Abstract:
The purpose of this review is to summarize the biological factors contributed to the development of the phenotype of Autistic Spectrum Disorders, by elaborating the published research on this topic. According to the researchers, an abnormal brain development due to alternative gene expression is the pattern of ASD. The genetic factor can be considered accepted, though the exact mechanism remains unknown. ASD has a polygenic background (several genes seem to contribute to the pathogenic process), but also environmental interactions (especially in utero) seem to have causal relationship with ASD. The candidate genes encode for proteins that act as neurotransmitters, cell adhesion molecules, or contribute to the brain developmental process in general. Also a latter large scale study strongly implies the role of auto antibodies in the pathogenesis of ASD.
The biological background of autism

By Athina D Papandreou

The term Autism Spectrum Disorders (ASD) or Pervasive Developmental Disorders (PDD) includes a wide spectrum of cognitive and behavioral disorders; individuals developing ASD show lack of communicational skills and impaired social interactions. The common underlying feature of the Autism Spectrum Disorders (including Autistic Disorder, Pervasive Developmental Disorder-Not Otherwise Specified (PDD-NOS), Asperger’s disorder, Childhood Disintegrative Disorder, and finally Rett’s syndrome), is a more or less deficit in social reciprocity skills (DSM-5, 2013). According to DSM-5, the diagnosis will be called Autism Spectrum Disorders (ASD), and there will no longer be subdiagnoses. These disorders are characterized by deficits in social level and difficulties in communication, behaviors and interests that are stereotyped or repetitive, and in some cases, cognitive delays. According to the new diagnostic criteria two areas exist in ASD: (1) social communication/interaction, and (2) restricted and repetitive behaviors (DSM-5, 2013). Along to the above features atypical response to sensory stimuli (affecting one or more sensory perceptual systems) is present (DSM-5, 2013), and displayed via hypersensitivity or underresponse to several aspects of the environment, such as light, noise, smells etc. This difficulty with the input and processing of sensory stimulation can lead to abnormal behavior, or stereotypic patterns (Ventola & Tsatsanis 2011).

The recent changes in DSM’s criteria rise controversies and concerns, about the impact on people on the spectrum; in UK the main set of criteria used for diagnosing is the World Health Organisation’s International Classification of Diseases (ICD).

According to ICD-10, ASD is categorized under the term Pervasive Developmental Disorders (PDD), a group of disorders characterized “by qualitative abnormalities in reciprocal social interactions and in patterns of communication, and by a restricted, stereotyped, repetitive repertoire of interests and activities” (ICD – 10 Version 2010)”. According to ICD, PDD belong to a group of disorders of physiological development. PDD include the syndromes: Childhood autism (symptoms emerging by the age of three), Atypical autism (independent of age, or does not fulfill all diagnostic criteria present in childhood autism), Rett’s syndrome, Other childhood Disintegrative Disorder, Overactive disorder associated with mental retardation and stereotyped movements, Asperger syndrome, Other pervasive developmental disorders and Pervasive developmental disorder, unspecified. As seen from the above, according to ICD diagnostic manual, PDD includes more subcategories, widening the spectrum of a possible diagnosis.

The main symptoms of “typical” (childhood) autism are lying in all three areas of psychopathology, and offer specific diagnosis features: reciprocal social interaction, communication, and restricted, stereotyped, repetitive behavior (ICD-10, 2010). The term “autism” was originally used by Eugen Bleuer (1911) to describe the phenomenon of his schizophrenia-suffering patients turning to their inner selves (Rau, 2003). Later the term “early infantile autism” was used by Leo Kanner, in order to describe a group of children socially isolated and showing a lack of communicational skills. Kanner described autism as an emotional disorder caused by parental absence or psychopathology. These parents were referred to as “refrigerator parents” (Kanner, 1943). It was not until 1960, when it was revealed that some autistic children were also having epileptic seizures, that a neurological background was suggested. Since then, ASD have been a continual and wide-ranging subject of research, lately focusing on the biological (genetic and epigenetic) factors leading to its appearance. It is now described as a neurodevelopmental disorder undergoing a genetic background (Rutter et al., 2000; Keller & Persico, 2003).
The most widely accepted view is that ASD follow a pathophysiological process which is a result of early environmental insult and a genetic predisposition. As a number of factors interfere with brain development, including - but not limited to- normal expression of several genes, environmental factors and viral infections, which can alter gene expression patterns, ASD turn out to be a multifactor neuropsychological disorder (Muhle et al., 2004; Newschaffer et al., 2002; Rapin & Katzmann, 1998; Folstein & Rutter, 1977).

During the last 15 years the incidence of ASD has shown a dramatic increase. The recent studies estimate the prevalence of autistic disorder at around 20 / 10,000, and the prevalence of Pervasive Developmental Disorders Not Otherwise Specified (PDD–NOS, former diagnostic label) at around 30 / 10,000 (Fombonne, 2009). With Asperger disorder and Childhood Disintegrative Disorder taking into consideration, the prevalence of the whole autistic spectrum rises up to 60-70 /10,000, establishing ASD as a not so rare developmental disorder, and one should keep in mind that several cases remain undiagnosed (Kim et al., 2011). Whether this increase is due to improved awareness or actual incidence increase remains unclear at the time (Baird et al., 2006).

Genetic background

The ASD's genetic basis is supported by findings which show that ASD or related disorders appear to exist among individuals of the same family. First-degree relatives of autistic patients can be 'detected' with features belonging to a wider spectrum of autistic traits (Keller & Persico 2003). The “broad autism phenotype” (BAP) implies that relatives can have some of the factors leading to a susceptibility to autism. It is related to personality, language, and social-behavioral impairments, such as anxiety related features, pragmatic language use, unctactful reactions (Losh et al., 2008), decreased interest in reciprocal social interactions and a focus on special interests as a single conversational topic (Gerds & Bernier 2011).

According to the recent study of Ulijarević et al (2014), a high percentage of mothers of children on autistic spectrum, also show unusual responses to sensory stimuli, a feature common to autistic children. Sensory atypicalities have also found in siblings of autistic children, who themselves were not diagnosed with autism (Wouter De la Marche et al., 2012). BAP strongly implies a genetic mechanism in developing ASD features.

Main evidence for the implication of genetic background to the phenotype of ASD provide the twin concordance studies. The conclusive increase of concordance in ASD from dizygotic to monozygotic twins strongly points out the heredity of ASD. A 60% concordance for autism was found in monozygotic twin pairs versus 0% in dizygotic twins, while the heritability at the same time was estimated over 90% (Hallmayer et al., 2002; Bailey et al., 1995). According to a recent study (Hallmayer et al., 2011) though the concordance regarding the monozygotic twins remains the same (estimated at 58 - 60%) the correspondence between dizygotic twins is slightly higher, ranging from 21-27%. Especially when the whole spectrum of ASD was taken into account the concordance rates were assessed at 77% (male twins) and 31 – 36%, for monozygotic and dizygotic siblings respectively (Hallmayer et al., 2011). The proposed concordance rates among dizygotic twins (30%) are consistent with other studies (Rosenberg et al., 2009; Taniai et al., 2008); they imply a strong impact of genetic implication (maybe overestimated), while emphasizing on the environmental influences (non genetic factors).

As mentioned above, the symptoms of ASD lie within a wide spectrum. This means that many different genes are associated, excluding the possibility of a simple Mendelian pattern of inheritance. The currently accepted genetic model of ASD involves 3-15 genes, but also includes gene-gene or gene-environment effects (Risch et al., 1999; Szatmari, 1999) and/or epistatic interactions between several susceptibility genes (Keller & Persico, 2003). This specific model (“multilocus model with epistasis”) produces the clinical phenotype of the disorder.
It is worth mentioning though, that Rett’s Disorder (cerebroatrophic hyperammonemia), which is categorized under the ASD spectrum, shows a specific genetic basis. It is caused by aberrations (mostly de novo) in MECP2 (methyl-CpG-binding protein 2) gene, located on X chromosome, and thus affecting mostly females (although it has been described also in male patients) (Jellinger, 2005). Due to its specific etiology, researchers argue that Rett’s syndrome should be classified together with syndromes such as fragile X syndrome, tuberous sclerosis, or Down syndrome that present autistic features, though themselves are non-autistic spectrum disorders (Tsai1992).

Evidence for genetic basis

Although several chromosomal regions are known to be associated with the prevalence of ASD, the spotting of specific candidate genes proved to be a challenge. Several genes seem to be implicated, but none of them is considered the major susceptibility gene. According to Betancur (2011) autism spectrum disorders are highly genetic disorders; over 1,000 genes are estimated to contribute to ASD risk (Betancur C & Buxbaum JD, 2013)

The candidate genes are not always altered due to mutations, but sometimes present common polymorphisms that are found also in the normal population (Keller & Persico, 2003). The heterogeneity observed across the population, insinuates that the genetic factors triggering ASD are similarly diverse, a conclusion consistent with the majority of the results from recent genetic studies (Werling et al., 2014). The phenotype of ASD often coexists with specific diseases (in about 20% of cases), which have a well-characterized genetic background. Such diseases may cause intellectual disabilities, developmental delays, seizures and other symptoms that are common to ASD. So ASD is often originating from these clinical syndromes, such as untreated phenylketonuria (PKU), tuberous sclerosis, fragile-X syndrome (Smalley 1997), Angelman and Prader-Willi syndromes (Folstein & Rosen-Sheidley, 2001). For example, Fragile X syndrome co-appears in about a quarter of cases of autism (Trottier et al., 1999).

Genes affected

According to the studies available, there seems to be a strong interconnection between gene ENGRAILED 2 (EN2-7q36.2 locus), leading to cerebellar development and ASD (Gharani et al., 2004; Benayed et al., 2005). EN2 is found to contribute in 40% of the ASD cases studied.

Also, genes encoding for gamma-aminobutyric acid (GABA) receptor subunits, such as GABRB3 and GABRA4 genes, seem to contribute to the autism phenotype. GABA is an inhibitory neurotransmitter of the human brain involved in early developmental processes of the cerebral cortex (Keller & Persico, 2003). GABRB3 gene is also responsible for the pathogenesis of Angelman syndrome.

Another protein known to have been associated with ASD is Reelin glycoprotein, a protein that controls neuronal migration in the developing brain by intermediating cell-cell interactions. In an adult brain Reelin associates synaptic plasticity, and so is involved in memory formation. According to Serajee et al., (2006), and Skaar et al., (2005), mutation affecting Reelin gene (RELN) contributes to the phenotype of autism. RELN is located in 7q22, within a region that has shown linkage with autism in several genome-wide scans (Keller & Persico, 2003)

Genes encoding cell adhesion molecules (CAD’s), such as cadherin 10 and cadherin 9 (CDH9, CDH10 -5p14.1 locus), which are neuron-specific cadherines, were associated with autism (Wang et al., 2009). The affected genes demonstrated six single-nucleotide polymorphisms.
Cell adhesion molecules at the synapse are affected also by neuroligines, a well conserved family of proteins. Frame shift mutations affecting two X-linked genes, NLGN3 and NLGN4, which are encoding for neuroligins, are reported in siblings with autism-spectrum disorders (Jamain et al., 2003).

Another protein that seems to contribute to the development of ASD is neurexin 1. This protein is involved in communication between neurons, thus contributes to the development of the brain. It is expressed very early, already in utero or in the first months of life. In 2007, the largest scale genome scan in autism research took place, and reported several aberrations in this particular gene as a cause of some cases of autism (Autism Genome Project Consortium, 2007). The NEUREXIN 1 gene is located in 2q32 region.

It is worth mentioning that many proteins take part in the neuroligin-neurexin interaction at the glutamate synapse. Apart from the above mentioned, SHANK3 protein (SH3 and multiple ankyrin repeat domains 3) also known as proline-rich synapse-associated protein 2 (ProSAP2), a scaffolding protein part of the multi-protein complex that includes also neuroligins, neurexins and the other SHANK family members (SHANK1 and SHANK2), contributes to the cell-to-cell communication and to the formation and maturation of dendritic spines. SHANK3 plays a significant role in recruiting functional molecules to the synapse, including components of α-amino-3-hydroxy-5-methyl-4-isoxazole-propionic acid (AMPA), metabotropic glutamate (mGlu) and N-methyl-D-aspartic acid (NMDA) glutamate receptors (Bozdaqi et al., 2010). Shank3 gene on chromosome 22 has been studied because disruptions leading to loss of function and lower levels of SHANK3 lead to the 22q13 deletion syndrome (Phelan-McDermid syndrome). This syndrome displays several features common in ASD such as developmental delay, intellectual disability, and absent or severely delayed speech.

SHANK3 is found in many of the body’s tissues but is most abundant in the brain in the postsynaptic regions. Shank3 tissue-specific expression is regulated in humans by DNA methylation, which particularly affects protein levels in hippocampal neurons (Boccuto et al., 2013).

In order to reveal the critical role SHANK3 protein plays in the synaptic development, transmission and plasticity, as well as on social behaviors, knock-out mice models have been developed. According to Peca et al., 2011, a mice with shank3 gene deletions exhibited self-injurious repetitive movements and disrupted social interpretations. The loss of a functional copy of shank3 gene (haploinsufficiency) led to reduced reciprocal social interactions (Bozdaqi et al., 2010) in mouse, providing evidence for the contribution of this specific gene to the neurobehavioral deficits present in ASD. The deficits in plasticity are reflected in both electrophysiological and structural abnormalities.

In another study (Boccuto et al., 2013) screened shank3 gene in 221 ASD cases, and revealing pathogenic alterations. A single nucleotide polymorphism (rs76224556) appeared to be significantly associated with PDD-NOS cases, although several variants were present any ASD subtypes. The above studies enhance the causality between aberrations leading to loss of function in the shank3 gene and the manifestation of autistic-like behaviours in mice.

Since hyperserotoninemia is present in 25–45% of patients across studies, the serotonin transporter gene on chromosome 17q was accused of contributing in ASD (Hranilovic et al., 2007; Anderson et al., 1990). Devlin et al., (2005) has found the Serotonin Transporter to be associated with compulsive behaviors, depression and autism in male.
Other genes that affect the development of embryonic brain are HOX genes. HOXA1 and HOXB1 genes (multiple loci), when altered lead to developmental abnormalities. HOX1 gene is specifically associated with increased head circumference, a common feature in autistic children (Conciatori et al., 2004).

Genes suspected of contributing to ASD development are also genes associated with the Oxytocin-Signaling pathway (Keller & Persico, 2003), the c-Harvey-ros oncogene (Herault et al., 1993; Comings et al., 1996) and genes responsible for Angelman’s syndrome (UBE3A). UBE3A(15q) gene, as well as RFWD2 and PARK2 genes are implicated in ubiquitin signaling pathways (Glessner et al., 2009) and protein degradation through proteasome, thus controlling the synaptic plasticity (Yi & Ehlers, 2005).

In addition, some researchers indicate a role of autoimmune diseases, since the presence of autoantibodies to neural antigens, such as myelin, are often found in children with autism (Singh et al., 2002). According to the latest relevant study, mothers of ASD children were more likely to harbor anti-brain antibodies in their serum (Brimberg et al., 2013). These antibodies don't affect the mature brain, due to the blood–brain barrier. In fetuses the blood-brain barrier isn’t functional, and thus can't filter these antibodies. These antibodies can pass to the fetuses' brains, causing damage, possibly by combining with brain tissue antigens. Mothers of an ASD child were four times more likely to have anti-brain antibodies (10.5 vs 2.6% in control group) (Brimberg et al. 2013). Due to the wide scan (the sample includes approx. 2600 women), the effect of these unspecified antibodies to the ASD pathogenesis considers to be indisputable.

Environmental factors

Although it is nowadays widely accepted that the phenotype of ASD is merely due to genetic factors, the evidence of genetic background of ASD cannot exclude the environmental contribution to the appearance of the disorder. Especially factors acting during prenatal period, that affect neurodevelopment, can enhance the possibility for ASD (Lyall et al., 2014). The mechanism is still poorly characterized. It remains unclear whether the genetic background can alone lead to the pathogenesis of the syndromes, or environmental factors may also contribute to the process. The genetic liability seems to be prerequisite for developing the autistic syndromes, but environmental factors often trigger the pathogenic process.

Several environmental factors have been associated with ASD. Prenatal exposure to chemical reagent (such as thalidomide), viral infections and antiepileptic drugs are responsible for independent cases, but are not always the case (Hertz-Picciotto et al., 2006). For some environmental factors, such as thalidomide-induced embryopathy and antiepileptic drugs taken during pregnancy, there is preliminary evidence of such causation (Szatmari P, 2008).

Furthermore there is a continuous debate about the implication of vaccination to the appearance of autism. According to one theory, the MMR (Mumps-Measles-Rubella) vaccine leads to the development of autism. This theory found fertile ground to grow, after the evidence that mother’s immune response can be activated when there is prenatal exposure to rubella, influenza or cytomegalovirus which can increase the risk for autism (Lyall et al., 2014); Libbey et al., 2005; Chess, 1977). This risk seems to be increased at early prenatal stages (first and second trimester) (Atladóttir et al., 2010).

Briefly, the literature suggests a prominent genetic background, but also a conceivable chemical and microbial influence. Whether the environmental impact by itself can give birth to ASD, or can only affect and alter only the severity of the symptoms, need to be further elucidated.

Cellular and neurochemical level
Several macroscopic and microscopic abnormalities of development were revealed using structural and functional imaging of the central nervous system (DiCicco et al., 2006). About half of the autistic patients have abnormal electroencephalograms (Trottier et al., 1999), whereas brain scans show differences in the shape and structure of the brain of minors with autism versus neurotypical children.

A consistent finding retrieved from structural imaging of the ASD brain is an abnormal enlargement. During birth, the brain size does not differ between ASD patients and normal children, but by 3-4 years of age, brain size exceeds normal average by approx. 10 % (Courchesne & Pierce, 2005; Courchesne et al., 2003; Sparks et al., 2002). An approx. 5% difference remains also at older ages (Schultz et al., 2005), showing overall that there is a brain growth phenotype in ASD. The enlargement influences most of the brain regions including the cerebellum, cerebrum, amygdale and hippocampus (Hazlett et al., 2005; Carper & Courchesne, 2005; Sparks et al., 2002; Carper et al., 2002, Aylward et al., 2002; Courchesne et al., 2001; Hashimoto et al., 1995).

Evidence provided by electroencephalograph (EEG) recordings of individuals diagnosed with ASD show a dysfunction in the mirror neuron system. Mirror neurons lie at the premotor cortex and trigger when a person watches the actions of others. Apart from the imitation process mirror neuron system play a critical role in perception and understanding of other peoples actions (Rizzolatti & Fabbri – Destro 2010), but also in higher cognitive processes such as language development. The inability to imitate and learn from others’ actions, or decode their intentions, traits that are found in ASD, can be explained by disabilities affecting particulary this brain area.

At the neuronal level, findings including diverse cell migration, altered synaptogenesis, uncontrolled cell proliferation or cell death, are associated with ASD (Keller & Persico, 2003). Limited neuron integration may lead to autism, according to a theory called "underconnectivity theory" (Just et al., 2012). Functional magnetic resonance imaging (fMRI) gave evidence of poor coordination between brain areas, especially in cortical brain area. Areas that are mostly affected where Broca’s and Wernicke’s areas, the two main language areas. These areas where shown to be less synchronized and especially Broca’s area was also less active, compared to the similar areas of the control group (Just et al., 2012). This evidence was emerged during cognitive demanding tasks, and was established in several studies (Koshino et al., 2008; Kana et al., 2007; 2006; Just et al., 2007; 2004). On the other hand, in local circuits increased connectivity was found, leading to enhanced reactions, during a resting - state cognitive function (Keown et al., 2013; Supekar et al., 2013). Excessive sensory responses, a common feauture present in ASD, (Rudie & Dapretto, 2013) can be interpreted via this mechanism The diversity in areas affected can explain the heterogeneity in symptoms, as well as the fact that some autistic children show superior skills in some areas, while total deficit in others. Since each autistic person experiences different pattern of brain impairing, a wide range of behaviors can be produced among people on the autistic spectrum. The outcomes brought in light by fMRI rise a debate among the researchers concerning the legitimacy of over – or underconnectivity theory. The results can be partly explained, due to the different methods that were used in the studies (Maximo et al., 2014), but still more light should be shed on this aspect, since parameters such as the age of the patients may play a role. According to some researchers hyperconnectivity may be the case in younger patients, but in puberty the pattern seems to change towards underconnectivity (Rudie & Dapretto, 2013). Additionally, there seems to be a correspondence between brain hyperconnectivity and symptom severity, since children who manifested excessive functional connectivity were more severely impaired in the social domain (Superkar et al., 2013). At the neurotransmitting level, the serotoninergic system, the GABA-ergic and the cholinergic system seem to be involved in the pathogenic process. Other neurochemical studies implicate opioid systems and changes in oxytocin neurotransmission (Trottier et al., 1999).
Conclusion

The biological basis of ASD is indisputable. Altered gene expression pattern underlie this disorder; several genes seem to contribute more or less to the manifestation of the symptoms, more of them involving brain cell adhesion molecules, receptors and scaffolding proteins, provoking brain developmental distortions. The altered neurobiological mechanisms give rise to the behavioral and communicational deficits. Considering the number of the genes implicated, and the different brain areas affected, the heterogeneity of the symptoms isn’t surprising. Altogether with the environmental factors that can trigger the syndrome taking into account there is much space for research to elucidate the parameters that contribute to the manifestation of ASD. Establishing the environmental effects can play a critical role in prevention through abstinence from substances that are suspected for contributing to ASD susceptibility, At the same time evaluation of the degree the genes actually play role in the pathogenesis can lead to easier and earlier diagnosis by genome screen, and to the development of personalized efficient treatment.

REFERENCES


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