bul. Krste Misirkov 2, 1000, Skopje, Tel: + 389 (0)2 3 23 54 00, Fax: + 389 (0)2 3 23 55 00, E-mail: popjordanova.nadica@gmail.com

Abstract
Autism is a severe and the most heritable developmental disorder, whose pathogenesis is still largely unknown. The rising incidence of autism in the last decade has increased the scientific interest and research. More than a thousand papers concerned with information about the etiology of this "static disorder of the immature brain" can be found on Pub Med. The aim of this paper is to give a review of published genetic chromosomal anomalies associated with autistic spectrum disorders, as well as to discuss common syndromes associated with autistic traits. In addition, some of our own findings in genetics, as well as in quantitative electroencephalography and neurofeedback training in autistic children, will be presented and discussed. Generally, the subsequent analyses indicate that the causes of autism include fewer common single-gene mutations and chromosomal abnormalities, as well as multiple interacting genes of weak effect. Genome-wide linkage analysis has identified several susceptibility loci and positional and functional candidate genes which appear to represent possible risks of the autistic spectrum. Electrophysiological findings showed high delta/theta activity in frontal-central regions, while in 25% high beta activity was detected as a result of anxiety. Neurofeedback is a promising therapy for symptom mitigation.

Key words: autism, genetics, chromosomes, candidate gene, quantitative EEG, neurofeedback.

Introduction
Autism is characterized by impairments in three behavioural domains: social interactions, language impairments and stereotyped, repetitive patterns of behaviour. Symptoms are manifested by the age of 3 years, and affected individuals often require constant care from family members and professionals. Other disorders that are included in the autism spectrum include atypical autism, Asperger disorder, Rett disorder, childhood disintegrative disorder, and pervasive developmental disorder not otherwise specified [DSM-IV-TR].

It was previously reported that autism affects approximately 5 of every 10,000 children, but prevalence rates of both autism and autism spectrum disorders (ASDs) have increased markedly in the past decade. Two population-based studies conducted by the Centers for Disease Control and Prevention in the United States reported ASD prevalence of 3.4 and 6.7 per 1000 children [1, 2]. In UK the incidence is estimated to be much higher (1 : 75 children) [3]. Although this increase may be to some degree artifactual, it may also reflect a true increase in the incidence of ASD and implies an important role of environmental causes.

Clinically, it is important to distinguish "idiopathic" autism, of no known specific cause, and "secondary" ASDs, in which a known environmental agent, chromosome abnormality, or single-gene disorder can be identified as causative. Approximately 5–10% of individuals with autism can be more specifically diagnosed with secondary autism, and the remaining 90–95% have idiopathic autism. It is also important to
distinguish between what has been termed as "complex autism" and "essential autism" [4]. Complex autism has been defined by the presence of dysmorphic features and/or macrocephaly in an individual with an ASD diagnosis. Approximately 20–30% of children with idiopathic autism have complex autism. Essential autism has been defined by the absence of both macrocephaly and dysmorphic features. Approximately 70% of children with idiopathic autism have essential autism. Individuals with complex autism will include cases of autism arising from de novo genetic variants [5, 6].

The diagnosis is made clinically, through Autism Diagnostic Interview–Revised (ADI-R) [7] and the Autism Diagnostic Observational Schedule (ADOS) [8]. In addition, photographic dysmorphology, physical and neurological examination, and medical and family history are needed. The evaluation also comprises use of some neuroimaging techniques such as CT scan, MRI, fMRI, SPECT, and BOLD to evaluate the morphology and functional ability of the brain.

Possible etiology

Most plausible neurodevelopmental theories of autism focus predominantly on genetic factors which imply high heredity. The heritability of autism is mainly confirmed by twin and family studies. It was demonstrated that monozygotic twins have a higher incidence than dizygotic twins. The sibling recurrence risk in families with an autistic child is 2–6%, which is much higher than in the general population (< 0.5%). Also confirmed were the heritability of IQ, skills for nonverbal communication, social adaptability, repetitive behaviour and spoken language. In addition, in some families with an autistic child a broader phenotype is common in non-autistic family members such as social phobia, obsessive-compulsive disorder, stereotypic behaviour, language problems, etc. [9, 11].

However, studies of monozygotic twins indicate that fewer than 70% of twin pairs are concordant for autism and approximately 90% are concordant for a broader spectrum of related cognitive or social abnormalities. This finding suggests the influence of other non-heritable, prenatal, and perinatal risk factors for autism, a possibility supported by studies that have shown an association between autism and obstetric complications, prenatal or intrapartum use of medications, and parental preconception chemical exposures. In addition, the roles of vaccines, heavy metals and other environmental factors are still being discussed [12–14].

The parental characteristics associated with an increased risk of autism and autism spectrum disorders include advanced maternal and paternal age, and maternal place of birth (underdeveloped countries). The obstetric conditions that emerged as significant fell into two categories: (1) birth weight and duration of gestation and (2) intra partum hypoxia.

When talking about genetic contributions to the ASDs, one can distinguish between genetic factors, including chromosomal abnormalities and specific genetic mutations, which by themselves appear to cause autism (with or without other associated conditions), and susceptibility loci, which are neither necessary nor sufficient by themselves to cause ASDs. It is currently thought that most ASDs are caused by the interaction of several susceptibility loci and the minority of ASDs are caused by causal genetic variations [15–22].

Generally, known genetic causes of autism 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%)

a. Cytogenetically Visible Chromosomal Abnormalities

Structural variants known as balanced chromosome abnormalities (BCAs), in which DNA segments are moved into different locations in the same chromosome or exchanged with segments in other chromosomes, leaving the overall size of the chromosomes unchanged are known to be significantly more common in individuals with autism spectrum disorders than in a control population. Different gene variations – deletion, duplication or inactivation — can result in very similar effects, while two similar changes at the same site might have very different neurodevelopmental manifestations. It is suspected that the genetic causes of autism and other neurodevelopmental abnormalities are complex and likely to involve many genes.
A significant portion (~5%) of ASDs results from chromosomal abnormalities and specific gene mutations that involve multiple genes [15]. Specific chromosomal abnormalities that are associated with ASDs include, most commonly, chromosomal deletions or duplications in the 15q11-q13 region (also called the Prader-Willi/Angelman syndrome [PW/AS] region), as well as Down syndrome, Smith-Magenis syndrome, 22q13 deletion syndrome (recently attributed to haploinsufficiency of a single gene, SHANK3/PROSAP2), and other more rare conditions and syndromes.

Several genes previously associated with X-linked or autosomal mental retardation have been found in children who have an ASD diagnosis [18]. These include the RS MeCP2 gene, the fragile X-syndrome FMR1 gene, the tuberous sclerosis TSC genes, the Smith-Lemli-Opitz syndrome DHCR7 gene, the neuroligin genes (NLGN3 and NLGN4), ATR-X, neurotrypsin and other genes as well.

Aneuploidy, found with high-resolution chromosome analysis, is found in 5% of children with autism. Using Fish methodology, 3–5% other chromosomal abnormalities have been identified. Unbalanced chromosomal abnormalities are found in children with certain dysmorphic characteristics. Practically, some abnormality has been found in every chromosome, but only in a few of them were possible loci related especially with autism.

As we said previously, the most commonly observed chromosome abnormality in autism is maternally-derived duplication of the region 15q11-q13. In the majority of cases this duplication is the result of de novo supernumerary isodendris 15q chromosome and less commonly the result of segregation of a paternal chromosome translocation or maternally derived interstitial 15q duplication. The maternally derived 15q11-q13 interstitial duplication is a highly penetrated cause of autism.

### b. Single Gene Disorders

Some known chromosomal abnormalities with single gene mutation can be associated with autism. As we mentioned previously, the most common association is with fragile-X syndrome, tuberous sclerosis complex, Rett syndrome (in girls), Sotos syndrome, etc. [23–27].

**Fragile-X syndrome** – The association between autism and fragile-X is different in different reported studies (incidence from 2% to 25%). Indeed, some patients with fragile-X syndrome manifest behavioural characteristics of autism, but the exact function of the FMRI gene related to the pathophysiology of autism remains unclear. However, it is important to consider fragile-X in differential diagnosis of autistic spectrum disorders.

**Sotos syndrome** – Sotos syndrome is characterized by the cardinal features of typical facial appearance, overgrowth (height and head circumference ≥ 2 SD above the mean), and learning disability ranging from mild to severe. Sotos syndrome is associated with the major features of behavioural problems, congenital cardiac anomalies, neonatal jaundice, renal anomalies, scoliosis, and seizures. Though Sotos syndrome is probably not a significant cause of classic autism, children with Sotos syndrome and behavioural problems such as difficulty with peer group relationships and lack of awareness of social cues may be referred to autism clinics. Eighty to 90% of individuals with Sotos have a demonstrable mutation or deletion of NSD1; inheritance is autosomal dominant.

**Rett syndrome** – Rett syndrome satisfies practically all the criteria for autism according to DSM-IV TR. The possible misdiagnosis of this syndrome in early childhood implies careful neurological assessment for diagnostic clarification. It is believed that MECP2 mutation causes Rett syndrome. There are some other phenotypic groups where diagnoses of X-linked mental retardation and female sex are associated with autism. However, screening for MECP2 in girls with autistic symptoms is highly indicated.

**Tuberous sclerosis complex** – It is well known that 40–70% of patients with tuberous sclerosis (TSC) manifest autistic behaviour. In addition, epilepsy and mental retardation as clinical manifestations can be assumed as risk factors for the development of autism. The prevalence of tuberous sclerosis in autistic patients is estimated to be 1–4%, but much higher (8–14%) in autistic patients with epilepsy. Tuberous sclerosis is related to TSC1 and TSC2 genes located on chromosomes 9q34 and 16p13 respectively, which underlie the development of cen-
central nervous lesions. There are various explanations of the association between autism and tuberous sclerosis. First, that the abnormal brain organization caused by tuberous sclerosis gene mutations occurs in brain areas involved in autistic behaviour; second, that a separate autism susceptibility gene exists in linkage disequilibrium with genes of tuberous sclerosis; and third, that downstream effects of the TSC gene (e.g. seizure, mental retardation, cortical tubers) are responsible for the development of autism. Fig. 1 shows a typical MRI finding in tuberous sclerosis.

**Fig. 1 – MRI in patient with tuberous sclerosis**

**Neurofibromatosis type 1 (NF1)** – Although NF1 has been diagnosed in children with autism, it is unclear whether this is a true association or the chance simultaneous occurrence of two relatively common childhood disorders. No genetic overlap between mutations in the NF1 gene and autism has been demonstrated.

**Timothy syndrome** – Timothy syndrome, a disorder of calcium channels caused by a mutation in the CACNA1C gene, is characterized by severe QT prolongation, syndactyly, cardiac defects, dysmorphic faces, developmental delays and autistic symptoms. Inheritance is autosomal dominant.

**Joubert syndrome** – This autosomal recessive disorder is characterized by partial or complete agenesis of the cerebellar vermis, seen as the "molar tooth sign" on MRI, abnormal breathing, abnormal eye movement, cognitive impairment, and behavioural problems. A subset of Joubert syndrome appears related to the AHI1 gene, encoding the ‘jouberin’ protein. Some authors [28] delineated behavioural and genetic differences between autism and Joubert syndrome, implying that they are etiologically distinct disorders. In a report by Muhle et al. (2004) of monozygotic twins with Joubert syndrome, the twin with the more severe cerebellar abnormality had autism, suggesting that some disorders may have the potential to cause the autism phenotype if as-yet unidentified autism regions or circuits of the brain are affected.

Some **metabolic disorders** such as phenylketonuria, Smith-Lemli-Opitz syndrome and others are also associated with autism. It is very important to have a clear diagnosis of these metabolic disorders because their treatment can mitigate the autistic symptomatology [29].

In Table 1 the association between autism and some rare syndromic disorders is summarized.

Table 1

<table>
<thead>
<tr>
<th>Syndrome</th>
<th>ASD co-diagnosis</th>
<th>Gene mutation</th>
<th>Cell function</th>
</tr>
</thead>
<tbody>
<tr>
<td>Angelman</td>
<td>&gt; 50%</td>
<td>UBE3A(maternal)</td>
<td>protein degradation</td>
</tr>
<tr>
<td>Down syndrome</td>
<td>&gt; 5%–15%</td>
<td>chromosome 2 triplication</td>
<td>Multiple</td>
</tr>
<tr>
<td>Fragile X</td>
<td>&gt; 15%–70%</td>
<td>FMR1</td>
<td>RNA trafficking</td>
</tr>
<tr>
<td>Neurofibromatosis</td>
<td>&gt; 4%</td>
<td>NF1/NF2</td>
<td>PI3K signaling activity</td>
</tr>
<tr>
<td>Macrocephaly</td>
<td>&gt; 75%</td>
<td>PTEII</td>
<td>PI3K signaling activity</td>
</tr>
<tr>
<td>Potocki-Lupski</td>
<td>&gt; 90%</td>
<td>17p duplication</td>
<td>Unknown</td>
</tr>
<tr>
<td>Smith-Lemli-Opitz</td>
<td>&gt; 50%–75%</td>
<td>DHCR7</td>
<td>cholesterol biosynthesis; RAS mediated</td>
</tr>
<tr>
<td>Timothy</td>
<td>&gt; 75%</td>
<td>CACNA10</td>
<td>calcium signalling</td>
</tr>
<tr>
<td>Tuberous sclerosis</td>
<td>&gt; 40%–50%</td>
<td>TSC1/T3C2</td>
<td>PI3K/mTOR signalling</td>
</tr>
<tr>
<td>22q13 deletion</td>
<td>&gt; 90% PDD–NOS</td>
<td>Microdeletions</td>
<td>Multiple</td>
</tr>
<tr>
<td>Rett syndrome</td>
<td>&gt; 50%</td>
<td>MECP2</td>
<td>transcriptional regulation, inducing MET, CD11 and SPI</td>
</tr>
</tbody>
</table>
c. Searching for other genes

Generally, there are at least two distinct genetic etiologies for autism: rare, private (de novo) single gene mutations that may have a large effect in causing autism, and inherited, common functional variants of a combination of genes which have a small to moderate effect in increasing autism risk [29–34].

Although structural variants with large effect sizes may explain up to 15% ASD, genome-wide association studies have failed to uncover common single nucleotide variants with large effects on phenotype. The focus within ASD genetics is now shifting to the examination of rare sequence variants of modest effect, which is most often achieved via exome selection and sequencing. This strategy has indeed identified some rare candidate variants; however, the approach does not capture the full spectrum of genetic variation that might contribute to the phenotype.

A recent study has implicated two X-chromosomal neuroligin genes, NLGN3 and NLGN4, as having an etiological role in autism, having identified a frameshift mutation in the one gene and a substitution mutation in the other in multiplex autism spectrum families [27]. The function of neuroligin as a trigger for synapse formation suggests that such mutations would likely result in some form of pathological manifestation. To identify candidate genes, the physical mapping of balanced chromosomal aberrations is a powerful strategy, since several genes have been characterized in numerous disorders [35, 36].

The Autism Genomic Project (AGP) co-funded by Autism Speaks and other international public-private funding agencies, found several new autism risk genes by identifying copy number variants (CNVs), rare tiny insertions and deletions in the genome, which appear to disrupt more genes in individuals with autism than in the general population. Some of these CNVs are considered "highly penetrant" or sufficiently disruptive to cause autism on their own, while others only raise the risk of autism and may interact with other genetic and/or environmental risk factors to cause the disorder. Furthermore, while many CNVs are inherited, some are "de novo", meaning that they are found only in the child but not in the parents [37–39].

Like other genetic risk factors reported in the recent years, some of the new genes, such as SHANK2 and SYNGAP1, are detected as working in the synapse, the communication hub between neurons. Others, however, appear to cluster around specific biochemical pathways in the brain, including those involved in cell growth and motility. This novel finding suggests new biological mechanisms that could shed light on how autism develops [40].

Some research has revealed that the KCNMA1 gene, which encodes the alpha-subunit of the large conductance Ca\(^{2+}\)-activated K\(^+\) (BK\(_{Ca}\) ) channel, a synaptic regulator of neuronal excitability, is physically disrupted in autism. Further molecular and functional analyses showed the haploinsufficiency of this gene as well as decreased activity of the coded BK\(_{Ca}\) channel. This activity can be enhanced in vitro by the addition of a BK\(_{Ca}\) channel opener (BMS-204352). Further mutational analyses on autistic subjects has led to the identification of an amino acid substitution located in a highly conserved domain of the protein not found in comparison subjects [41]. These findings support the hypothesis that autism is associated with defects in neuronal circuitry and excitability which are the main characteristics of brain functioning in autism.

Further research showed that excessive Ca\(^{2+}\) levels are responsible for boosting aspartate/glutamate carrier (AGC) activity, mitochondrial metabolism and, to a more variable degree, oxidative stress in autistic brains. AGC and altered Ca\(^{2+}\) homeostasis play a key interactive role in the cascade of signalling events leading to autism: their modulation could provide new preventive and therapeutic strategies [42–44].

Xq12-q13.3 duplication is another novel global developmental delay and autism-predisposing chromosomal aberration, the pathogenesis of which may be mediated by increased dosage of genes contained in the duplication, including NLGN3, OPHN1, AR, EFNB1, TAF1, GJB1, and MED12.

The neuroligin-neurexin interaction at the glutamate synapse: NLGN3, NLGN4, NRXN1, CNTNAP2, and SHANK3 are shown to be involved in a study [44]. The mutated neuro-
ligin 3, neuroligin 3(R451C), as a recent molecular focus, in gain-of-function studies and its role in induced impairment of synaptic function has been found, but endoplasmic reticulum (ER) stress induced by mutated molecules also deserves investigation. Two missense mutations, H246N and Y251S, have been found in the gene-encoding synaptic cell adhesion molecule-1 (CADM1) in ASD patients, including cleavage of the mutated CADM1 and its intra-cellular accumulation. In addition, the mutated CADM1 showed slightly reduced homophilic interactions in vitro but most of its interactions persist. The mutated CADM1 also showed morphological abnormalities, including shorter dendrites, and impaired synaptogenesis in neurons. Wild-type CADM1 was partly localized to the ER of C2C5 cells, whereas mutated CADM1 mainly accumulated in the ER despite different sensitivities towards 4-phenyl butyric acid with chemical chaperone activity and rapamycin with promotion activity for degradation of the aggregated protein. Modelling analysis suggested a direct relationship between the mutations and the conformation alteration. Both mutated CADM1 and neuroligin 3(R451C) induced upregulation of C/EBP-homologous protein (CHOP), an ER stress marker, suggesting that in addition to the trafficking impairment, this CHOP upregulation may also be involved in ASD pathogenesis.

SLC25A12 is a gene found on human chromosome 2, in an interval that has shown a linkage to ASDs in multiple studies. This gene is involved in the mitochondrial function and is responsible for maintaining the levels of ATP (a critical source of energy) in the cell [45].

There are multiple studies of GABA receptors in ASDs. One reason for this is the association of chromosomal abnormalities in the PW/AS region with ASDs. The genes in the PW/AS region include several GABA receptor sub-units. In addition, GABA receptors are the major inhibitory transmitter receptors in the brain and have a central role in anxiety, and disruption of these receptors can lead to epilepsy, a common, but not diagnostic, feature of ASDs [46].

There have been several studies of the SLC6A4 transporter in ASD [47]. The interest in serotonin in ASDs arises because blood serotonin levels are elevated in about 30% of individuals with ASD. Additional evidence for a role for serotonin in ASDs comes from the beneficial effects of selective serotonin re-uptake inhibitors for certain core symptoms in ASD. Multiple groups have investigated a functional promoter variant in SLC6A4, termed HTTLPR, which is known to modulate the expression of the gene. There are two predominant forms of the HTTLPR variant, a short and a long form, and one would anticipate that in studies showing an association of HTTLPR with ASD, this same variant (long or short) would contribute to risk.

All these data demonstrate the power of massively parallel, targeted sequencing studies of affected individuals for identifying rare, potentially disease-contributing variation. However, they also point out the challenges and limitations of current methods of direct functional testing of rare variants and the difficulties of identifying alleles with modest effects.

**Genetic testing**

It must be pointed out that the most common abnormalities in autism are not detected with routine karyotyping or cytogenetic analysis. Special tests are required for fragile-X, MECP2 mutation or others. In a routine karyotyping, the finding of 15q duplication must be assumed as a marker chromosome, but other analyses are needed for detecting the Willi-Prader, or Angelmen area. In addition to the dysmorphic characteristics of patients, the presence of mental retardation and seizures, the mentioned analyses are needed.

Many researchers focus on multiplex families with more than one affected member to perform linkage analysis to assess whether a region of genome that seems to segregate with the disorder in the families can be identified. The most frequent findings are related to 7q, 2q and 17p regions.

Fig. 2 shows steps which are needed for experimental approaches to determining genetic etiologies for autism.
Beyond multiple genetic elements implicated in autism risk, there is high vulnerability to environmental influence. The findings from some studies involving cell stressors and toxic chemicals suggest additional ways in which genetic risk may combine with environmental factors to shift a system closer to disease threshold.

For a decade researchers have announced the promise of stem cells as the basis of new medical advance. With the discovery of new stem cell reprogramming techniques after 2006, the creation of stem cells taken directly from living people finally became a reality, and scientists all over the world started the race to create stem cells from individuals with a variety of specific conditions. For autism, stem cells made their mark in October 2010 when scientists in California generated stem cells (called inducible pluripotent stem cells, or iPSCs) from skin samples of people with Rett syndrome. The stem cells offered some of the first clues to what autism may look like at a cellular level, and provide a remarkable new way to test autism treatments.

The stem cell field is in its infancy, but with new techniques making this complicated process easy, stem cell researchers are beginning to model autism and its related disorders. In 2010 scientists also published initial stem cells findings on Fragile X, Angelman and Prader-Willi syndrome, all conditions that have autism as a feature. The significance of having these stem cells is not just that we are now closer to revealing the biological mysteries of autism, but that the stem cells can directly be used as a way to screen for therapeutics based on the specific deficit found in a person's cells. This opens up the future to design of individually-tailored treatments [48, 49].

**Main Macedonian results**

a. **Genetic testing for autism spectrum disorders**

At present, molecular genetic testing for several autism spectrum disorders is available at the Research Centre for Genetic Engineering and Biotechnology (RCGEB) in the Macedonian Academy of Sciences and Arts.
Genetic testing for Fragile X syndrome has been introduced following the research project performed in the period from 1994 to 2000 that aimed to determine the prevalence of Fragile X syndrome among mentally retarded individuals from the Republic of Macedonia, to determine the allelic and genotypic frequency of FMR1 CGG normal alleles in our population and to determine the molecular basis of Fragile X and the genotype phenotype correlation. The methodology included nested polymerase chain reaction (PCR) and Southern blot analyses. Among the total of 273 patients with mental impairment included in this study, five patients with full mutation of the FMR1 gene were detected. Two patients with full mutation expressed somatic variability and in one patient the process of reverse mutation was detected, i.e. the proband had a shorter mutation than his mother [51]. This study estimated that Fragile X syndrome participates with 1.8% in the mental retardation etiology in our country. The prevalence of Fragile X syndrome in the general population was estimated at 1 in 5456 individuals. The most frequent FMR1 CGG normal alleles were those with 30 repeats, followed by 29 and 31. These results corresponded to the data for other populations published in the literature. Our results suggested that there is no clear relation between the length of the expansion and the degree of mental retardation. Namely, two brothers with equal repeat length manifested different mental impairment (one brother had severe and the other moderate mental retardation).

Since the completion of the Fragile X syndrome project, approximately 150 patients have been referred for testing to our laboratory among whom two additional patients with CGG expansion were diagnosed. At present, the methodology includes PCR analysis of the promoter region of the FMR1 gene including the CGG repeats, followed by sizing of the PCR products by capillary electrophoresis on an ABI 3130 Genetic Analyzer. The methylation status of the CpG sequence of the FMR1 gene in males with CGG expansions is determined by Methylation-Specific Multiplex Ligation-dependent Probe Amplification (MS-MLPA) using kits from MRC Holland, Amsterdam, The Netherlands (www.mlpa.com). Southern blot analyses are nowadays only rarely performed.

The genetic testing for Rett syndrome was first introduced for the purpose of a prenatal diagnosis in a family with a Rett syndrome child with c.502C->T (p.R168X) mutation in the MECP2 gene diagnosed in a foreign laboratory. The sequencing of the exon 4 of the MECP2 in this family showed that the Rett syndrome was caused by a de novo mutation. Although aware that recurrence is very unlikely, the family decided to perform a prenatal diagnosis, which showed absence of the mutation in the foetus and brought relief to this family. The sequencing of the coding exons and associated intron junctions of the MECP2 (exons 2–4) in another female patient suspected of Rett syndrome showed no mutation in the MECP2 gene.

Genetic testing for tuberous sclerosis was introduced recently following a small pilot research study that aimed to determine the molecular basis of tuberous sclerosis in our country [52]. Mutational analysis of TSC1 and TSC2 genes was performed in eight Macedonian patients with a clinical diagnosis of TSC using MLPA analysis for the detection of large gene rearrangements and sequencing analysis of all exons and exon/intron boundaries of the TSC1 and TSC2 genes.

During the pilot study we detected five mutations, of which four were in the TSC2 and one in TSC1 gene. The TSC2 defects included two large deletions, i.e. deletion of exon 1 and upstream sequence and deletion of exons 1–15, one frameshift mutation (c.4318delC) and one missense mutation (c. 772A -> T). One frameshift mutation was detected in TSC1 (c.1431_1434del|AGAA). All mutations were de novo events and have been previously reported. The mutational spectrum among our patients suggested that the best approach for detection of mutations in TSC patients would be MLPA analysis of TSC genes for the detection of large rearrangements, followed by sequencing analysis for the detection of point mutations first in TSC2 then in TSC1 gene.

Following this pilot study, six other patients suspected of tuberous sclerosis were refer-
red for genetic testing at our laboratory. The mutational screening followed the aforementioned approach and one additional patient with TSC2 c. 1793A > G (p.Y598C) mutation was detected.

Recently we have also introduced genetic testing for several microdeletion syndromes, based on MLPA analysis. Several patients have been screened using an MLPA P245 kit that includes probes for the detection of 21 microdeletion syndromes, including some that are associated with autism, such as Prader Willi/Angelman, 22q11 (Phelan-McDermid), NF1 microdeletion syndrome, Sotos syndrome 5q35.3, etc. Thus far, using this kit we have detected two patients with Prader-Willi syndrome; the results were confirmed by a Prader Willi/Angelman specific MLPA probemix.

We have also introduced the array-based comparative genomic hybridization (array-CGH) using 180K Agilent Human Genome CGH Microarrays and an Agilent DNA Microarray system. The pilot study included array CGH analysis of eight mentally retarded patients, of whom two had autistic behaviour. The Database of Genomic Variants (DGV, http://projects.tcag.ca/cgi-bin/variation/) was used to compare our findings to previously reported CNVs. The results showed the presence of 6 to 18 CNVs in each patient, but none was pathological CNV.

A recent consensus statement proposed that microarray analysis should replace G-banded karyotype analysis as the standard genetic test for autistic patients [53]. This recommendation is based on the fact that microarrays have ten times higher resolution than karyotyped and six times greater diagnostic yield. We hope that in the near future the array-CGH analyses will become more widely available for patients in our country, including those with autism.

b. Electrophysiological findings in autism

Bearing in mind that the brain dysfunction is a main characteristic in autism, researchers tried to find some specific deficits in the brain functioning. In this context large neuro-imaging and electrophysiological studies have been performed. Not only one characteristic feature that distinguishes the brains of those with autism was found. The reason is that there are many different paths that lead to what we behaviourally define as ASD and each of those paths may correspond to distinct differences in the brain.

The electrophysiological research in autism confirms hyper-excitability and disturbed connectivity in the neuronal network as two main findings. In addition, six parts of the brain are selected to be involved in autism: 1) amygdala and its relation to the orbital and medial frontal area, 2) fusiform gyrus, 3) superior temporal gyrus, 4) the anterior insula and the anterior cingulate (both part of the limbic system, the so-called "emotional brain"), 5) frontal and parietal-temporal mirror neuron areas, and 6) the prefrontal cortex.

The main pattern obtained with quantitative electroencephalography (QEEG) are: 1) High Beta activity which corresponds to obses-sing, over focusing and anxiety, 2) High Delta/Theta activity which corresponds to cortical slowing and inattention, impulsivity and hyper-activity, 3) Abnormal EEG/Seizure activity, 4) Metabolic/toxic pattern of lower overall EEG activity (voltage), 5) Mu activity (high alpha range in central brain parts) which corresponds to social skills, and 6) Coherence Abnormalities [54–57].

In our study of only 15 autistic children [58–60] we obtained the following QEEG findings: In the frontal region we obtained high delta/theta activity; these patterns are not in linear correlation with the maturity processes. In 25% of patients we obtained high beta activity related to anxiety. These results correlate to other studies.

In addition, we calculated the brain-rate parameter as a measure of general mental activation [61, 62]. Our finding showed hypo arousal, especially in frontal and central brain regions (brain-rate was 5. 86). In Figs. 3 and 4 the QEEG obtained for a 3.5 year old male patient in two conditions (eyes open and eyes closed) is displayed. Fig. 5 presents brain-rate calculated in different disorders showing lower mental activation in autistic children which corresponds to the clinical presentation.
Neurofeedback has been found to have the capability of reducing some of the major deficits in ASD by individually targeting specific areas of the brain to increase or decrease its brain wave activity in certain frequency ranges [62–70]. We used training protocols based on all assessment information with an emphasis on initial QEEG which included analysis of absolute and relative power, as well as connectivity measures. Treatment protocols comprised primarily bipolar or interhemispheric montages individualized for each patient. The focus was on reducing hyperconnectivity which was frequently observed in posterior-frontal to anterior-temporal regions. The results of 20 sessions of neurofeedback training, including 5 previous sessions of EDR training for stress reduction, show a reduction in autistic symptoms, including a reduction in social interaction deficits, communication deficits, sensory/cognitive deficits and behaviour deficits. It must be said that it is very exhausting to obtain both QEEG as well as to perform neurofeedback in ASD patients. Only high functioning autistic persons can be candidates for this methodology.

In addition, there is empirical evidence that an ASD can respond to intensive behavioural interventions [71], and so identifying indi-
individuals with greater risks of ASDs at an earlier age is likely to have important clinical and practical implications.

As can be seen from the previous text, a wide number of scientific methodologies and disciplines are relevant to the study of autism, including studying genetically ‘at risk’ siblings, the development of novel neuroimaging techniques, and development and testing of screening instruments and interventions. The COST action BM 1004 entitled Enhancing the Scientific Study of Early Autism (ESSEA) (2010–2014) is aimed at establishing an interdisciplinary scientific network to advance the pace of discovery about the earliest signs of autism; combining techniques from cognitive neuroscience with those from the clinical sciences, and establishing European practice guidelines on early identification and intervention. The first author of this article, together with some collaborators from R. Macedonia, is a member of this action.

Some concluding remarks

There is extensive evidence that indicates that ASDs are genetic disorders. Whereas 5–10% of ASDs result from chromosomal abnormalities and causal mutations, a larger proportion is a result of the interaction of multiple susceptibility loci. The exact nature of the genes that are implicated in ASDs, whether they are common variants or rare variants, will impact on the feasibility of defining specific genetic assays as predictors for increased risk of ASDs, as well as the exact form the assays.

Most cases of autism are not caused by a single genetic mutation. However, several disorders with autism-like symptoms, including the Fragile X syndrome, can be traced to a specific mutation which leads to overproduction of proteins found in brain synapses.

The investigations into single-gene diseases have given researchers new ways of evaluating the molecular basis of autism spectrum disorders. And for the first time, it seems possible that – one day – they may find ways to alleviate the devastating effects of neurodevelopmental disorders.

Until now, autism is an incurable disorder. Medication, behavioural tailoring and neurofeedback could promise some mitigation of the clinical picture.

The basic science, while promising, is still in the early stages. A particularly daunting challenge is finding a safe delivery mechanism (sometimes called a vector) for gene therapy in people. In this context stem cell therapy seems to be a new challenge.

REFERENCES


49. Vaughn A. Stem cell research may have application to treat autism. 2012; 13 Fox News, USA.

50. Raven K. Stem Cell Trial for Autism Launches in U.S. Stem cell treatment could lower inflammation levels and demonstrate whether autism is an autoimmune disease. 2012; Scientific American.

Резиме

АУТИЗАМ: ГЕНЕТИКА, ЕЛЕКТРОФИЗИОЛОГИЈА И КЛИНИЧКИ СИНДРОМИ

Нада Поп-Јорданова,
Дијана Плашевска-Каранфилска

Македонска академија на науките и уметностите, Скопје, Р. Македонија

Аутизмот претставува сериозна и многу наследена болест, чија патогенеза се уште не е позната. Зголемената инциденција на аутизмот во последната декада го зголемува научноистражувањето на оваа „статична болест на незрелиот мозок” може да се најдат на PubMed.

Цел на оваа статија е да се даде ревија на најновите излази на случаи на аутизмот и на наука во областа. Аутизмот е наследен и претставува сериозна и многу наследена болест, чија патогенеза се уште не е позната. Зголемената инциденција на аутизмот во последната декада го зголемува научноистражувањето на оваа „статична болест на незрелиот мозок” може да се најдат на PubMed.
зиолошките карактеристики и примената на неврофидбек.

Генерално, анализите покажале дека како етиолошки фактори за аутизмот може да се сметаат помалку познати единствени гени или хромозомски аномалии, како и многу губроно интерактивни гени со послаб ефект. Опсежните анализи по долга на геномот откривле многу сусептибилни локуси, како и позициони или функционални кандидати гени кои може да се сметаат како можен ризик фактор за аутистичниот спектар.

Електрофизиолошките наоди укажуваат на висока тета/делта активност во фронталните мозочни регии, а кај 25% се наоѓаат високи бета-фrekвенции во темпоралните регии како резултат на анксиозност. Неврофидбекот претставува метода која ветува ублажување на симптомите.

Ключни зборови: аутизам, генетика, хромозоми, гени-кандидати, електроенцефалографија, неврофидбек.